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# A novel carbon fiber bundle microelectrode and modified brain slice chamber for recording long-term multiunit activity from brain slices

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**Abstract**

The fabrication and characteristics of a novel multiunit recording electrode and modified brain slice chamber suitable for long-term recording from brain slices are described. The electrode consisted of an electrolyte-filled glass micropipette with a 20–50 μm thick wax-coated bundle of 5-μm diameter carbon fibers extending 2.5 cm from the tapered end and an AgCl-coated silver wire inserted into the open end and connected to a preamplifier. Both ends of the electrode were sealed with wax to prevent evaporation of the electrolyte. The brain slice was maintained over this extended period in an interface-type brain slice chamber modified to completely surround the slice with medium. Using this electrode, regular 24-h oscillations of spontaneous multiunit activity were recorded for 3 days from a single location in a 500 μm thick rat suprachiasmatic nucleus brain slice. Preliminary data suggest that this novel carbon fiber bundle electrode will be a favorable alternative to traditional metal electrodes for long-term recording of multiunit activity from brain slices.

*Keywords:* Electrode; Carbon fiber; Multiunit activity; Long-term recording; Brain slice; Suprachiasmatic nucleus; Hypothalamus; Circadian rhythm

## 1. Introduction

Long-term extracellular recording of spontaneous neuronal activity from brain slices in vitro poses special problems concerning recording methodology and maintenance of tissue viability. For example, the suprachiasmatic nuclei (SCN) are the site of an endogenous circadian pacemaker that has been studied extensively using the hypothalamic brain slice preparation (reviewed in Gillette, 1991). Continuous single-unit recordings lasting over 30 h have been performed on SCN brain slices (Satinoff et al., 1993). This single-unit recording method required constant electrode repositioning and many person-hours to accomplish. Additionally, the SCN brain slice endured repeated penetrations with a glass micropipette electrode over the course of these recordings. In order to improve upon this technique, we developed a multiunit recording technique that does not involve electrode repositioning and requires only minimal maintenance.

Early in the development of this technique, we used metal (platinum-iridium; 76 μm diameter) electrodes as described by Bouskila and Dudek (1993) for multiunit recording from SCN brain slices. Although metal electrodes can be used successfully for long-term recording, we have found it difficult to obtain consistent long-term recordings using them. As an alternative, we then tested electrodes made of carbon fiber bundles as a potentially more biocompatible alternative to metal electrodes. Single carbon fibers have been used for voltammetry (Adams, 1969) as well as for long-term recording from organotypic neonatal rat SCN explants (Bos and Mirmiran, 1990). The carbon fiber bundle (CFB) electrode proved to be highly compatible with long-term multiunit recording from SCN brain slices. The CFB electrode design incorporates features of both single-unit electrodes and multiunit electrodes. In essence, the CFB electrode pools the input from many single carbon fibers into a multiunit signal. In order to maintain tissue viability and neuronal activity for several days, an interface-type brain slice chamber was modified to perifuse the brain slice on all sides with fresh, oxygenated medium. The fabrication and recording characteristics of CFB multiunit electrodes as well as the design of the brain slice chamber are described here.

## 2. Electrode fabrication

A 20–50 μm thick, 13 cm long bundle of 5 μm diameter carbon fibers (Advanced Diversified Composites,

