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An economical multi-channel cortical electrode array for extended periods of recording during behavior

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Abstract

We report the development of a low-cost chronic multi-channel microwire electrode array for recording multi-unit cortical responses in behaving rodents. The design was motivated by three issues. First, standard connector systems tended to disconnect from the head-stage during extended periods of behavior. Disconnections resulted in a loss of data and an interruption of the animals' behavior. Second, the use of low insertion force connectors with locking mechanisms was cost prohibitive. Finally, connecting the head-stage to a skull-mounted connector on an unrestrained animal was highly stressful for both the researcher and animal. The design developed uses a high insertion force DIP socket separated from the skullcap that prevents inadvertent disconnects, is inexpensive, and simplifies connecting unrestrained rodents. Electrodes were implanted in layer IV of primary auditory cortex in 11 Sprague-Dawley rats. Performance of the electrodes was monitored for 6 weeks. None of the behaving animals became disconnected from the recording system during recording sessions lasting 6 h. The mean signal-to-noise ratio on all channels (154) following surgery was 3.9 ± 0.2. Of the 154 channels implanted, 130 exhibited driven activity following surgery. Forty percent of the arrays continued to exhibit driven neural activity at 6 weeks.

Keywords: Auditory cortex; Chronic; Unanesthetized; Behavior

1. Introduction

1.1. Overview

The development of multi-channel neural recording systems allows researchers to simultaneously record from multiple neurons in behaving animals. Chronic multi-channel recordings provide the opportunity to study ensembles to correlate population activity with specific actions during behavior and changes in population responses during learning (deCharms and Merzenich, 1996; deCharms and Zador, 2000; Donoghue, 2002; Nicolelis et al., 2003; Taylor et al., 2002, 2003; Sanes and Donoghue, 2000; Villa et al., 1999). Depending on the research question and animal model, chronic electrodes typically need to provide a stable interface with cortical neurons for weeks or months and in some cases years.

Two general electrode classes are currently being used. Standard microwire designs use an array of small diameter (25–50 μm) wires. The wires typically are made of stainless steel, tungsten, or platinum/iridium and are coated with various nonconductive polymers. The second class of electrodes is constructed from silicon.

Several microwire designs have been reported in the literature and provide viable recordings for extended periods (deCharms et al., 1999; Kralik et al., 2001; McNaughton et al., 1983; Porada et al., 2000; Schmidt et al., 1976, 1988, 1995; Venkatachalam et al., 1999; Westby and Wang, 1997; Williams et al., 1999). Despite the similarities between the microwire designs, the reported lifespan varies widely. Chronic implants have been reported to provide useable recordings ranging from weeks to years in a host of animal models including guinea pigs, cats, rats and primates.

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Westby and Wang (1997) reported 62% of electrodes were active at 5 weeks in rats. Schmidt et al. (1988) reported recordings from 3 electrodes in one monkey for 1144 days. Williams et al. (1999) obtained recordings in guinea pigs for up to 25 weeks. A skull-mounted hydraulic micro-drive reported by deCharms et al. (1999) provided over 18 months of recordings in primates. The variability in recording longevity is likely due to a plethora of differences between research paradigms including electrode design, species, surgical techniques, and electrode design. Despite the variability across designs, microwire arrays have proven to be a reliable, low-cost tool for performing basic research.

Several groups have developed more advanced silicon probes, which provide excellent recording quality due to the precise control over the recording site size and impedance (Nordhausen et al., 1994; Normann et al., 1999; Rousche and Normann, 1999; Rousche et al., 2001; Kipke et al., 2003; Vetter et al., 2004; Wise et al., 2004). Vetter et al. (2004) reported excellent results with the Michigan probe out to 18 weeks. However, the cost and level of expertise required to implant these devices can be prohibitive for smaller labs.

Common to both classes of electrode designs are the use of skull mounted connectors. Having the connector system on the skullcap can make it difficult to connect an unrestrained or naïve animal to the recording system. Typically the animal's head must be restrained to attach the head-stage, which can be highly stressful for both the animal and the researcher. Low insertion force connectors are often used to reduce the amount of force applied to the skull during connection of the head-stage. However, these types of connectors can become dislodged when used in a behaving model. Disconnection can result in a loss of data and possibly the destruction of wires and cables due to chewing. At a minimum, the animal has to be interrupted from its behavioral paradigm to be reconnected.

Another solution for reducing connection force is to utilize a connector system with a locking mechanism. These connectors provide low force insertion and remain connected during behavior, but they are fairly expensive for a small laboratory. For an electrode array, connectors can cost between $40 and $60 per animal depending on the manufacturer and number of connections required.

We developed a low-cost multi-channel micro-electrode array designed for chronic cortical recording in unrestrained non-primate animal models. This novel electrode design utilizes a high insertion force connector separated from the skull cap, providing several advantages over other designs. First, separation of the connector from the skull cap allows the use of high insertion force connectors which do not become disconnected during extended recording periods. Second, the animal does not need to be physically restrained to connect the electrode to the head-stage. Finally, the connector system is inexpensive. Overall the electrode array costs $10 and can be fabricated in approximately 3 h. This report describes the manufacturing process and reports on the recording properties of the electrode array over 6 weeks.

2. Methods

2.1. Electrode construction

The electrodes were constructed in three phases. During the first phase, the microwires and reference wires were attached to the connector. During the second phase, the microwires were arranged in the desired two-dimensional pattern using a custom built electrode jig (Fig. 1A). During the final phase, impedance measurements were taken and the array was sterilized.

