



Letter

A novel high-purity carbon-nanotube yarn electrode used to obtain biopotential measurements in small animals: flexible, wearable, less invasive, and gel-free operation

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ABSTRACT — Carbon-nanotube yarn (CNT-Y) made from high-purity, highly crystalized, double-walled carbon nanotubes is an advanced material with excellent electrical conductivity and flexibility; hence, it could potentially be used as a novel electrode for biopotential measurements. To our knowledge, the present study is the first in which CNT-Y electrodes were used to conduct electrocardiography (ECG) and electroencephalography (EEG) on experimental animals. All procedures and biopotential measurements were performed under isoflurane anesthesia. The CNT-Y electrodes were attached to the animals by creating a single interrupting suture on the skin. The lead II electrode configuration was used for ECG recording, i.e., the positive, negative, and body-earth electrodes were placed on the left apex of the auricular surface, the interscapular region, and the cervical region, respectively. The bipolar lead was used for EEG recording, with the exploring and reference electrodes on the bregma and base of the right auricular surface, respectively. Using CNT-Y electrodes, we obtained a clear ECG waveform from rats and a guinea pig; the QRS amplitude was ~1.4 mV. In rats, we obtained an EEG waveform with an amplitude of ~150 μ V; the peak frequency was 0.8 Hz and the range was ~3 Hz according to power spectral density analysis. In the guinea pig, we obtained an EEG waveform with an amplitude of ~500 μ V; the first peak was 0.1 Hz, the second peak was 1 Hz, and the range was ~3 Hz. These results show that CNT-Y could be used in toxicology studies to easily and inexpensively obtain high-resolution biological signals.

Key words: Biopotential measurements, Electrocardiogram, Electrode, Carbon-nanotube, Vital signs, Experimental animal

INTRODUCTION

Given the remarkable progress in digital technology in recent years, it is now possible to measure vital signs using small and wearable devices, which have become widely used in human medicine. To improve the performance of these wearable devices, it is necessary to con-

sider the sensor material; conventional devices are typically made of metal-based rigid materials that limit their shape and usage. In experiments using small laboratory animals, it is often important to measure vital signs, such as pulse, breathing rate, body temperature, blood pressure, and consciousness, which can help diagnose the health condition of animals; however, compared with

the results achieved in humans, the success of measuring vital signs in experimental animals has been limited. Although vital signs collected from conscious and unrestrained animals best represent the condition of the animal, most animals are restrained when taking these measurements; thus, major artifacts can be introduced into vital sign measurements, which can only be avoided by a skilled technician.

To solve this problem, telemetry methods have been established (Kramer and Kinter, 2003). For example, electrocardiography (ECG), a basic heart examination process, has been conducted in safety pharmacology studies related to pharmaceutical drug development and in basic physiology and pharmacology studies using telemetry transmitter systems attached to dogs, monkeys, and rodents (Chui *et al.*, 2009; Ewart *et al.*, 2013; Fish *et al.*, 2017). However, because the electrodes and transmitter (including a battery) must be surgically implanted into the body of an animal, this highly invasive procedure requires a recovery period that can delay the experiment. In addition, because the electrodes are generally made of metal, e.g., stainless steel or platinum, they may be subject to polarization after being embedded inside the body for a long period.

Carbon nanotubes (CNTs) are engineered nanomaterials with novel physicochemical properties that represent a promising new technology platform. Pristine CNTs are fibrous materials that are tens of micrometers in length. Incredible advances in this technology have made it possible for CNTs to be formed into yarn (CNT-Y) with a length of about 1 km. CNT-Y is electrically conductive and flexible, and it can be used like ordinary silk or cotton thread.

Here, we report the use of CNT-Y to produce surface electrodes that can be applied for ECG with small laboratory animals. To our knowledge, this is the first report in which CNT-Y was used as a material for electrodes to take biopotential measurements from rats and guinea pigs. The CNT-Y electrodes were attached to the animals by creating a single interrupting suture on the surface of skin using a monofilament suture. The CNT-Y electrode is easier to install and less invasive than an implantable telemetry system; thus, it has the potential to be used to collect various biopotential measurements.

