



A bundled microwire array for long-term chronic single-unit recording in deep brain regions of behaving rats

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ABSTRACT

Chronic single-unit recording in subcortical brain regions is increasingly important in neurophysiological studies. However, methods for long-term, stable recording of multiple single-units in deep brain regions and in dura-surrounded ganglion have not yet been established. In the present study, we propose a bundled microwire array design which is capable of long-term recording of the trigeminal ganglion and deep-brain units. This electrode set is easy to construct from common materials and tools found in an electrophysiological laboratory. The salient features of our design include: (1) short and separated tungsten microwires for stable chronic recording; (2) the use of a 30-gauge stainless steel guide tube for facilitating penetration and aiming for deep targets as well as electrical grounding; (3) the inclusion of a reference of the same microwire material inside the bundle to enhance common mode rejection of far field noises; and (4) an adjustable connector. In our case, we used a 90° backward bending connector so that implanted rats could perform the same hole-seeking behavior and their faces and the whiskers could be stimulated in the behaving state. It was demonstrated that this multi-channel electrode caused minimal tissue damage at the recording site and we were able to obtain good, stable single-unit recordings from the trigeminal ganglion and ventroposterior medial thalamus areas of freely moving rats for up to 80 days. This methodology is useful for the studies that require long term and high quality unit recording in the deep brain or in the trigeminal system.

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1. Introduction

Monitoring the change of activity of the same neuron for an extended period of time is needed for probing many physiological and pathological questions. For example, Herry et al. (2008) recorded the same amygdala neurons for 11 days in series of behavior tasks to test the role of single amygdaloid neuron in context-specific conditioning and extinction. In the field of long-term memory and chronic diseases, an even longer monitoring time is required.

Many types of electrodes, such as movable electrodes (Eliades and Wang, 2008; Haiss et al., 2010; Jackson and Fetisov, 2007; Wilson et al., 2003; Yamamoto and Wilson, 2008; Yang et al., 2010), tetrodes (Tolias et al., 2007), microarrays (Nicolelis et al., 2003), microelectrode bundles (Herry et al., 2008; Kubie, 1984; Nicolelis et al., 1997; Szymusiak et al., 1998), and silicone based probes (Suner et al., 2005; Vetter et al., 2004), have been successfully used to obtain good unit recordings chronically in rodents and primates. In the primate cortex, good quality neuronal recording could be extended to 1.5 years using microarray electrodes (Nicolelis et al.,

2003) and silicone based probes (Suner et al., 2005). However, high density array electrodes have limitedly used in deeper brain regions due to their tendency of causing severe brain damages. In addition, it is very difficult for the conventional linear microwire arrays to reach deep brain targets accurately and thus is of little practical use.

Bundled microwires, either singularly or in stereode/tetrode configurations, are better choices for making recordings in the nuclei of the deeper brain, and these were successfully used to record the nuclei in many regions, such as the amygdala (Chang et al., 2005; Herry et al., 2008), hypothalamus (Szymusiak et al., 1998), hippocampus (Kubie, 1984; Thompson and Best, 1990), and lateral thalamus (Nicolelis et al., 1997). It has been demonstrated in rabbits and primates that the temporal recording stability of bundle electrodes in visual cortex (Porada et al., 2000) and hippocampus (Thompson and Best, 1990) was over a year. However, one difficulty is the recording of trigeminal ganglion (TG) units. TG is located deep under the brain (~9 mm from the brain surface in a rat) while being surrounded by thick dura. Therefore, long thin microwires (~1 cm long), even when bundled together, cannot have sufficient rigidity to penetrate the layers of dura to reach it. Several studies successfully recorded the single-unit activity of TG using single-channel metal electrode with a wider shank (125 or 250 μm, to maintain the rigidity) and a sharp tip (to penetrate more easily) (Bermejo et al., 2004; Khatri et al., 2009; Leiser and Moxon, 2007).

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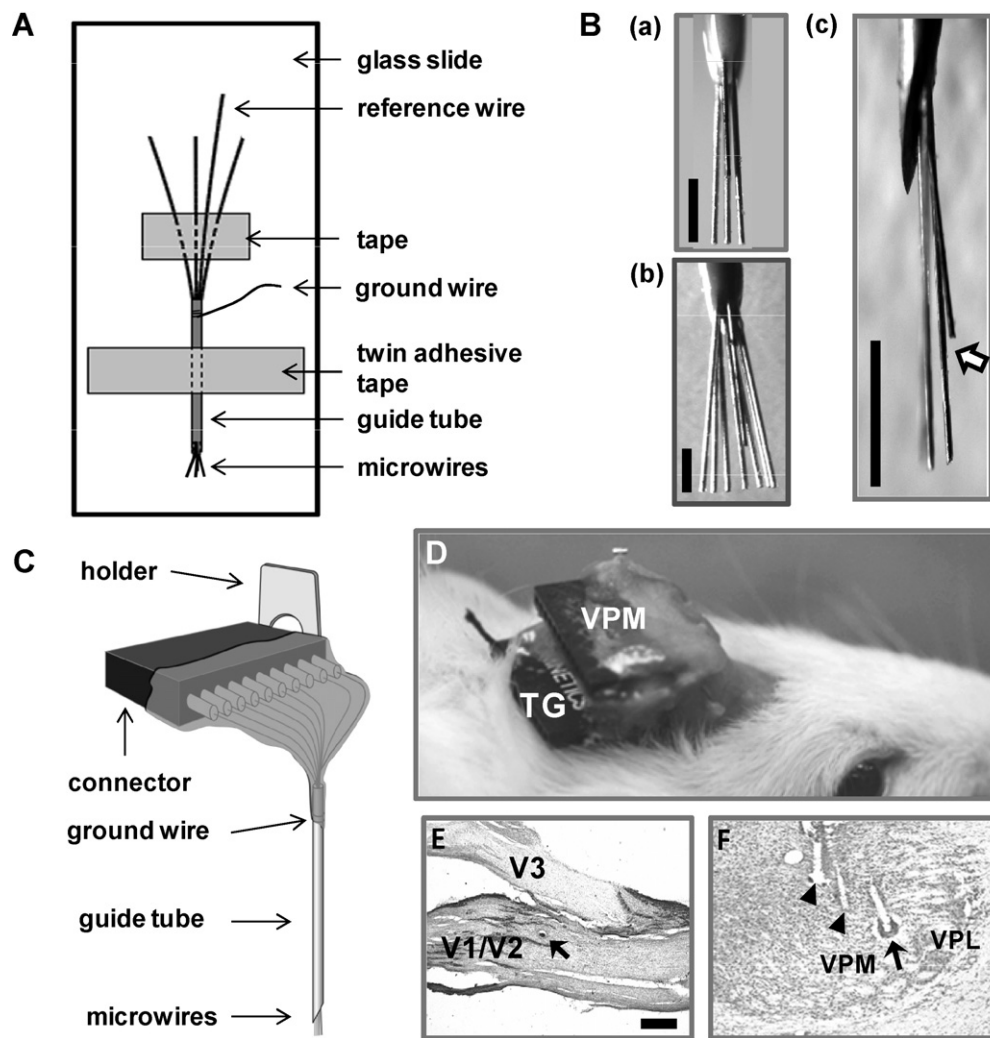