2.1.1. Phase I

Fourteen tungsten microwires (50 μm diameter, polyimide insulated; California Fine Wire Co.; Grover Beach, California) were attached to 14 pins of an 18-pin gold-plated DIP socket (Round 18-Pin Machined IC Socket; Pan Pacific). Microwires were cut to lengths, varying by 1/2 in. steps, decreasing from 18 in. (channel 14) to 11 in. (channel 1). The microwires were tightly coiled around the tip of forceps, with a minimum of 5 complete turns. Gripping the base of the coil with forceps, the insulation of the coils was stripped using an open flame. The forceps acted as a heat sink, preventing removal of the insulation past the coiled wire. The stripped, coiled wires were placed over the appropriate pins of the DIP socket and the length of the microwires ran down the center of the connector (Fig. 1B). Free pins from the manufacturer were placed over the DIP socket pin and coil to mechanically attach the microwires to the DIP socket. This resulted in the microwire coil being sandwiched between the pin on the DIP socket and the free pin. The pins were then cut to half height as shown in Fig. 1C. When cut correctly, a cavity was created that revealed the inner pin and coiled wire. The cavity between the two pins was filled with solder to ensure a consistent electrical connection between the pins and microwire. Once the fourteen electrode wires were attached, four pins remained on the DIP socket. These pins were used to connect four Teflon-insulated tungsten microwires (50 μm diameter). Teflon was used instead of the polyimide because it can be mechanically stripped to provide a low impedance reference. The four Teflon wires were attached, in the same manner mentioned above, to four remaining pins of the 18-pin DIP socket. The microwires and reference wires were threaded through heat-shrink tubing (0.2 in. diameter after heating, 1.5 in. long) and a spring (0.25 in. diameter, 1.5 in. long). The heat-shrink tubing and spring were pushed up to the bottom edge of the DIP socket to protect the microwires. Electrical tape was used to make a mold around the connector. The mold was filled with epoxy (Devcon 5-Minute Epoxy; Performance Polymers; Riviera Beach, Florida) to secure the microwires in place. After the epoxy cured, the heat-shrink tubing was filled with a silicone polymer (Silicone Mold-Making Rubber; Smooth-On, Inc.; Easton, PA) and allowed to set for 24 h.

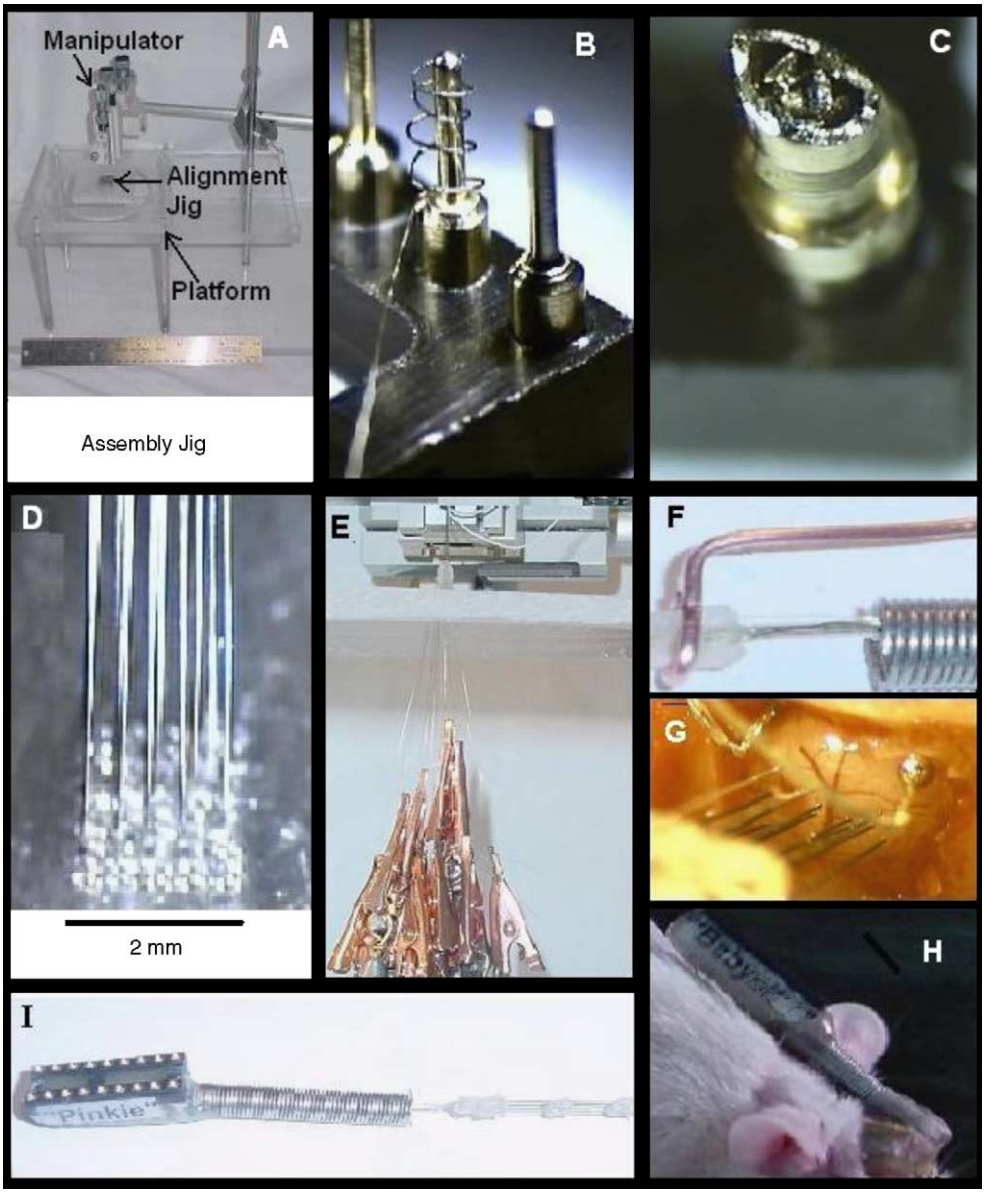

Fig. 1. (A) Electrode assembly jig, alignment jig and manipulator. The assembly jig is approximately 16 in. long and the platform is about 8 in. above the base. (B) Coiled microwire on DIP socket pin. (C) One of the pins after being cut to half height. The cavity is filled with solder to insure a solid connection. (D) Threaded microwires passing through the alignment jig. The alignment jig is a 10 × 10 array of 125 μm diameter holes separated 200 μm center-to-center. (E) Weights hanging on microwires below the jig. (F) Attachment of insertion wire to first acrylic "island." (G) Placement of electrode on primary auditory cortex. (H) Implanted subject. (I) Completed electrode array.