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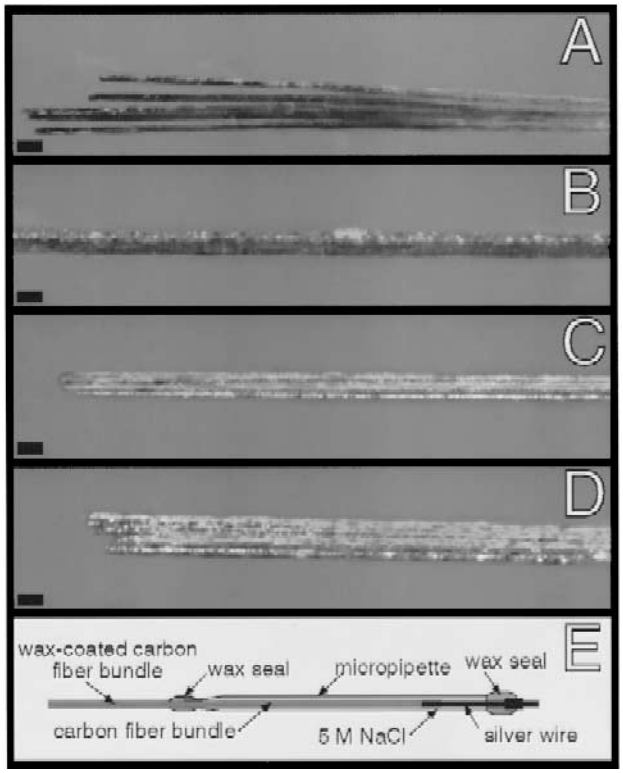


Fig. 1. CFB electrode. (A) Several 5 μm diameter carbon fibers. Most of these fibers are paired due to electrostatic attraction, although tips of individual fibers are discernible. (B) A wax-coated CFB. (C) A wax-coated, ethanol-treated CFB. (D) A wax-coated, ethanol-treated CFB with the tip cut off and fibers splayed. (E) Schematic diagram of a complete CFB multiunit electrode. Scale bar is 25 μm.

Orlando, FL) (Fig. 1A) was inserted into a 1 mm O.D.× 0.58 mm I.D.× 10 cm long glass filament micropipette (A-M Systems, Everett, WA) so that 1.5 cm of the CFB extended from each end of the micropipette. The micropipette was then pulled on a vertical micropipette puller (David Kopf Instruments Model 700C) so that a 5 cm length of the CFB spanned the distance between pipette tips. While still held in the micropipette puller, the 5 cm section of CFB was cut in the center with scissors, resulting in two micropipette-CFB electrode assemblies. The micropipettes were then removed from the puller and any CFB extending from the open end of the micropipettes was trimmed with scissors.

The tip of the micropipette and the length of exposed CFB were then coated with Sticky Wax (L.D. Caulk, Co., Milford, DE) (Fig. 1B), which is solid and brittle at room temperature, softens gradually upon heating, and adheres well to glass, plastic and carbon fibers. This wax coating prevented electrolyte from leaking and provided a layer of electrical insulation around the fibers. Coating was accomplished by melting 5 ml of wax in a 10 ml Pyrex beaker in a microwave oven (10 min on 'high') and dipping the entire tip of the electrode, including several millimeters of the tapered glass, into the liquid wax. The electrode was dipped in the wax several times until a uniform coating without beads was achieved. Care was taken to withdraw the electrode from the wax so that the CFB was straight. The thickness of the wax coating was then reduced by melting the wax with a soldering iron passed under the wax-coated bundle from the tip to the CFB-glass junction. The electrode was manipulated to position the resulting liquid wax bead over the CFB-glass junction.

The wax coating at the end of the electrode was then partially dissolved with boiling ethanol (Fig. 1C). This process served several purposes: (1) it thinned the wax coating so that individual wax-coated fibers separated from one another when the tip was cut off; (2) it produced the smallest possible fiber diameters, which minimized tissue damage; and (3) it may have perforated the wax coating and exposed the carbon fiber material, thus increasing biocompatibility. To accomplish this, 10 ml of 100% ethanol was heated to boiling (with boiling chips) in a 30 ml Pyrex beaker on a hot plate. The distal 5–10 mm of the wax-coated CFB was dipped several times in the boiling ethanol, dissolving away the outer layer of wax. Note that individual fibers are visible in Fig. 1C, in contrast to the thick wax coating shown in Fig. 1B.