Fig. 1. (A) Fabrication jig and assembly of the guiding tube (a 30-gauge needle) and Teflon-coated tungsten microwires (three for recording and one for reference). The three recording microwires were arranged separately and sandwiched between Scotch tapes. The reference microwire is longer than the other wires for easy identification. (B) The fan-shaped microwires at the recording end (TG, Ba and thalamus, Bb). Note that the reference microwire is in the middle position and shorter than the other microwires (Bc, arrow). (C) Sketch of a finished product for the thalamic recording. Seven microwires, one reference microwire and one ground wire were all soldered to a connector. The holder was removed after implantation. The connector was bent 90° backwards. (D) Photograph to show the final appearance of two electrodes implanted in a rat's head. Histological sections of TG (E) and ventroposterior medial nucleus of the thalamus (VPM) (F) show tracks of microwires (arrowheads) and electrolytic lesions (arrows) of selected recording sites. VPL: ventroposterior lateral nucleus of the thalamus; V1, V2, and V3 denote divisions of the trigeminal nerve. Scale bar: 500 μm .

The drawback of the above-mentioned method is using the wider shank as well as the limited channel number; accordingly only one or two electrodes can be implanted.

Ideally, it is preferable to have more channels while minimizing tissue damage within a certain brain region. We noted that a tungsten microwire maintains strong rigidity when it is shorter than 1.5 mm. In addition, at this length, if the adjacent microwires are separated by a minimal inter-electrode distance of 200 μm , they can resist the adhesion force of water to remain separated in the implantation process. Based on the above characteristics, we developed a multi-channel electrode set for multiple single-unit recording chronically in the deep brain region.

2. Materials and methods

2.1. Electrode construction

A glass slide with a piece of two-sided adhesive tape stuck on it was used as the fabrication jig. A 12-mm stainless steel needle with a sharp tip was cut to serve as a guide tube (30- and 29-gauge for TG

and thalamic recording respectively). Three or four circular notches were made with an electronic cutter at the blunt end, and a 15-mm copper wire (with a diameter of about 150 μm) was tightly wound two or three times on the notches and soldered onto the needle to serve as the ground wire. The guide tube was fixed to the fabrication jig by sticking it onto the two-sided adhesive tape. The preparation described above made it transferable between the microscope and the bench for soldering, and the following processes for microwire slotting, gluing, and adjusting would be easily carried out with a well-fixed guide tube.

Several pieces of 4-cm Teflon-insulated tungsten microwires (with diameters of 35 μm bare and 50 μm insulated, California Fine Wire Co., Grover City, CA; #100211) were cut and served as recording electrodes. The number of channels of the electrode used for the TG and thalamic recording were three and seven respectively. These microwires were slotted one by one through the guide tube from the sharp end of the needle, and about 10 mm of each wire was left protruding out of the guide tube from the sharp tip. The free ends of these microwires that protruded out of the blunt end were separated and laid out in a fan-like array, and all were sandwiched between two small pieces of tape (Fig. 1A), which favored

moving these microwires together as a unit. The microwire bundle that protruded out of the sharp end was then cut at a site near the free end to make all microwires at the same length from the sharp tip of the needle. Further, one 5-cm microwire, of which 1 mm of its insulation at the tip was stripped by a brief burn, was also slotted through the guide tube with the end of de-insulation protruding out of the sharp tip of the needle. This microwire was used as a reference channel, and the longer length was for being distinguished from the other recording microwires (Fig. 1B).

Tiny spots of plastic steel epoxy glue (#3344 Powerbon, Taipei County, Taiwan) were applied to the microwires at the site where they protruded out of the sharp end. By pulling the sandwich tape, the microwires that originally protruded out of the sharp end slot would move into the guide tube. This process also simultaneously dragged the epoxy glue into the guide tube and helped to fix the arrangement of the microwires. With the aid of a dissecting microscope and fine forceps, the protruding bundled microwires were adjusted to protrude only by 1–1.5 mm from the tip of the sharp end, and were separated and slightly bent at the edge protruding out of the needle to form a fan-like spreading conformation. However, the reference microwire was adjusted to protrude shorter than the other microwires by 0.5 mm and remained straight (Fig. 1Bc, arrow). After this adjustment, an additional tiny spot of epoxy glue at the sharp end was molded to form a streamlined shape. The molding process was carefully done to keep the sharp tip of the needle free of glue, otherwise, a glued tip would lose its sharpness and it could cause serious dural dimpling during the dura penetration.

Some epoxy glue was further applied to the blunt end of the needle to fix the arrangement of microwires. When the glue solidified, the free ends of the recording and the reference microwires were cut to about 8 and 10 mm respectively. And the insulations of the free ends were all removed about 1 mm by a brief burn before soldering them to a 10-pin subminiature headpiece connector (Omnetics Connector, Minneapolis, MN), in which soldering paste (Taiyo Electric, Japan) was used to assist the soldering process. After all the microwires were tightly soldered, every soldering point was sealed and stabilized with a thin layer of epoxy glue (Tsai and Yen, 2003). When the thin layer of glue had solidified, more epoxy glue was applied to the base and sides of the connector. When the glue nearly hardened, the connector was bent 90° toward the guide tube (Fig. 1C), and this configuration was maintained until the glue had solidified completely. A piece of plate cut from the top of a barrel of a 1-ml syringe (made of polypropylene) was vertically glued to the side of the headpiece connector (Fig. 1C), which was used as a holder for attaching the micromanipulator. The tip impedance of the electrode was 0.5–1 M Ω at 1 kHz.

2.2. Functional evaluation of the electrode

Adult female Sprague-Dawley rats (250–320 g) were used. All animal care and experimental procedures performed in this study were approved by the Institutional Animal Care and Use Committee of National Taiwan University, and were in accordance with guidelines specified in the “Codes for Experimental Use of Animals” of the Council of Agriculture of Taiwan, based on the *Animal Protection Law* of Taiwan.