2.1.2. Phase II

The microwires were arranged in the desired two-dimensional pattern using a custom alignment grid (Fig. 1D). The manufacturing jig (Fig. 1A) consisted of three parts: the platform, the alignment grid, and the micro-positioner. The platform was machined from 3/8 in. Plexiglas and aluminum rods. The alignment grid (0.00125 in. thick, stainless steel; Laser Rod, California) was a 10 × 10 array of laser-trepanned holes (125 μm diameter, 250 μm center-to-center). The 3-axis micro-positioner (KITE-R; World Precision Instruments, Inc.; Sarasota, Florida) was attached to a rod extending up through the platform.

A 2 × 2 in. piece of low-density polyethylene (Glad Cling-Wrap) was stretched taut over the top of the alignment grid and taped down to prevent acrylic from filling the holes. The DIP socket was placed in the micro-positioner directly above the alignment grid. Using a binocular microscope, the microwires, including the reference wires, were fed individually through the holes of the alignment grid and pulled taut by attaching weights (Fig. 1D and E). Care was taken to prevent stripping the insulation from the wires.

After all microwires were threaded and weighted, the micro-positioner was lowered so that the bottom of the spring was approximately 1.5 cm above the alignment grid. One

large hourglass shaped "island" of acrylic (Kiss Acrylic; Port Washington, New York) was placed approximately 1 cm below the bottom of the heat-shrink and spring protective covering. The hourglass shape was accomplished by first placing a 4 mm ball of acrylic on the wires and then a smaller ball on top of the first. Two or three small diameter "islands" at least 3 mm tall were placed 7 to 10 mm apart down the length of the wires. Finally, the microwires were cut just above the weights and the electrode assembly was removed from the micro-positioner.

2.1.3. Phase III

The excess epoxy on the DIP socket was machined down and smoothed to reduce the weight and roughness of the connector (Fig. 1I). Copper wire (18 AWG) was bent into a u-shaped hook and then crimped around the hourglass "island" for use as an insertion rod (Fig. 1F). Impedance measurements were taken to ensure a good connection. The microwires had an average impedance of 60 kΩ at 1 kHz in saline. Finally, the entire electrode assembly was gas-sterilized using ethylene oxide. Finished electrodes weighed approximately 6 grams on average.

2.2. Implantation

The subjects were 2- to 4-month-old Sprague-Dawley female albino rats with weights of 200–300 g obtained from Charles River Labs (Dallas, TX). The subjects were individually housed and exposed to a 12:12 h light-to-dark cycle. Recording sessions were performed during the light portion of the cycle. All animals were habituated to handling for at least 1 week prior to implantation.

Eleven animals were implanted with the described 14 channel microwire electrode arrays. Surgery was conducted using standard sterile procedures in accordance with the University of Oklahoma's Laboratory Animal Resources and Institutional Animal Care and Use Committee regulations. Animals were anesthetized using Ketamine, Xylazine, and Acepromazine (50, 20, 5 mg/kg respectively). Their heads were shaved and cleaned using a triple application of alcohol and Betadine. Atropine, dexamethazone, and antibiotics were administered subcutaneously prior to and following surgery.

Lidocaine was injected under the scalp and a midline incision was made caudally to the animal's eyes and ending between the ears. The connective tissue was blunt-dissected from the skull, and the top of the skull was exposed and cleaned using hydrogen peroxide. The right temporalis muscle was partially dissected exposing the temporal bone and lateral suture. The temporal bone was cleaned with hydrogen peroxide. A #55 stainless steel drill bit was used to drill two holes in both parietal bones and one hole in both frontal bones. Care was taken to avoid drilling near the underlying sinuses.

A 2 × 3 mm portion of the temporal bone was removed adjacent to the lateral suture just above primary auditory cortex. A #26 hypodermic needle was used to slit the dura, and dura scissors were used to resect the dura, exposing the pia. The micro-electrode array was cut between the last two "islands" with sterile scissors. The Teflon-coated reference wires were stripped using forceps. Using a spring-loaded insertion device mounted to a micro-drive (Rennaker et al., 2005), the array was implanted to a depth of 600 μm at a rate of 1.49 m/s in primary auditory cortex (Fig. 1G).

A silicone elastomer (Kwik-Cast; World Precision Instruments, Inc.; Sarasota, Florida) was used to form a rubber-like gasket to seal the craniotomy and cover the exposed brain. Once the silicone cured, acrylic was used to cover the elastomer and the surrounding temporal bone and to secure the first "island" to the bone screws. Once the acrylic dried, the electrode was removed from the insertion device, and the insertion rod was carefully disconnected. The connector was oriented caudally above the midline and the heat-shrink and spring protective covering was secured using acrylic. The connector was positioned such that it did not touch the animal's back when sitting. Finally, sutures were sewn at the front and back of the incision, pulling the skin tight around the skull cap (Fig. 1H). Prophylactic antibiotics were administered orally for 5 days post-implantation. Placement of the connector does not appear to aggravate the animal post-implantation.