Finally, the distal 1 mm of the CFB shaft was trimmed off with iridectomy scissors. If the wax coating was thin enough, the tip splayed and individual fibers were visible (Fig. 1D). Splaying the fibers at the tip of the CFB increases the sampling volume within the tissue, which may be advantageous for some applications. The amount of splay at the tip of the electrode can be increased by bending the fibers with a fine forceps and breaking the wax holding the fibers together. The electrode was then back-filled with electrolyte (5 M NaCl), an AgCl-coated silver wire was inserted into the open end of the micropipette (leaving enough wire exposed to connect to the amplifier electrode lead), and the wire was sealed into the pipette with wax to prevent electrolyte evaporation. A completed electrode is schematically illustrated in Fig. 1E. A single electrode could be used repeatedly, providing the tip was kept clean. Additionally, a fouled tip could be trimmed off and the electrode reused.

## 3. Brain slice chamber design

An interface-type brain slice chamber (Haas et al., 1979) was used to maintain the tissue during recording. The portion of the chamber housing the brain slice consisted of an inclined Plexiglas ramp with vertical walls on either side (Fig. 2A). Tissue medium consisted of EBSS (Earle's Balanced Salt Solution, Sigma) supplemented with 2.2 g/l sodium bicarbonate, 3.42 g/l D-glucose, and 5 ml/l gentamicin solution (Sigma), maintained at pH 7.4. Brain slices were constantly perifused with medium by a peristaltic pump at a rate of 0.5 ml/min. The brain slice chamber was maintained at 33°C. Medium flowed from a shallow trough at the top edge of the ramp and was drawn

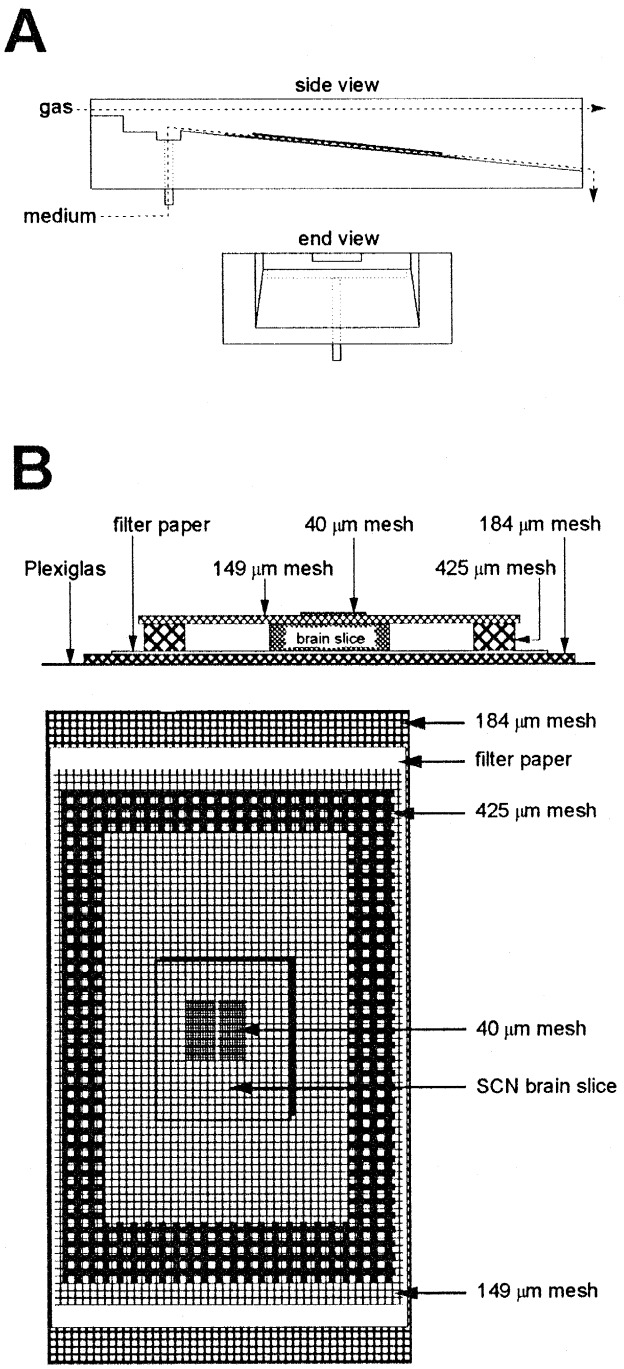


Fig. 2. Brain slice chamber. (A) Schematic diagram showing a cross-sectional side view and end view of the brain slice environment of an interface-type brain slice chamber. The nylon mesh enclosure housing the brain slice is pictured resting on the Plexiglas ramp. (B) Schematic diagram showing a cross-sectional side view and top view of the nylon mesh enclosure comprising the brain slice microenvironment.