2.3. Behavioral test

Partial damage of trigeminal nerve may induce sensitization of the face for 40–120 days postoperatively (Iwata et al., 2001; Kitagawa et al., 2006; Saito et al., 2008; Vos et al., 1994). Therefore, it is necessary to test whether our electrode set caused prolonged TG damage. The behavioral test followed a method described by Tsuboi et al. (2004). In daily sessions, rats were trained to remain stable in a

carton and keep their snouts protruding through a hole in the wall during mechanical stimulation of the left and right whisker pad with a series of 8 von Frey filaments (1, 4, 6, 8, 10, 15, 26 and 60 g; Touch-Test, North Coast Medical, CA). The criterion performance was when rats could withstand 60-g mechanical stimuli on their whisker pad without escape behavior. When rat achieved the criterion performance, electrodes implanting surgery was performed the next day. The threshold for escape behavior to mechanical stimulation of whisker pad was then measured 3, 6, 10, 13 and 17 days after implantation. Quantitative mechanical stimuli were applied in ascending-descending-ascending order to evaluate the median escape threshold. We started with the lowest intensity. Each von Frey filament was applied 10 times at 1 Hz to the whisker pad. When rats showed brisk withdraw response, the intensity of that filament was defined as the first escape threshold. Then, the filaments in the descending order were applied sequentially to determine the strongest filament that rats would not escape at all 10 trials, and this intensity of filament was the second escape threshold. The same ascending procedure of defining first escape threshold was used to determine the third escape threshold. The median escape threshold was determined from these three escape threshold values.

2.4. Implant surgery

Rats were anesthetized with sodium pentobarbital (50 mg/kg, ip). A supplementary dose (16 mg/kg, ip) of pentobarbital was given if necessary. Four stainless-steel screws were placed on the skull to be anchors for the electrodes. Limited craniotomies were performed to expose the brain surface vertical to the recording sites within TG (L 2.5–3.5, P 5.5–6.5) and ventroposterior medial nucleus of the thalamus (VPM, R 2–3, P 3.5–4.5). The electrode for TG was inserted perpendicularly and located at the depth of 9.5 mm or so while the one for the thalamus at the depth of 5–6 mm or so. Once the electrodes were in the target region as determined by standard somatosensory mapping, the craniotomy was sealed using dental cement, and the plastic holder on the connector was removed by a flaming needle. The final appearance of the implanted electrode on the skull is shown in Fig. 1D. After the surgery, lincomycin hydrochloride (30 mg/kg) was administered intramuscularly to the rats for preventing infection.

2.5. Recordings

TG and thalamic unit activities were transmitted via the connecting cable to a multi-channel acquisition processor system (MAP, Plexon, Dallas, TX). Signals were amplified and filtered (250–13 kHz). Extracellular single units were recorded in real time using time-voltage windows and a principle component-based template-matching algorithm (Sort Client, Plexon). Selected high-pass-filtered raw signals were recorded simultaneously by another data acquisition system (Model ML870, PowerLab 8/30) at a rate of 20 kHz per channel using PowerLab software (version 5, AD Instruments) to capture and analyze continuous signals. For the unit stability analysis, neuronal activities were recorded in several separate daily sessions over 80 days. In each daily session, a rat was placed in a transparent chamber. After 30 min habituation, the single-unit activities of TG and thalamus were recorded together with the rat's behavior (CinePlex, Plexon) for 10 min. Then, the rat was anesthetized with continuous inhalation of 1% isoflurane. After confirming the receptive field, the spontaneous activities were recorded for 10 min under anesthesia; then it was followed by recording the evoked response to mechanical stimuli of the receptive field. General mechanical stimulations consisted of hair stimulation with a puff of air, cutaneous stimulation with a soft brush, and noxious stimulation with a mechanical pincher with a stimulus area of 20 mm². Additional mechanical stimula-

tions were applied as follows: (1) von Frey filaments (1, 6, 15, 26, and 60 g); (2) a hand-held fine stick was used to stimulate guard hairs and whiskers; and (3) a cotton swab was used to stimulate the tooth with scratching movements. Each stimulation was applied for 5–10 s and the minimum inter-stimulus interval was set to 30 s. The border of the receptive field was determined using the minimum intensity of stimulation under an operation microscope.

In order to test whether the grounding and reference method of our electrode could reduce noise, one each of the two types of electrodes (the first was the needle-ground plus local reference electrode reported here and the second type was an electrode fabricated similarly but grounded to the skull screw and without local reference) were implanted respectively into the left and right side of thalamus in the same rat. After one week for recovery, signals from these two types of electrodes were recorded with the rat in freely moving state. Spike waveform data, continuous multi-unit data and video of rat's behavior were acquired simultaneously as described in the previous section.

2.6. Data analysis

Saved waveforms were re-sorted using Offline Sorter (Plexon), based on principle-component clustering, with a user-defined template. For each unit, the spike waveform data from all subsequent days after implantation were applied to the same template. The unit identity across multiple days was defined by similar waveform shapes and receptive fields. To evaluate the stability of the unit recording, the similarity in the shape of spike waveforms, as described by Jackson and Fetz (2007), was calculated by a linear correlation (r) value between a waveform from the initial recording and that from each subsequent day for over 80 days. As for each unit, the maximal r value across time slots of two averaged and normalized waveforms obtained from different days was calculated ($r = 1$ indicates identical waveforms). Long-term single-unit stability was also evaluated by the principle component stability tube (PCST) (Wang et al., 2011; Williams et al., 1999), computed from a single file merged from consecutive recording files (PlexUtil; Plexon), through Wavetracker (Plexon). Straight tube suggests that the same set of units was recorded across days.

All units were tested with somatosensory stimuli in each session to determine their sensory properties. Evoked responses to mechanical stimulation of each unit were analyzed using NeuroExplorer (Nex Technologies, Winston-Salem, NC). The responsive units were determined if three continuous bins of the histogram during stimulus exceeded the threshold, calculated as the mean + 2.33 SD during 10-s data before each stimulus. If the responsive property or receptive field of unit was different from that of previous session, we defined the unit as a new one.

Statistical analysis was performed by a two-way repeated-measures analysis of variance (ANOVA) followed by Tukey's post-hoc test for the behavioral test. Results are presented as the mean \pm standard error. Differences were considered significant at $p < 0.05$.