2.3. Auditory stimuli and recording

Following surgery and at the beginning of each of the following 5 weeks animals were placed in a double-walled acoustic chamber. Naïve animals tended to sit in one of the back corners during attachment to the head-stage. The elasticity of the spring and silicone sleeve allowed the animal to move its head from side to side without disruption of the connection process. New undergraduate researchers were able to quickly master connection of the animals. Disconnection of the animals required the use of a small standard screwdriver. The screwdriver was placed between the electrode and head-stage and rotated. This process provided quick (<1 min), reliable disconnection of the animal. The animals appeared to become habituated to this process within a few days.

Auditory stimuli were generated using a calibrated free-field speaker in a double-walled acoustic chamber using Tucker-Davis Technologies (TDT) System 3 hardware. The pure tone stimuli consisted of 25 repetitions of a set of 45 tones. The tones were 35 ms in duration at 55 dB SPL, and the frequencies ranged from 1.3 to 32 kHz, at intervals of 0.03125 octaves, spanning most of the rat hearing range. The tones had a 5 ms onset and offset cosine ramp. The broadband clicks had a frequency range from 1.3 to 23 kHz at 50 dB (SPL). All stimuli were characterized using an ACO Pacific 1/4 in. microphone and the spectral analysis was plotted on a digital oscilloscope. Multi-unit neural responses were recorded using Brainware (TDT) software. The neural data were digitized at 25 kHz and band-pass filtered from 500 to 5000 Hz, 12 dB/oct. A custom-made head-stage amplifier (TDT) was directly attached to the electrode connector. Spike detection

was accomplished using a simple manual threshold detector. The threshold was set above the noise and below spike peaks by zooming in on the raw recording.

2.4. Analysis

Multi-unit responses to auditory stimuli on each channel were plotted as Peri-Stimulus Time Histograms (PSTHs) with bin sizes of 1 ms. Spikes were not sorted for the individual channels. Spontaneous rates were measured 30 ms prior to stimulus presentation and averaged over the stimulus set. Onset response rates were measured from 10 to 30 ms after the stimulus onset and averaged over the stimulus set. Channels were defined as driven if the onset response rate was significantly greater than the spontaneous rate (*t*-test, $\alpha = 0.05$). Driven responses were calculated by subtracting the spontaneous rate from the onset response rate. All data were analyzed using custom software written in MatLAB or Visual Basic. Tuned multi-unit responses were defined by frequency selectivity with a best frequency peak response at least 50 Hz greater than off-peak responses. Two-second raw signal recordings were taken from each animal at least once per week for 6 weeks, and the signal-to-noise ratio (SNR) was calculated for all electrodes. The signal-to-noise ratio was defined as the peak-to-peak value divided by the RMS value of the signal. The RMS value was calculated from the entire 2-s recording. Channels that did not exhibit unit activity were assigned a SNR of 1. All data presented were collected from unrestrained awake animals.

3. Results

None of the electrodes became disconnected from the head-stage during extended recording periods lasting up to 6 h. The electrode arrays took a practiced researcher about 3 h to manufacture and 3 h to implant. The entire array cost less than $10 in materials, making them reasonable for studies requiring large numbers of animals.

3.1. Recording quality

Responses to auditory stimuli were obtained from animals immediately following the surgical procedure, and again every week for a total of 6 weeks. All data reported after the first week were collected from awake freely-moving rats. Fig. 2 is an example of a raw recording at 6 weeks in a behaving rat. The dashed line represents a typical threshold setting. The waveform on the right side of the figure was selected from the 4.8 to 5.6 ms post-stimulus onset portion of the recording as annotated by the black bar. The dots on the spike denote the actual samples for this waveform (25 kHz). The typical peak-to-peak voltage using these electrodes ranged from 100 to 200 μV with a 40–50 μV RMS noise level. Two or three distinct waveform shapes can be identified in the 60 ms recording in Fig. 2. The threshold is set manually and is annotated by the dashed line. Typically, as represented in this figure, the threshold value was set to avoid capturing noise, resulting in some of the smaller spikes not being recorded.

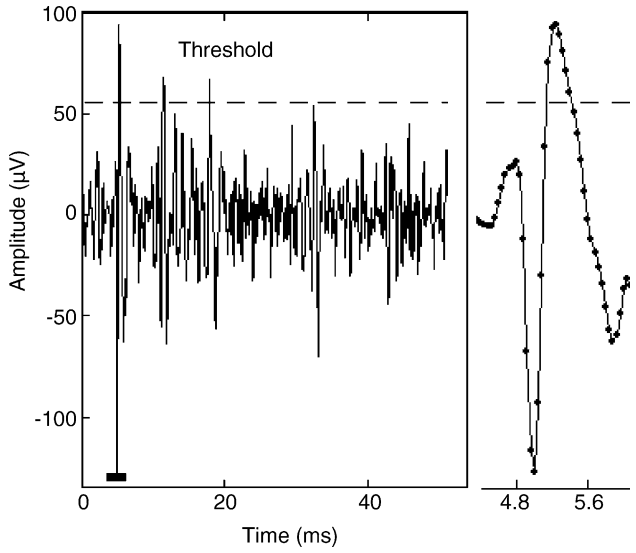

Fig. 2. A 60 ms raw recording from one electrode at week 6. The dashed line represents the typical threshold setting. The waveform to the right of the raw recording was extracted from the recording at 4 ms. The dark box below the spike on the raw recording denotes this time segment. The dots on the waveform are the actual samples taken by the recording system at 25 kHz. At least two other spikes can be seen in this figure.