by a wick into a reservoir at the bottom of the ramp. A humidified 95% $O_2$/5% $CO_2$ atmosphere was maintained within the chamber. The chamber was covered with glass coverslips to minimize drying of the slices and filter paper was placed between the coverslips and the top of the chamber to prevent water drops from condensing and falling into the chamber. Condensation droplets were never observed on the CFB electrodes.

Special measures were taken to provide the tissue with a favorable environment and to preserve cell viability since slices were maintained in vitro for several days. Fig. 2B schematically illustrates the brain slice microenvironment. The brain slice rested on a piece of Whatman no. 42 filter paper which in turn was supported by a piece of 184 mm nylon mesh (all nylon mesh was supplied by Small Parts, Inc., Miami Lakes, FL). The slice was covered on top by a piece of 149 μm nylon mesh which was supported at its edges by a frame of 425 μm nylon mesh with the center cut out. Finally, after the recording electrode was placed in the SCN, two pieces of 40 μm nylon mesh were placed on either side of the electrode, adjacent to, but not touching the electrode. This was necessary to increase the depth of the medium covering the slice near the electrode and prevent drying of the tissue. This microenvironment provides the brain slice with constant perifusion of fresh medium on all sides and ensures that the slice will remain physically stable over extended periods. It is comparable to a submerged environment since the tissue is exposed to a liquid environment on all sides.

### *3.1. Brain slice preparation*

Animal care and brain slice preparation were performed in accordance with PHS and USDA guidelines for the humane treatment of animals. Both male and female Long-Evans rats from an inbred colony were used in this study. Animals were between 7 and 9 weeks old and were housed under a 12 h light/12 h dark schedule. Brain slices were collected between 8 a.m. and 11 a.m. (lights on = 7 a.m.). Animals were killed by rapid decapitation and 500-μm thick coronal hypothalamic brain slices containing the central portion of the SCN were prepared using a mechanical tissue chopper (Hatton et al., 1980) and placed in the brain slice chamber.

## 4. Multiunit recording protocol

The tip of the CFB electrode was placed in the ventral region of the SCN. Precise placement of the electrode was possible since the square openings in the 149 mm nylon mesh were ~100 mm. If the region of the SCN where the electrode was to be placed was occluded by a strand of the 149 μm nylon mesh, the mesh was gently moved to expose the desired area. After the electrode was positioned, it was advanced μ200 mm into the slice and left stationary for the duration of the recording. A DAM 60 differential preamplifier (WPI, Sarasota, FL) in single-ended mode provided a gain of 10 000 times and band-pass filtering of 300–3000 Hz. This signal was monitored on an oscilloscope and also passed to a Dell 50 MHz 80486

computer running LabVIEW for Windows (National Instruments). A program written in the LabVIEW graphical programming language by one of the authors (T.T.) was used to collect and display multiunit activity (MUA) data over the course of experiments lasting up to several days. Raw data were sampled at 20 kHz and passed through a 3rd order highpass Butterworth filter with a cutoff frequency of 100 Hz to remove baseline fluctuations. Multiunit activity was defined as the average number of times per s that the voltage signal exceeded a threshold level. Three separate thresholds were set 1 μV apart at the beginning of an experiment so that the highest, usually set just above the background noise level at 5 μV, detected activity at 0–1 Hz and the lowest detected activity at 20–50 Hz. Neuronal firing is inhibited immediately after brain slice preparation, probably due to anoxia and associated spreading depression, and gradually recovers over the following 90 min (Lipton and Whittingham, 1984). This electrically inactive state provided a convenient reference for setting the thresholds. Three separate thresholds were used to investigate whether changes in MUA could be observed intermingled with the background noise. Additionally, the standard deviation (SD) of the digitized signal was used to measure relative changes in the aggregate of neuronal activity detected by the electrode. Multiunit activity and signal SD were collected at 1-s intervals and averaged into non-overlapping 1-min bins. Data were plotted using SigmaPlot for Windows 2.0.