2.7. Histology

After completing all experiments, the rats were anesthetized with sodium pentobarbital (60 mg/kg). Following an electrolytic lesion (30 μ A for 30 s) of recording sites, the rats were perfused with saline followed by a 4% formalin solution. The brain and TG were removed and postfixed in the same fixative. The tissue was transferred to 20% sucrose in 0.1 M phosphate buffer 2 days before frozen sectioning. The tissue was frozen-sectioned into 50- μ m slices and processed with Nissl staining. Implantation tracts and lesion sites

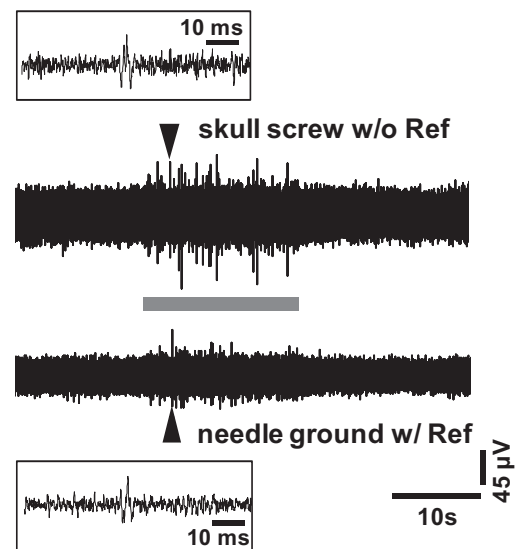


Fig. 2. Comparison of recordings obtained by the two types of grounding-reference methods. In the upper trace, the microwire was grounded to a skull screw without local reference. In the lower trace, the microwire was grounded to the needle guide tube with local reference. Continuous recording traces were obtained simultaneously from the same time segment in the same rat. Note that while rat was moving around in the testing box (gray horizontal line), the recording through the microelectrode with conventional grounding method (inset of upper trace) had more movement artifacts than that through microelectrode with needle ground and reference wire method (inset of lower trace).

were observed and recorded with a Zeiss microscope (Axioplan 2, Germany) equipped with a Nikon digital camera (Fig. 1E and F).

3. Results

Both TG and thalamic single-unit recordings were successfully obtained from 6 rats. The recorded noises were effectively attenuated by the needle-grounding with local reference method as shown in Fig. 2. These included smaller peak-to-peak background noise, and fewer and smaller far-field noise from animal movements.

Behavioral evaluation of the rats showed that escape thresholds of the ipsilateral and contralateral sides of the whisker pad significantly dropped to 22 ± 8.7 and 20.2 ± 8.8 g respectively on day 3 after implantation, and rose back to 54.3 ± 5.7 g on day 17 (Fig. 3). This indicates that although electrode implantation may have tem-

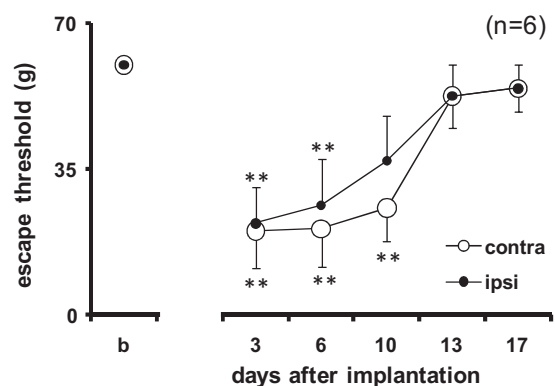


Fig. 3. Changes in the escape threshold after implantation. $**p < 0.01$, compared to the value before implantation, by two-way repeated-measures ANOVA with Tukey's test. ipsi, ipsilateral side to ganglion implantation; contra, contralateral side to ganglion implantation.

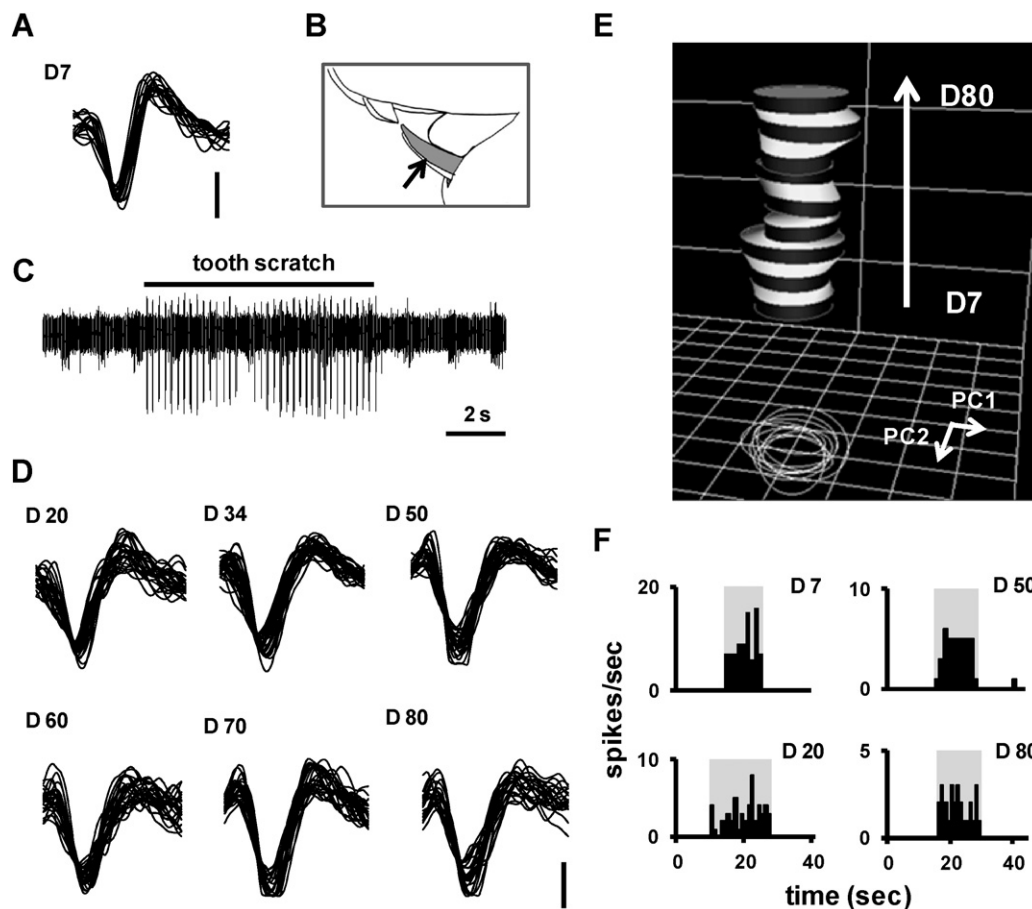


Fig. 4. Exemplary neuronal recording from one microwire implanted in TG. (A) Superimposed waveforms (0.8 ms) of the action potential of a single unit on day 7 after implantation in wakeful condition. (B) The receptive field of this unit is a lower tooth (arrow). (C) Single-unit recording of this unit activated by tooth scratching under anesthesia. (D) Superimposed waveforms (0.8 ms) on day 20 and five other subsequent days while the animal was conscious. (E) Principle component stability tube representation of waveforms from day 7, 20, 27, 34, 40, 50, 60, 70 and 80. Each tube is a principle component cluster of the unit in daily recording session. (F) Example of an evoked response of this neuron by scratching the tooth under anesthesia on four postoperative days after implantation. The gray square denotes the stimulus period. The bin size is 1 s. The vertical scale bars in A and D are 50 μ V.

porarily injured TG and caused whisker pad allodynia, the damage was reversible and the rat recovered within 2–3 weeks.