Raw recordings were taken once a week to determine the signal-to-noise ratio. These data were not collected from three animals due to scheduling errors and are not included in the calculations. The mean SNR was calculated for the 8 remaining arrays and is displayed in Fig. 3. The horizontal bars at the bottom of the plot denote arrays with active channels. The labels at the end of the bars denote the animal. The post-implantation SNR was 3.9 ± 0.2 following surgery and re-

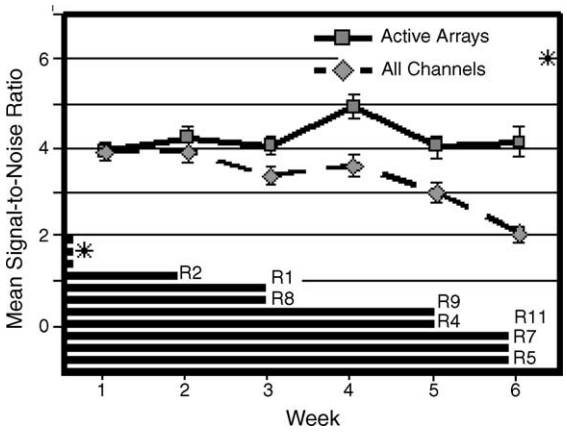

Fig. 3. A plot of the mean signal-to-noise ratio (SNR) over 6 weeks. The solid line represents the mean SNR for all active arrays. Active arrays are defined as arrays with at least one driven channel. Non-active arrays are not included in that week's measure of SNR. The dashed line is the mean SNR for all channels. The error bars shown are standard error of the mean. (*) SNR data were not available for three of the arrays.

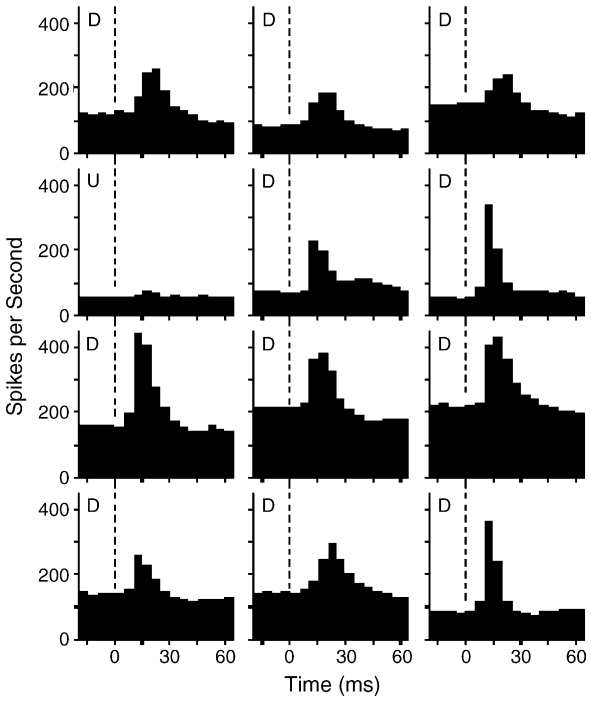

Fig. 4. Simultaneous recordings from 12 channels on one array were plotted as PSTHs. The PSTHs demonstrate the typical driven response to auditory stimuli. The dashed line marks the start of the auditory stimulus. The "D" and "U" in the upper left corner denote channels defined as driven or undriven, respectively.

mained near 4:1 for the following 5 weeks (Fig. 3). The solid line represents the SNR for all channels on active arrays, while the dashed line is the SNR for all 84 channels. The plot shows that those arrays that exhibit neural activity remain relatively stable. Much of the decrease in SNR is due to loss of discriminable neural activity from entire arrays. The loss of discriminable spiking activity from an array tended to occur rapidly.

3.2. Driven response properties

Investigations into plasticity and learning in primary auditory cortex require that the functional response properties (driven neural activity in response to auditory stimuli) remain relatively stable. The driven response was monitored over 6 weeks to assess the usefulness of this electrode for those types of studies.

Fig. 4 displays an example of simultaneous PSTH responses to pure tone stimuli taken 1 week post-implantation from a single array. All but one channel on this array display significant driven responses, and it is denoted by a "U" in the upper left hand corner. The two channels not shown also display significant driven responses similar to the others but were not shown to conserve space. The dashed line denotes the start of the stimulus. All of the driven channels exhibit short latencies and strong onset responses. It was not uncommon to find channels with multi-unit spike rates between 200 and 400 spikes per second in response to pure tones. Onset

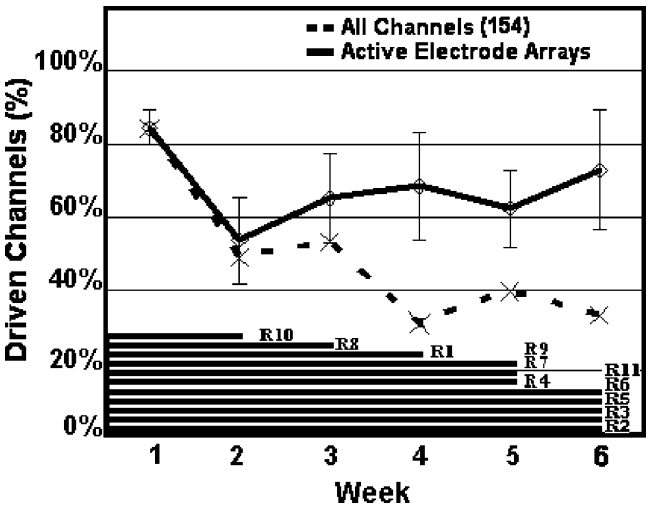

Fig. 5. The percent of driven channels was recorded for 6 weeks. The horizontal bars denote active arrays. Active arrays are defined as having at least one driven channel. The solid line is a plot of the percent of driven channels for all active arrays. The dashed line represents the percent of driven channels for all arrays. Error shown is standard error of the mean.

response latencies were typically between 8 and 15 ms to tones and clicks.