## 5. Electrode recording characteristics

### *5.1. Electrode impedance*

An electrode can be modeled as a resistor in series with a capacitor. Total impedance is a function of both resistance and capacitance. Using this model, impedance ($Z$) is expressed in the form '$Z\Omega$ at $\omega$ capacitive', where $\omega = 0°$ is a pure resistor and $\omega = 90°$ is a pure capacitor.

The impedance of a 20 μm diameter CFB electrode with only the tip immersed in electrolyte was 26 kΩ at 66° capacitive at 1 kHz. Impedance with ~500 μm of the CFB immersed in electrolyte was 10 kΩ at 61° capacitive at 1 kHz.

### *5.2. Electrode conditioning*

When not in use, the tip of the CFB electrode was immersed in tap water. For 1 h prior to recording, the tip of the electrode was submerged in the medium within the nylon mesh enclosure that was to contain the brain slice and a mock recording was initiated. These electrode conditioning procedures served two purposes. First, any static charge on the electrode was dissipated, facilitating placement of the electrode into the SCN. An unconditioned electrode was electrostatically attracted to strands of the nylon mesh, making precise placement of the electrode very difficult. Second, an unconditioned electrode did not produce a stable, consistent recording. During the initial 1 h mock recording, the spike activity count obtained from the bath during the trial recording was noisy at first and contained transient peaks lasting up to several minutes. After 10–30 min, these transient peaks disappeared and were replaced by a steady, consistent spike count. The brain slice was then prepared and the conditioned electrode was used for multiunit recording.

### *5.3. Recording artifacts*

Successful multiunit recording using a CFB electrode required constant conditions in the recording chamber. Variations in the rate of perifusion caused changes in the level of the medium surrounding the slice and produced recording artifacts. A lowering of the medium level could cause the tissue to recede from the electrode. This left the electrode positioned at or above the surface of the tissue where it recorded artifactually large voltage fluctuations as demonstrated by transient peaks in the MUA record. Similar artifacts were also caused by dehydration of the brain slice, often the result of a drop in medium level. Dehydration could be identified by the dry appearance of the surface of the slice. Unusual recording artifacts were caused by tissue adhering directly to the carbon fibers, a testament to their biocompatibility. For example, tissue from the brain slice adhered to raw carbon fibers and traveled up the shaft of the CFB and out of the medium where it dehydrated. Before the tissue adhering to the electrode dehydrated, a sustained increase in the size of the MUA signal was observed. Adhesion of the tissue to the electrode was prevented by coating the electrode with wax and cutting off the tip.

### *5.4. Raw multiunit data*

Six examples of raw multiunit data are shown in Fig. 3A. These data were collected from a single electrode position in the ventral SCN at various times after brain slice preparation, indicated by the vertical dotted lines in the Fig. 3 inset labeled SCN Multiunit Activity. The ground trace is included as a reference and contains no contribution from the electrode. The 5-min trace was obtained while the tissue was electrically inactive and its similarity to the ground trace shows that the electrode is intrinsically quiet. The 1 h trace shows partially recovered neuronal activity. The remaining samples were recorded at the indicated intervals after brain slice preparation and represent fully recovered SCN neuronal activity. The jagged appearance of the digitized waveforms is due to temporally overlapping action potentials and noise which sum or cancel to produce irregularly shaped waveforms.