Thirty-six and 66 units were successfully acquired from TG and thalamus respectively. Among them, 42% (15/36) of the TG units and 53% (35/66) of the thalamic units had receptive fields and survived for at least 2 days. This population included the stable units, which emerged in the initial recording and survived up to 80 days after implantation, and the new units. Fig. 4 shows an example of a stable TG unit. This single unit could be activated by tooth scratching under anesthetized condition (Fig. 4C). Comparing 20 superimposed spike waveforms on day 7 with those on the subsequent days (D20, 34, 50, 60, 70, and 80) in the conscious condition, the waveforms showed high similarity (Fig. 4D) and the PCSTs kept straight across days (Fig. 4E), indicating that the recording was stable. Furthermore, mechanical stimulation of the same receptive field activated this cell across different days (Fig. 4F). Highly stable units, which survived 80 days, were also seen in the VPM recordings. As shown in Fig. 5, the spike waveforms of a unit with a receptive field on one whisker were highly similar among day 6 and the subsequent days (D20, 32, 40, 50, 70, and 80) after implantation.

Peak-to-peak amplitude of stable and new units in TG and thalamus was computed from collections of averaged waveforms during a 10-min period in the conscious condition, as shown in Fig. 6. Averaged amplitude of all stable and new thalamic units across days was $156.6 \pm 4.1 \mu$ V and $194.1 \pm 17.0 \mu$ V respectively (Fig. 6A). In

the final session of an 80-day experiment, we were able to obtain clear thalamic units with amplitude up to 132.0 μ V (stable unit) and 285.4 μ V (new units). Fig. 6B shows the amplitude of all the stable and new TG units. Averaged amplitude of all the stable and new units across days was $128.3 \pm 9.2 \mu$ V and $109.9 \pm 18.9 \mu$ V respectively. On the last day of the recording, good TG units with amplitude up to 128.5 μ V (stable unit) and 87.4 μ V (new units) was able to be obtained. Following the experiment, the background noise of the recording systems was calculated by the root mean square of 10-s continuous data without spike, which ranged from 15 to 40 μ V. The recording quality for each of the 35 thalamic units and the 15 TG units was quantified by calculating the signal-to-noise ratio (SNR), defined as the peak-to-peak amplitude divided by the noise level. The SNR for all these units in the initial recording session was ~ 8 , not different from the SNR of ~ 7 in the final session. It represents high stability and quality of the recordings in TG and thalamus from our electrodes.

11 of the 15 TG units and 25 of the 35 thalamic units were acquired on the first day of recording after implantation. The overall stability of these units is shown as a percentage of the original population retained in each 10-day session (Fig. 7). Approximately 60% of the thalamic and TG units were still retained on day 50 after initial recording, and 40% of them survived to the final recording day (day 80). Waveform similarity between the waveform recorded on the first day and that of same unit obtained on each subsequent

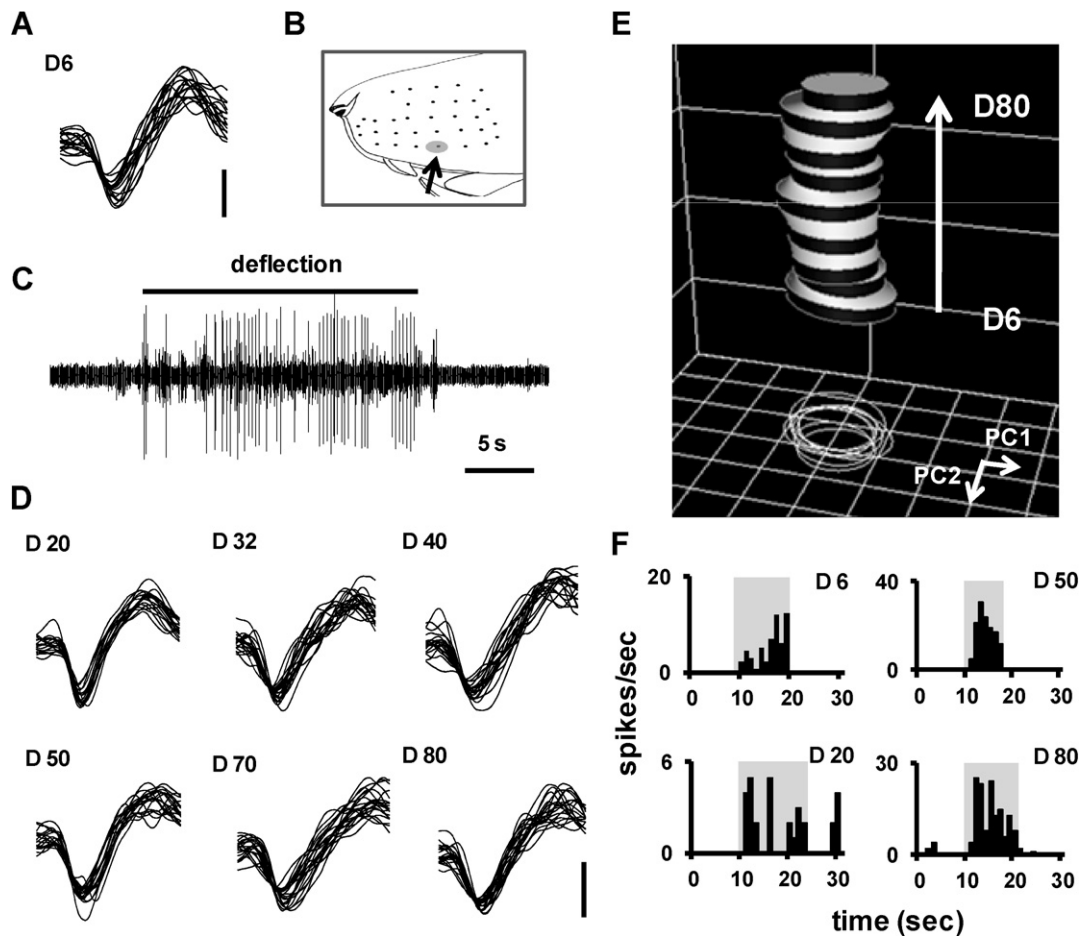


Fig. 5. Exemplary neuronal recording from a microwire implanted in the VPM. (A) Superimposed waveforms (0.8 ms) of the action potential of a single unit on day 6 after implantation in a wakeful condition. (B) The receptive field of this unit was a single whisker (E3, arrow). (C) Single-unit recording of this neuron activated by deflecting a whisker under anesthesia. (D) Superimposed waveforms (0.8 ms) on day 20 and five other subsequent days while the animal was conscious. (E) Principle component stability tube representation of waveforms from day 6, 20, 27, 32, 40, 50, 60, 70 and 80. Each tube is a principle component cluster of the unit in daily recording session. (F) Example of an evoked response of this neuron by deflecting a whisker under anesthesia on four postoperative days after implantation. The gray square denotes the stimulus period. The bin size is 1 s. The vertical scale bars in A and D are 100 μ V.