In the first week following implantation 82% of the 154 channels implanted exhibited significant driven responses to auditory stimuli (Fig. 5). The horizontal bars at the bottom of the figure denote arrays exhibiting driven neural activity that week and are included in the active electrode array calculations (solid line). At week two, the percent of driven channels dropped from 82 to 54%. The percent of driven channels continues to decrease across the 6 weeks when looking at all channels implanted. However, the plot of driven channels on arrays with driven responses illustrates that 60% of channels remain driven. This percentage remains relatively stable from week 2 to 6. Eight of the 11 arrays provided 5 weeks of recordings while 5 of the 11 lasted the entire 6 weeks.

Fig. 6 displays the mean number of channels per electrode exhibiting tuned responses to auditory stimuli. The horizontal bars represent arrays with driven activity. Initially there were 8 tuned channels per array on average. The average number of tuned channels drops to approximately 4 at week 4 and continues to decrease when considering all channels implanted. Analysis of only those arrays exhibiting any driven activity decreases out to week 4, but then goes back up to 8 at week 6.

Fig. 7 displays the functional responses of a representative single electrode over a 6 week period. As can be seen, recording quality is relatively stable until the sixth week, when there is a marked decrease in driven response. Degradation of functional responses typically occurred abruptly, and could be attributed to a decrease in signal-to-noise ratio which impaired spike extraction with threshold detection.

PSTH properties over time are reflective of the effect of signal-to-noise decreases on spike detection, and driven responses are seen to drop abruptly before loss of multi-unit

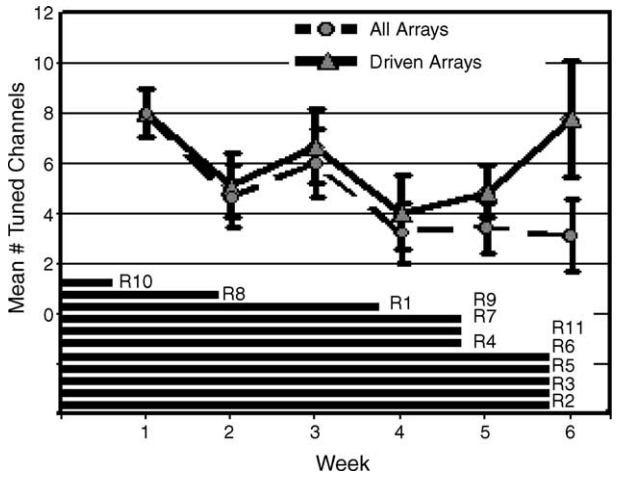

Fig. 6. The mean number of tuned channels was recorded for 6 weeks. The horizontal bars denote active arrays. Active arrays are defined as having at least one driven channel. The solid line is a plot of the percent of tuned channels for all active arrays. The dashed line represents the percent of tuned channels for all arrays. Error shown is standard error of the mean.

recordings. In turn, tuning properties are dependent on the strength of driven responses and become less sharp as SNR decreases. For the first 5 weeks of recording for the electrode shown in Fig. 7, the best frequency of the multi-unit cluster varied by approximately 0.125 octaves and showed strong responses to a relatively narrow band of frequencies, but the effect of the degradation of driven responses in week 6 can be seen in the increased variability of tuning. Functional responses on electrodes in this study lasted anywhere between one and 18 weeks, but showed similar patterns of failure regardless of functional life.

4. Discussion

This report documents the manufacturing methods and recording properties of a novel electrode design that separates the connector system from the skullcap. The design developed uses a high insertion force DIP socket separated from the skullcap, which prevents inadvertent disconnects, is inexpensive, and simplifies connecting unrestrained rodents. These novel features provided up to 6 h of continuous behavioral recordings. During this 6-week study and in the 6 months following, none of the electrodes have become disconnected from the head-stage. The electrode was easily attached to the head-stage on an unrestrained rat. The electrode's flexibility allowed the animal to move its head while being connected. This feature reduced the stress on the animals and researchers. Finally, the use of off-the-shelf connector systems significantly reduces the cost. The DIP sockets used in this design cost $.50 per animal versus $20 to $40 per animal with dual row locking connectors. The design is appropriate for studies requiring three to 6 weeks of viable recordings from non-primate behaving animals.

Westby and Wang (1997) reported the development and testing of a floating electrode array in the superior colliculus of rats. They followed the forty-two best electrodes of the 252 implanted. They reported 62% of 42 electrodes provided single-unit recordings for 5 weeks, with 24% of the 42 electrodes recording from the same cell at week 5 (Westby and Wang, 1997). One implant was reported to last 9 months. Venkatachalam et al. (1999) reported useful recordings for 1–2 month periods in rat somatosensory cortex using a skull-mounted microdrive. The longevity reported with the current device is consistent with other reports in rats.

Williams et al. (1999) demonstrated viable recordings in guinea pigs for 25 weeks, using the same materials and general surgical procedure as reported in this electrode design except for the connector system. Schmidt et al. (1988) and deCharms et al. (1999) reported recordings for more than a year. Differences including behavioral activity, skull thickness, depth of insertion, surgical procedures, and immune responses may account for the disparity in longevity. Without histological evidence or a direct comparison between species it is difficult to determine the actual cause for decreased longevity in rats.

The design reported does have several features that could decrease recording longevity. The design is likely to place added stress on the microwires at the attachment points. Specifically, the stress could occur where the wires enter the acrylic skullcap and also the epoxy on the connector. Bending of the flexible portion of the connector could cause the wires to break. The electrode was positioned on the skullcap to minimize motion and to prevent the connector from constantly rubbing the animal. However, the connector did bend during excessive movements: when it hit the side of the cage or when the animals aggressively groomed themselves. Broken wires typically resulted in large 60 Hz noise on a channel. While impedance measures were not taken to verify broken wires, typically very few channels exhibited significant changes in noise levels after failure. This suggests that failure in most cases was likely due to some other mechanism such as encapsulation of the electrode or insulation degradation. Only one of the arrays in this study exhibited failure due to visible breaking of the wires resulting in large 60 Hz noise on the entire array, and this occurred after the sixth week.