### 5.5. Estimate of sampling volume and number of neurons sampled

The size of the three-dimensional volume sampled by the electrode was estimated using two independent methods. First, the lower limit of the sampling volume can be estimated as the volume of space circumscribed by the splayed electrode tip in the tissue. For example, the 50 μm diameter splayed tip of a 25 μm thick carbon fiber bundle inserted 200 μm into the brain slice describes a 392 700 $\mu m^3$ cylinder. The size of the sampling volume was also estimated by dividing the number of neurons sampled by the density of neurons (neurons per unit volume) in the SCN.

The number of neurons present in the signal was estimated by analyzing 6-s samples of SCN MUA. Fig. 3 illustrates this analysis and Table 1 enumerates some of the relevant statistics. Each peak in these samples, defined here as any sampled voltage value flanked by either lower (relative maximum) or higher (relative minimum) values, represents either noise (dominant between −2 and 2 μV; Fig. 2C) or neuronal activity (dominant below −2 and above 2 μV; Fig. 2C) or a combination of both (present at all amplitudes). The histograms in Fig. 3B show the number of peaks counted in 0.1-μV bins within the 6-s digitized samples. Note that the *y*-axis is the $\log_{10}$ number of peaks so the maximum bar height is $\sim 10^3$. Subtracting the 5-min histogram (equipment/environment and background noise) from the 1-, 2-, 4- and 6-h histograms produced the histograms in Fig. 3C, which represent an estimate of the neuronal contribution to the sampled signals.

Table 1
Statistics for 6-s digitized samples shown in Fig. 3

| Statistic | Ground | 5 min | 1 h | 2 h | 4 h | 6 h |
|---|---|---|---|---|---|---|
| Total peaks [a] | 29138 | 28962 | 28736 | 28639 | 28373 | 28294 |
| Neuronal peaks [b] | – | – | 1042 | 1610 | 2540 | 2418 |
| Single unit average (Hz) [c] | – | – | 2.5 | 3.0 | 5.0 | 6.0 |
| Number of neurons [d] | – | – | 34.7 | 44.7 | 42.3 | 33.5 |

[a] Fig. 3B.
[b] Fig. 3C.
[c] Satinoff et al. (1993).
[d] Calculated see text.

The number of neurons in the sampling volume can be calculated by dividing the number of neuronal peaks in Table 1 first by 2 (because action potentials are biphasic) and then by 6 (each sample was 6 s in duration) and finally by the average single unit firing rate (Hz) determined by