day by linear correlation was used to evaluate recording stability. An example of thalamic single-unit recording of one microwire on day 7, 20, 40 and 80 after implantation is shown in Fig. 8A. This microwire was able to capture two stable units 3a and 3b from day 7 to day 40. Unit 3a responded to whisker E1, and unit 3b responded to whisker gamma. Unit 3a was still present on day 80, but instead of unit 3b, there was a new unit, unit 3c that responded to movement of small hairs under the nose. Principle component clusters and spike shapes (inset) of these two stable units remained stable across days. The maximum r values (given at the bottom right of each subfigure) of two time shift-averaged waveforms of these units were 0.98–0.99, indicating the stability of the unit waveform. Recording stability of the 11 TG and 25 thalamic units across different days was calculated and shown in Fig. 8C and B, respectively. In total, there were 50 TG unit pairs and 167 thalamic unit pairs, of which 70% and 83% of corresponding r values were >0.96 , respectively. Among comparisons of the thalamic units, 69% of D60–80 pairs were with high similarity at r value >0.98 , and 83% were >0.96 . Because there were only 3 TG neurons survived from day 50 to day 80 after implantation, pair numbers in the group D40–60 and D60–80 suddenly dropped. But the r values of these comparisons were still above 0.9. These results demonstrate that the recordings remained stable over 80 days in TG and thalamus.

4. Discussion

Chronically tracking neuronal signals in the same electrode over a period of 1.5 years has been reported using silicone-base micro-electrode in primate motor cortex (Suner et al., 2005). In rodents, high quality cortical single-units could be recorded for over 127 days (Vetter et al., 2004). It is unclear, however, whether the same single-units were followed. Recently, the prevailing technique for chronic recording in the awake primates and rodents is the movable electrode (microarray or tetrode) (Eliades and Wang, 2008; Haiss et al., 2010; Jackson and Fetzi, 2007; Wilson et al., 2003; Yamamoto and Wilson, 2008; Yang et al., 2010). The movable electrode can acquire many single-units in the same animal with large advancements, or enhance the quality of the same unit across days with fine adjustments. The longest recording period of the stable cortical neuron found in the literature is over 1 year (Porada et al., 2000; Swadlow, 1985).

A tetrode, consisted of four microwires twisted tightly together, is also a good tool for chronic recording of multiple single-unit activities (Emondi et al., 2004; Tolia et al., 2007). It has been reported that such electrode successfully followed the same neuron for 23 days in the cortex (Tolia et al., 2007). A tetrode equipped with a micro-driver has been used for the recording of thalamic units for 4–5 days (Yamamoto and Wilson, 2008; Yang et al., 2010).

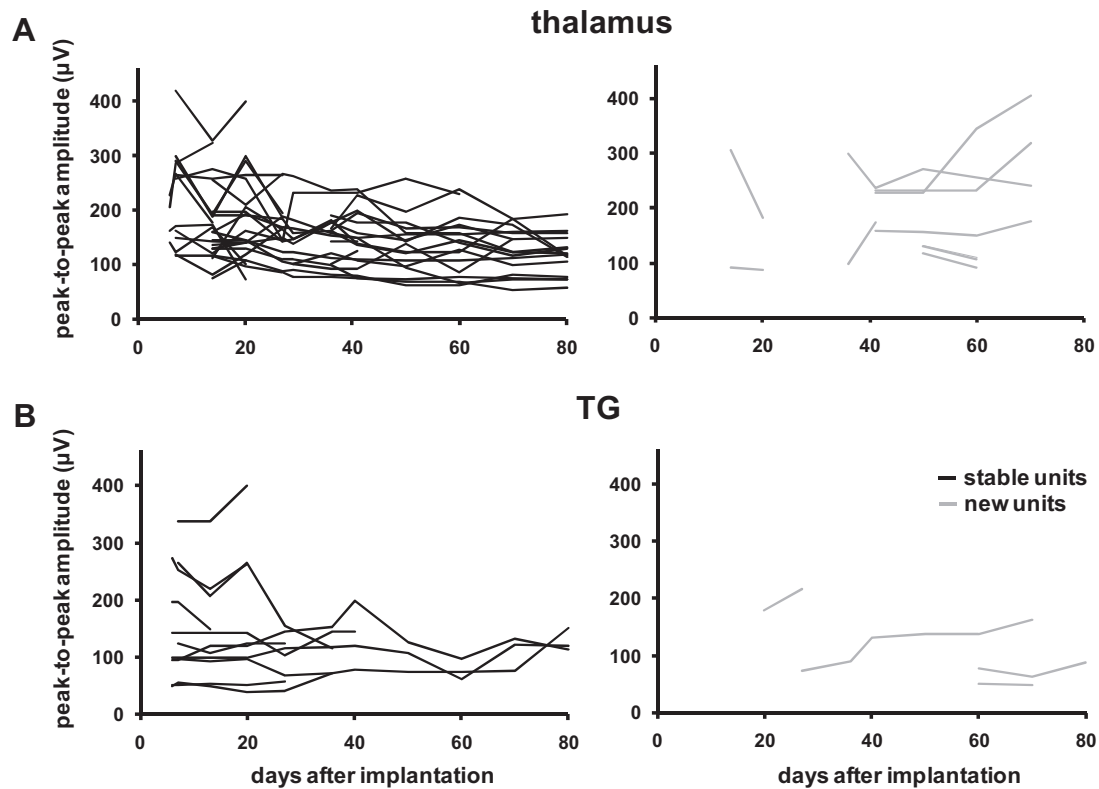


Fig. 6. Peak-to-peak amplitude of all stable and new thalamic (A) and TG (B) units followed longitudinally across recording days. Each line indicates a unit recorded from the same wire and had high waveform and receptive field similarity.

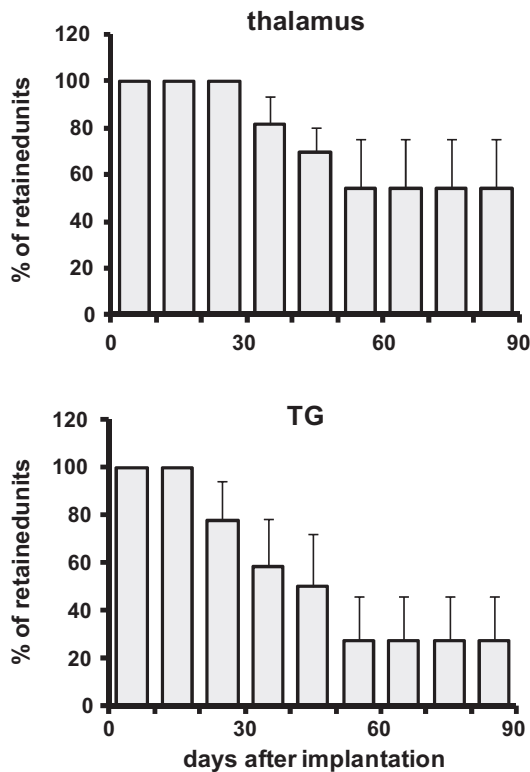


Fig. 7. Percentage of stable single units remained throughout the 80 day recording period. New units were not included.