Walking around the cage or simple head movements induced very little noise. However, the noise could saturate the amplifier during periods of extreme restlessness. Quantification of the noise is difficult, but typically lasted 10–20 ms and was low frequency in nature. The use of silicone in the heat-shrink tubing minimizes the noise by restricting motion of the connector. Fig. 2 displays a recording from an animal actively moving in the recording booth. The spikes are easily discriminable from the noise. Behavioral tasks using nose poke holes have been designed to reduce motion of the animals during behavior. Several design changes, such as the incorporation of field effect transistors into the skullcap, and shortening of the flexible portion of the array could be implemented to further reduce noise. It may be possible to

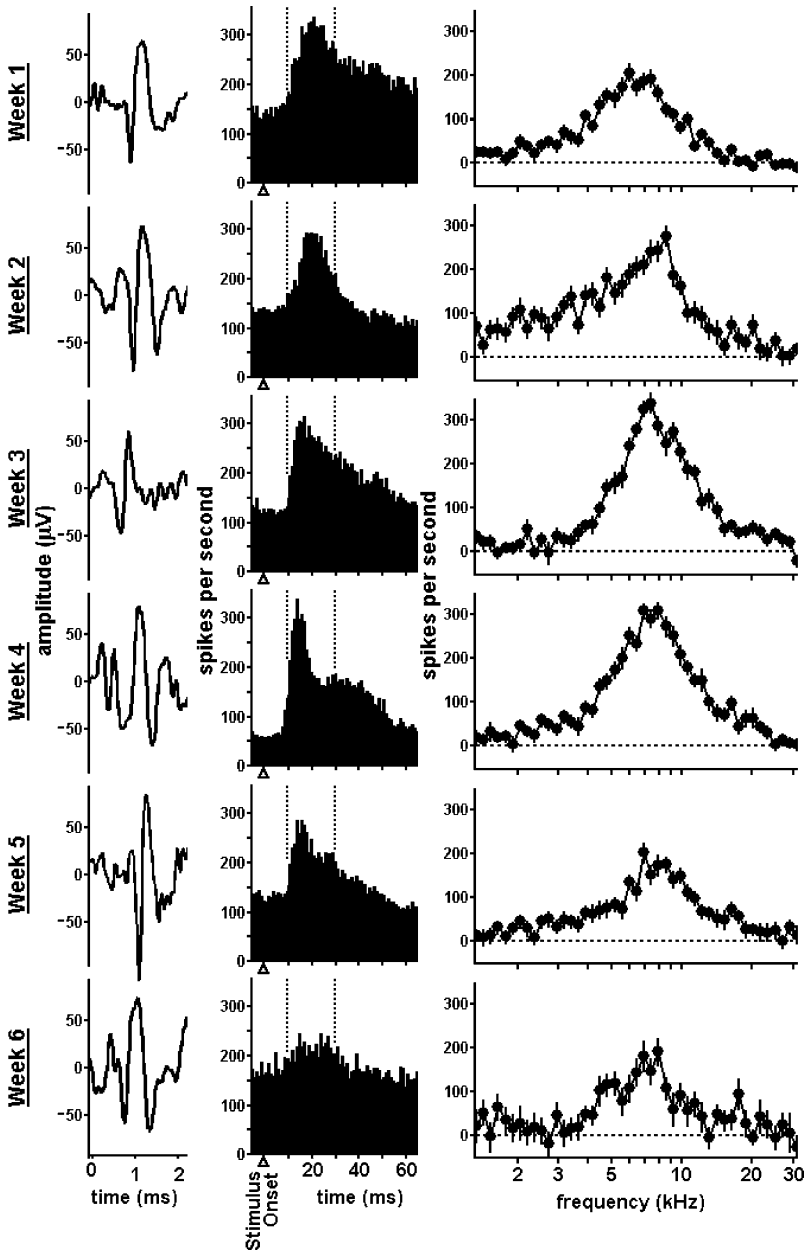

Fig. 7. Data from a single electrode are displayed for 6 weeks. The left column displays one of the waveform shapes recorded from the electrode for each week. The middle column displays the PSTH to all pure-tones of an iso-intensity tuning curve (IsoTC). The right column displays the tuning properties of the multi-unit cluster in response to the IsoTC. Dashed lines annotate the onset response window.

extend the recording life to 12 or 15 weeks given the recording longevity seen with Rat #2 in this study. Low production cost and manufacturing ease make it possible for standard research labs to fabricate large quantities of multi-channel arrays with a minimum time investment. Arrays can be readily made into a variety of two-dimensional patterns not offered by some of the other reported techniques (Tsai and Yen, 2003; Williams et al., 1999). The flexibility of this design permits the manufacture of custom arrays for recording from specific structures.

Thirty animals were implanted during the development of the electrode using a manual insertion method. Manual implantation, however, resulted in poor electrode yield and performance over time. None of the animals implanted with the manual technique provided recordings for more than 3 weeks (Rennaker et al., 2005). It is believed that the manual insertion procedure induced traumatic brain injury as a result of cortical compression during insertion, limiting the recording longevity. The 11 animals in the present study were implanted with a custom-built mechanical insertion device which implanted the array at a velocity of 1.49 m/s with no cortical compression as viewed under a digital microscope. The velocity of the insertion devices was slower than the 8.3 m/s used by Rousche and Normann (1992). A slower ve-

locity could be used due to the decreased density and number of electrodes with this electrode design. Other techniques for reducing the amount of cortical compression, such as etching the electrodes, attaching the pia to the skull with cyanoacrylate, or using vacuum pressure to hold the cortex in place have been reported elsewhere (Bai et al., 2000; Kralik et al., 2001; Rousche and Normann, 1992; Venkatachalam et al., 1999).