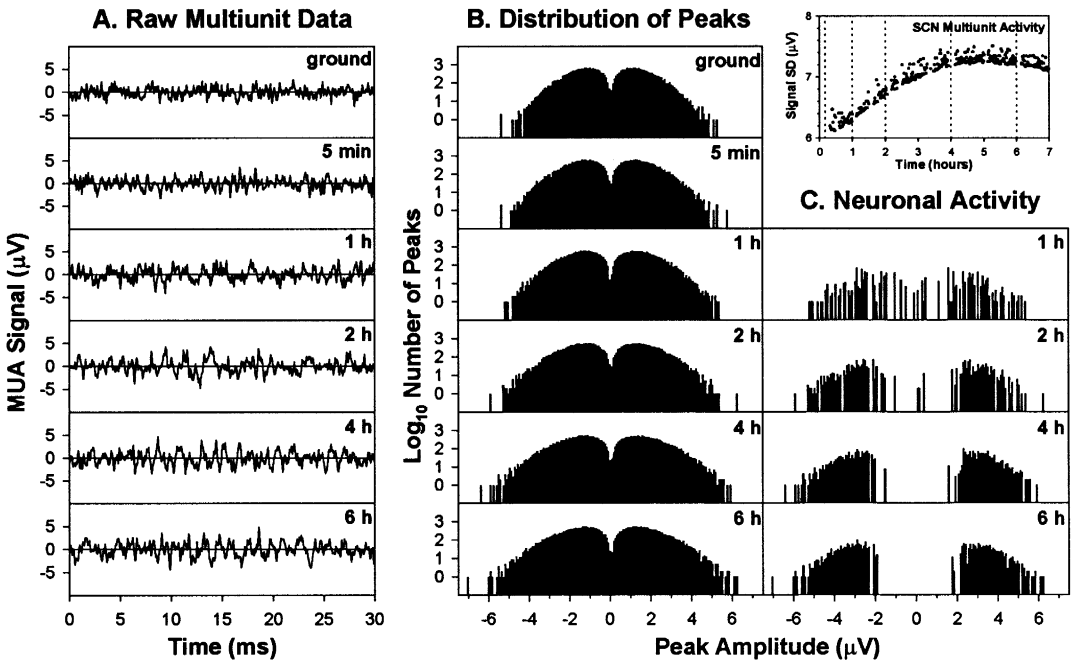


Fig. 3. Analysis of 6-s samples of raw multiunit data after filtering and digitizing at 20 kHz. The inset labeled SCN Multiunit Activity shows the long-term multiunit record during which the 6-s samples were taken. The gradual rise and decline are typical of the SCN MUA circadian rhythm. The vertical dotted lines indicate the times at which the 6-s samples were acquired. (A) Raw multiunit data: 30-ms segments of the 6-s raw multiunit data. The ground trace originated from the amplifier's internal ground circuit and represents equipment/environment noise; it does not include any contribution from the tissue. The 5 min trace was recorded 5 min after brain slice preparation while neuronal activity was still inhibited (see text) and represents a combination of environment/equipment and background noise present in the 1-, 2-, 4- and 6-h samples. The 1 h trace shows recovering neuronal activity. The 2-, 4- and 6-h traces show fully recovered neuronal activity. (B) Distribution of peaks: Histograms of the number of peaks (relative maxima or minima) detected at each voltage amplitude in the 6-s samples. Note that the *y*-axis is the $\log_{10}$ number of peaks so that the maximum bar height is $\sim 10^3$ peaks. (C) Neuronal activity: These histograms are the result of subtracting the 5 min histogram in panel B from the 1-, 2-, 4- and 6-h histograms in panel A. They represent an estimate of the neuronal component of the histograms in panel A.

extracellular single unit recording for the time-of-day that each sample was obtained (Satinoff et al., 1993), as shown in Table 1. This operation gives a conservative estimate of the number of neurons in the sampling volume because it only considers active neurons. The values for number of neurons given in Table 1 were calculated by this method. Averaging these values for the 2-, 4- and 6-h samples (neuronal activity may not be fully recovered in the 1 h sample) gives an estimate of ~40 neurons in the sampling volume. There are ~8000 neurons in 0.068 $mm^3$ in the SCN (van den Pol, 1980), or one neuron per 8500 $\mu m^3$. Multiplying this value by 40 gives a conservative estimate of 340 000 $\mu m^3$ for the sampling volume. This is in general agreement with the 392 700 $\mu m^3$ calculated above. These estimated sampling volumes represent 0.5–0.6% of the total volume of the SCN.