However, long-term stable recording of a unit for weeks to months in deep brain regions has not been reported to our knowledge. It may result from a blocking of the tiny recording sites by glia processes (Biran et al., 2005). Another possibility is that a long thin electrode might be inherently unstable and prone to displacements in an active animal.

Herein, we report a novel bundle-like microwire array that afforded stable high quality thalamic and dura-surrounded TG single-unit recordings across weeks to months in freely moving and behaving rats. This was achieved with a short, separated microwire design. This design included appropriate extent of protrusion of guide-tube array and fan shape arrangement of the microwires (Fig. 1B). The short length of protruding microwires contributes to their rigidity. This helped keeping the position of the microwires fixed during the whole experiment session. The fan-shaped arrangement minimized local tissue damage at the recording site produced by a big bundle.

Removing the dura before implantation is an important process due to the blunt end and non-rigid nature of a microwire array or bundle. However, removing the dura usually damages the underlying tissues. Therefore, reducing the amount of dura that has to be removed is beneficial for implantation. In our design, the use of the tungsten microwire with a short protruding length produced strong rigidity, retained the array conformation, and prevented the microwires from sticking together due to capillary forces at a length of <1.5 mm. This conformation is advantageous for directly penetrating the dura.

In order to create a fan-like spreading conformation, the site where the microwires protruded out of the guiding needle was reinforced with epoxy glue. This could cause serious dura dimpling and damage the brain tissue underneath. Two designs were used to overcome this problem. First, care was taken to keep the sharp tip of the needle free of epoxy glue so it could easily penetrate the dura;

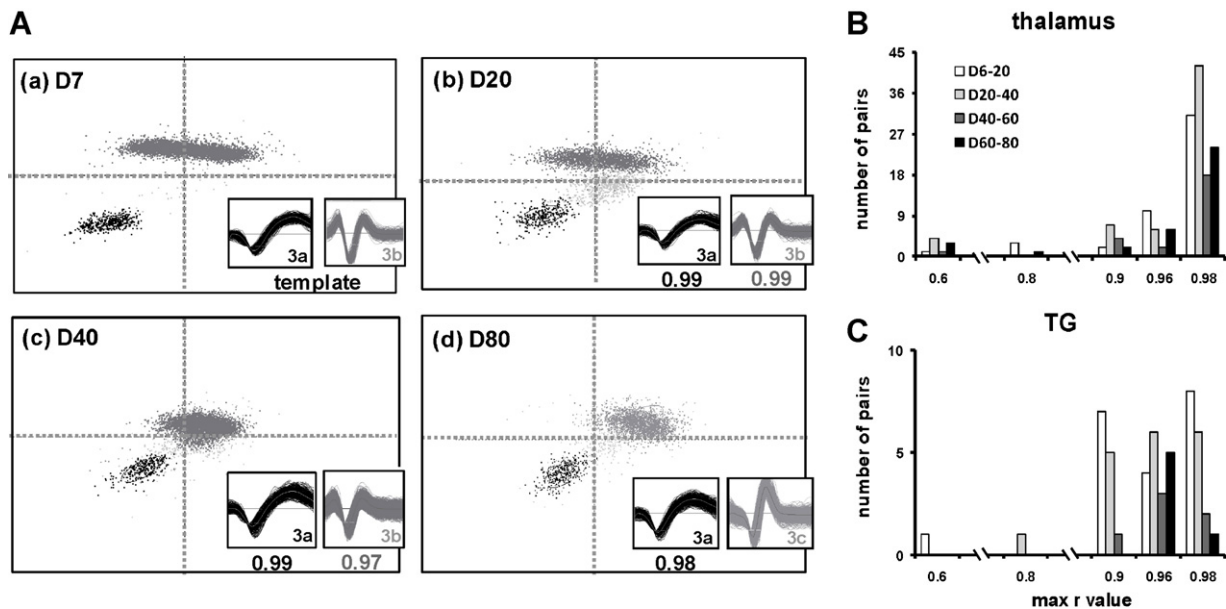


Fig. 8. Waveform similarity of the stable units. (A) An example of all thalamic units recorded from a single microwire. Two units were recorded for 40 (unit 3b) and 80 (unit 3a) days, respectively, from this microwire. Unsorted (light gray) and sorted (black and dark gray) waveforms (inset) from each recording session projected onto first (x-axis) and second (y-axis) principle components. The cluster centers of unit 3a and 3b were stable across days. The correlation coefficient of averaged waveform on day 20 (Ab), 40 (Ac), and 80 (Ad) of the units compared to their respective templates on the first recording session was all above 0.97 (numbers shown below the insets). A new unit, unit 3c, appeared on day 80. (B and C) Distribution of waveform similarity of all stable thalamic and TG units pairs across different recording days. Correlation coefficient (r value) was computed by linear correlation from TG (50 pairs) and thalamic (167 pairs) unit waveforms. Pairs were categorized into four groups based on the day acquired after initial recording, including 6-, 20-, 40-, 60- and 80-day.

and second, we molded the epoxy glue at the sharp end to form a streamlined shape, which allowed the needle tube to be smoothly inserted into a small incision.

Comparing with other brain region, the deep location and the surrounding dura layers make TG a difficult target for implantation and for acquiring high quality single-unit activity. Although previous reports demonstrated that it was feasible to record single-unit activity in TG from wakeful rats using single-channel microelectrode (Bermejo et al., 2004; Khatri et al., 2009; Leiser and Moxon, 2007), we improved the efficiency of TG unit recording even further through multi-microwires and minimized tissue damage by a reduction of size. The longest stable recording period of the single-unit with our electrode was 80 days, a significant improvement from the period of 1–2 weeks reported previously.

By a simple step, the connector can be bent to variable angles according to the user's need. In our case, it was bent 90° backward to allow the rats to go through a behavioral carton and be able to protrude their snouts through the hole. Later, investigators could give stimulations on the rats' face using von Frey filaments or other instruments. Hence, there is a potential to apply the electrode designed herein to the study of various trigeminal functions in behaving, unrestrained rats in the future.