This study documented the recording properties of a novel microwire design developed for low-cost chronic recordings in behaving rodents. The array is designed to minimize inadvertent disconnections between the electrode and head-stage during behavior. The array provides 5–6 weeks of driven multi-unit recordings on 70% of the electrodes. The design provides a customizable, low-cost solution for labs interested in extended chronic recording sessions in behaving rodents.

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References

Bai Q, Wise KD, Anderson DJ. A high-yield microassembly structure for three-dimensional microelectrode arrays. IEEE Trans Bio-Med Eng 2000;47:281–9.

deCharms RC, Merzenich MM. Primary cortical representation of sounds by the coordination of action-potential timing. Nature 1996;381:610–3.

deCharms RC, Zador A. Neural representation and the cortical code. Annu Rev Neurosci 2000;23:613–47.

deCharms RC, Blake DT, Merzenich MM. A multielectrode implant device for the cerebral cortex. J Neurosci Methods 1999;93:27–35.

Donoghue JP. Connecting cortex to machines: recent advances in brain interfaces. Nature Neurosci 2002;5(Suppl 1):1085–8.

Kipke DR, Vetter RJ, Williams JC, Hetke JF. Silicon-substrate intracortical microelectrode arrays for long- term recording of neuronal spike activity in cerebral cortex. IEEE Trans Neural Syst Rehabilitation Eng 2003;11:151–5.

Kralik JD, Dimitrov DF, Krupa DJ, Katz DB, Cohen D, Nicolelis MA. Techniques for long-term multisite neuronal ensemble recordings in behaving animals. Methods 2001;25:121–50.

McNaughton BL, O'Keefe J, Barnes CA. The stereotrode: a new technique for simultaneous isolation of several single units in the central nervous system from multiple unit records. J Neurosci Methods 1983;8:391–7.

Nicolelis MA, Dimitrov D, Carmena JM, Crist R, Lehew G, Kralik JD, et al. Chronic, multisite, multielectrode recordings in macaque monkeys. Proc Natl Acad Sci USA 2003;100:11041–6.

Nordhausen CT, Rousche PJ, Normann RA. Optimizing recording capabilities of the Utah intracortical electrode array. Brain Res 1994;637:27–36.

Normann RA, Maynard EM, Rousche PJ, Warren DJ. A neural interface for a cortical vision prosthesis. Vision Res 1999;39:2577–87.

Porada I, Bondar I, Spatz WB, Krüger J. Rabbit and monkey visual cortex: more than a year of recording with up to 64 microelectrodes. J Neurosci Methods 2000;95:13–28.

Rennaker RL, Street S, Ruyle AM, Sloan AM. A comparison of chronic multi-channel cortical implantation techniques: manual vs. mechanical insertion. J Neurosci Methods 2005 (in press). doi:10.1016/j.neumeth.2004.08.009.

Rousche PJ, Normann RA. A method for pneumatically inserting an array of penetrating electrodes into cortical tissue. Ann Biomed Eng 1992;20:413–22.

Rousche PJ, Normann RA. Chronic intracortical microstimulation (ICMS) of cat sensory cortex using the Utah Intracortical Electrode Array. IEEE Trans Rehabilitation Eng: A Publication of the IEEE Engineering in Medicine and Biology Society 1999;7:56–68.

Rousche PJ, Pellinen DS, Pivin DP, Williams JC, Vetter RJ, Kipke DR. Flexible polyimide-based intracortical electrode arrays with bioactive capability. IEEE Trans Bio-Med Eng 2001;48:361–71.

Sanes JN, Donoghue JP. Plasticity and primary motor cortex. Annu Rev Neurosci 2000;23:393–415.

Schmidt EM, Bak MJ, McIntosh JS. Long-term chronic recording from cortical neurons. Exp Neurol 1976;52:496–506.

Schmidt EM, McIntosh JS, Bak MJ. Long-term implants of Parylene-C coated microelectrodes. Med Biol Eng Comput 1988;26:96–101.

Schmidt EM, Bak MJ, Christensen P. Laser exposure of Parylene-C insulated microelectrodes. J Neurosci Methods 1995;62:89–92.

Taylor DM, Tillery SI, Schwartz AB. Direct cortical control of 3D neuroprosthetic devices. Science 2002;269:1817–8.

Taylor DM, Tillery SIH, Schwartz AB. Information conveyed through brain-control: Cursor versus robot. IEEE Trans Neural Syst Rehabilitation Eng 2003;11:195–9.

Tsai ML, Yen CT. A simple method for fabricating horizontal and vertical microwire arrays. J Neurosci Methods 2003;131:107–10.

Venkatachalam S, Fee MS, Kleinfeld D. Ultra-miniature headstage with 6-channel drive and vacuum-assisted micro-wire implantation for chronic recording from the neocortex. J Neurosci Methods 1999;90:37–46.

Vetter RJ, Williams JC, Hetke JF, Nunamaker EA, Kipke DR. Chronic neural recording using silicon-substrate microelectrode arrays implanted in cerebral cortex. IEEE Trans Biomed Eng 2004;51:896–904.

Villa AE, Tetko IV, Hyland B, Najem A. Spatiotemporal activity patterns of rat cortical neurons predict responses in a conditioned task. Proc Natl Acad Sci USA 1999;96:1106–11.

Westby GW, Wang H. A floating microwire technique for multichannel chronic neural recording and stimulation in the awake freely moving rat. J Neurosci Methods 1997;76:123–33.

Williams JC, Rennaker RL, Kipke DR. Long-term neural recording characteristics of wire microelectrode arrays implanted in cerebral cortex. Brain Res Protocols 1999;4:303–13.

Wise KD, Anderson DJ, Hetke JF, Kipke DR, Najafi K. Wireless implantable microsystems: high-density electronic interfaces to the nervous system. Proc IEEE 2004;92:76–97.