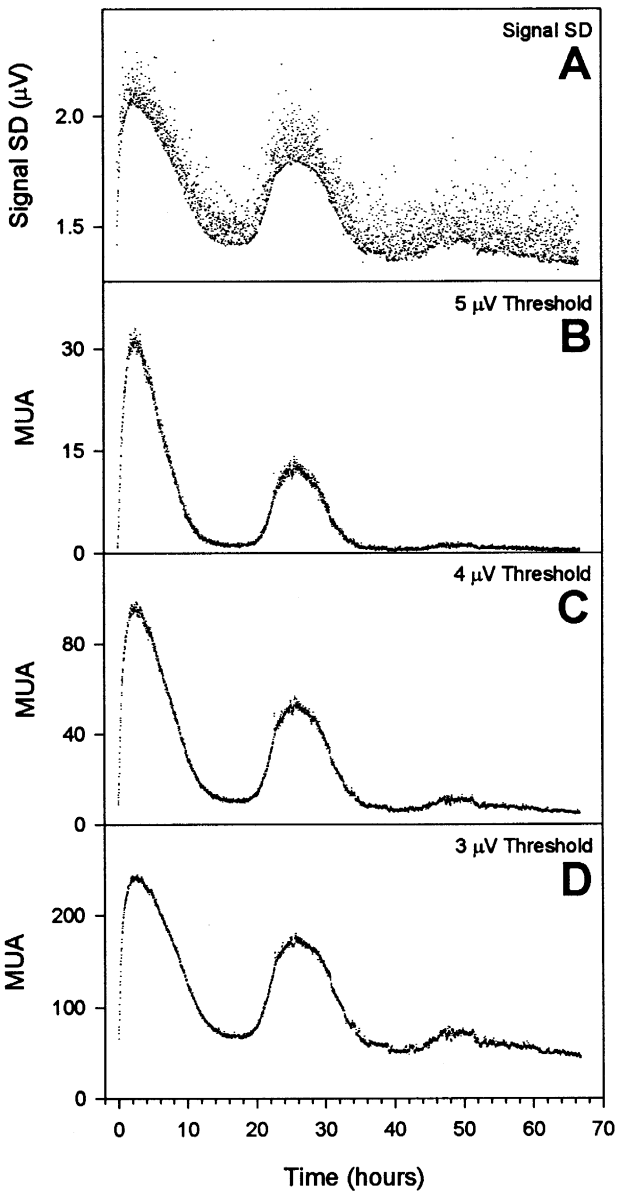


Fig. 4. Long-term record of spontaneous activity from a single electrode position in a suprachiasmatic nucleus. Each point represents a 1-min average of 60 1-s measurements. (A) The SD of the signal, illustrated in Fig. 3A, follows the same general waveform as the MUA. (B–D) Multiunit activity measured using 5-, 4-, and 3-μV thresholds, respectively.

## 6. Long-term multiunit recording

In a single experiment, long-term MUA was recorded from the ventral region of a suprachiasmatic nucleus for ~68 h using thresholds set at 3, 4, and 5 μV as well as recording the SD of the signal. Fig. 4A shows that the signal SD follows the nearly 24 h oscillation typical of this tissue both in vivo (Inouye and Kawamura, 1979) and in vitro (Prosser and Gillette, 1989). This suggests that the signal contains a large component of neuronal activity that dominates the SD statistic. The upward deflections in the SD plot are due to high amplitude activity and/or noise which, because of their large amplitude, affect the SD statistic more than the MUA spike count averages in which they would be counted as single spikes.

Fig. 4b–d shows MUA measured using 3-, 4-, and 5-μV thresholds. Note that as the threshold is lowered below the background noise level and progressively deeper into the signal, the three peaks in the record become broader and larger. This is presumably because more neurons are being sampled at the lower threshold levels. The similarity between Fig. 4D, corresponding to the lowest threshold, and Fig. 4A, the signal SD, further supports this speculation since the signal SD samples the entire signal and because the lower thresholds sample progressively more of the available signal. These observations support the notion that sampling using a threshold set below the background noise level can yield useful information concerning neuronal activity.

## 7. Concluding remarks

The CFB multiunit electrode described here has great potential for long-term multiunit recording from brain slices. This method stably records in vitro circadian oscillations of spontaneous neuronal activity that have been observed in vivo using metal multiunit electrodes and in vitro using glass single-unit and metal multiunit electrodes. Multiunit recording improves upon our earlier single-unit long-term recording method because it does not involve labor-intensive repetitive repositioning of the electrode and associated tissue damage. Multiunit recording also avoids the risk of subjective bias in selecting individual neurons from which to record.

The CFB multiunit electrode can also be used to record acute multiunit responses to various stimuli. The response of a local population of neurons to a stimulus, which is often more physiologically relevant than individual neu-

ronal responses, can be quickly and easily measured without the painstaking effort involved with sampling large numbers of individual neurons.

Finally, the apparent biocompatibility of the CFB electrode suggests that it may be adaptable to a range of electrophysiological applications including in vivo recording and use with multielectrode arrays.

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