Our results of the behavioral test demonstrated that on days 3, 6, and 10 after implantation, the escape thresholds of both sides of the rat face decreased significantly (Fig. 3). It is likely that the implantation had injured the left TG, which caused mechanical allodynia of the whisker pad. However, this injury was reversible as inflammation was maintained for only 2 weeks (Fig. 3). In addition, the time course along which the escape threshold lowers after implant surgery may be a useful reference for other researchers who use the same methodology to implant the electrode reported in the present study. The recovery time after implant surgery is recommended to be 2 weeks.

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References

- Bermejo R, Szwed M, Friedman W, Ahissar E, Zeigler HP. One whisker whisking: unit recording during conditioned whisking in rats. *Somatosens Mot Res* 2004;21:183–7.
- Biran R, Martin DC, Tresco PA. Neuronal cell loss accompanies the brain tissue response to chronically implanted silicon microelectrode arrays. *Exp Neurol* 2005;195:115–26.
- Chang CH, Liang KC, Yen CT. Inhibitory avoidance learning altered ensemble activity of amygdaloid neurons in rats. *Eur J Neurosci* 2005;21:210–8.
- Eliades SJ, Wang X. Chronic multi-electrode neural recording in free-roaming monkeys. *J Neurosci Methods* 2008;172:201–14.
- Emond AA, Rebrink SP, Kurgansky AV, Miller KD. Tracking neurons recorded from tetrodes across time. *J Neurosci Methods* 2004;135:95–105.
- Haiss F, Butovas S, Schwarz C. A miniaturized chronic microelectrode drive for awake behaving head restrained mice and rats. *J Neurosci Methods* 2010;187:67–72.
- Herry C, Ciocchi S, Senn V, Demmou L, Muller C, Luthi A. Switching on and off fear by distinct neuronal circuits. *Nature* 2008;454:600–6.
- Iwata K, Imai T, Tsuboi Y, Tashiro A, Ogawa A, Morimoto T, et al. Alteration of medullary dorsal horn neuronal activity following inferior alveolar nerve transection in rats. *J Neurophysiol* 2001;86:2868–77.
- Jackson A, Fetisov EE. Compact movable microwire array for long-term chronic unit recording in cerebral cortex of primates. *J Neurophysiol* 2007;98:3109–18.
- Khatri V, Bermejo R, Brumberg JC, Keller A, Zeigler HP. Whisking in air: encoding of kinematics by trigeminal ganglion neurons in awake rats. *J Neurophysiol* 2009;101:1836–46.
- Kitagawa J, Takeda M, Suzuki I, Kadoji J, Tsuboi Y, Honda K, et al. Mechanisms involved in modulation of trigeminal primary afferent activity in rats with peripheral mononeuropathy. *Eur J Neurosci* 2006;24:1976–86.
- Kubie JL. A driveable bundle of microwires for collecting single-unit data from freely-moving rats. *Physiol Behav* 1984;32:115–8.
- Leiser SC, Moxon KA. Responses of trigeminal ganglion neurons during natural whisking behaviors in the awake rat. *Neuron* 2007;53:117–33.
- Nicoletis MA, Dimitrov D, Carmena JM, Crist R, Lehw G, Kralik JD, et al. Chronic, multisite, multielectrode recordings in macaque monkeys. *Proc Natl Acad Sci U S A* 2003;100:11041–6.

- Nicolelis MA, Ghazanfar AA, Faggini BM, Votaw S, Oliveira LM. Reconstructing the engram: simultaneous, multisite, many single neuron recordings. *Neuron* 1997;18:529–37.
- Porada I, Bondar I, Spatz WB, Kruger J. Rabbit and monkey visual cortex: more than a year of recording with up to 64 microelectrodes. *J Neurosci Methods* 2000;95:13–28.
- Saito K, Hitomi S, Suzuki I, Masuda Y, Kitagawa J, Tsuboi Y, et al. Modulation of trigeminal spinal subnucleus caudalis neuronal activity following regeneration of transected inferior alveolar nerve in rats. *J Neurophysiol* 2008;99:2251–63.
- Suner S, Fellows MR, Vargas-Irwin C, Nakata GK, Donoghue JP. Reliability of signals from a chronically implanted, silicon-based electrode array in non-human primate primary motor cortex. *IEEE Trans Neural Syst Rehabil Eng* 2005;13:524–41.
- Swadlow HA. Physiological properties of individual cerebral axons studied in vivo for as long as one year. *J Neurophysiol* 1985;54:1346–62.
- Szymusiak R, Alam N, Steininger TL, McGinty D. Sleep-waking discharge patterns of ventrolateral preoptic/anterior hypothalamic neurons in rats. *Brain Res* 1998;803:178–88.
- Thompson LT, Best PJ. Long-term stability of the place-field activity of single units recorded from the dorsal hippocampus of freely behaving rats. *Brain Res* 1990;509:299–308.
- Tolias AS, Ecker AS, Siapas AG, Hoenselaar A, Keliris GA, Logothetis NK. Recording chronically from the same neurons in awake, behaving primates. *J Neurophysiol* 2007;98:3780–90.
- Tsai ML, Yen CT. A simple method for fabricating horizontal and vertical microwire arrays. *J Neurosci Methods* 2003;131:107–10.
- Tsuboi Y, Takeda M, Tanimoto T, Ikeda M, Matsumoto S, Kitagawa J, et al. Alteration of the second branch of the trigeminal nerve activity following inferior alveolar nerve transection in rats. *Pain* 2004;111:323–34.
- 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.
- Vos BP, Strassman AM, Maciewicz RJ. Behavioral evidence of trigeminal neuropathic pain following chronic constriction injury to the rat's infraorbital nerve. *J Neurosci* 1994;14:2708–23.
- Wang CM, Yang L, Lu D, Lu YF, Chen XF, Yu YQ, et al. Simultaneous multisite recordings of neural ensemble responses in the motor cortex of behaving rats to peripheral noxious heat and chemical stimuli. *Behav Brain Res* 2011;223:192–202.
- Williams JC, Rennaker RL, Kipke DR. Stability of chronic multichannel neural recordings: implications for a long-term neural interface. *Neurocomputing* 1999;26–27:1069–76.
- Wilson FA, Ma YY, Greenberg PA, Ryou JW, Kim BH. A microelectrode drive for long term recording of neurons in freely moving and chaired monkeys. *J Neurosci Methods* 2003;127:49–61.
- Yamamoto J, Wilson MA. Large-scale chronically implantable precision motorized microdrive array for freely behaving animals. *J Neurophysiol* 2008;100:2430–40.
- Yang S, Cho J, Lee S, Park K, Kim J, Huh Y, et al. Feedback controlled piezo-motor microdrive for accurate electrode positioning in chronic single unit recording in behaving mice. *J Neurosci Methods* 2011;195:117–27.