MATERIALS AND METHODS

Materials

Commercially available CNT-Y was purchased from Siddarmark LLC (Kanagawa, Japan); it is made of double-walled CNT and has a diameter of approximately

50 μm . The electrical resistance value of this CNT-Y, as measured using a resistance meter (RM3542-01; HIOKI E.E. CORPORATION, Nagano, Japan), was 1.204 Ω/m . Detailed information of the production method and physicochemical properties of CNT-Y can be found elsewhere (Iijima *et al.*, 2011).

Animals

Rats and a guinea pig were used in this study, i.e., two species with different electrophysiological characteristics of the heart (Joukar, 2021). Hairless female rats (HWY/Slc) and a male guinea pig (Slc: Hartley) were purchased from Japan SLC, Inc. (Shizuoka, Japan). Hairless rats were selected because they have no fur; thus, it is easy to attach the CNT-Y and acquire electrical signals from the surface of the body and to observe the CNT-Y for a couple of days after its placement without the need to clip the rat's fur.

The animals were housed in a conventional environmentally controlled animal care facility at the National Institute of Health Sciences (NIHS). The room temperature, relative humidity, and light cycle were $25 \pm 2^\circ\text{C}$, $50\% \pm 20\%$, and 12/12-hr light/dark, respectively. The rats and the guinea pig were kept in individually ventilated cages (Lab Product Inc., Seaford, DE, USA) and allowed free access to water and standard laboratory food (CRF-1 for rats and LRC-4 for the guinea pig; Oriental Yeast Co., Ltd., Tokyo, Japan). After acclimation for 2 weeks, 11-week-old rats ($n = 3$) and the 10-week-old guinea pig ($n = 1$) were subjected to the experimental procedure.

The guidelines established by the ethical committee for animal experiments of the NIHS were followed for the care and use of animals. The animal facility has been approved by the Health Science Center for Accreditation of Laboratory Animal Care, Japan (from 21 March 2009 valid until 24 March 2024). All the experimental protocols involving the laboratory rats used in this study were reviewed and approved by the Committee for Proper Experimental Animal Use and Welfare, a peer review panel established at the NIHS, with the experimental code #717 for the guinea pig and #727 for rats.

Biopotential measurements from the body surface of animals

We conducted ECG and electroencephalograph (EEG) to obtain biopotential measurements from the body surface of animals. The animals were subjected to these measurements under isoflurane (MSD Animal Health, Tokyo, Japan) anesthesia mixed with oxygen administered using an inhalation anesthesia apparatus (RC^2 + Anesthesia sys-

CNT yarn as a biopotential electrode for use in small animals

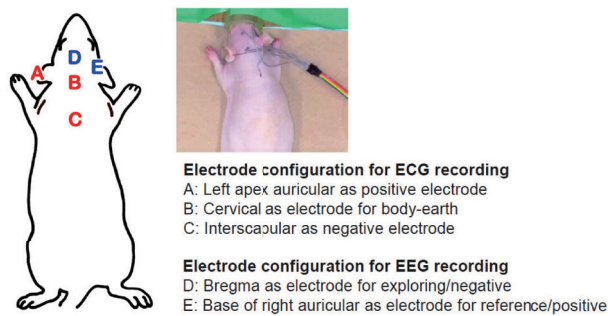


Fig. 1. Overview of the CNT-Y placement and electrode configuration for ECG and EEG recordings.

tem, VetEquip, Pleasanton, CA, USA). The animals were anesthetized in a tight-box using 4% isoflurane for anesthesia induction and then via a facemask using 2% isoflurane for the entire experiment. They were placed on a heater mat (KN-475-3-35; Natsume Seisakusho Co., Ltd., Tokyo, Japan) to prevent hypothermia.

The CNT-Y were attached to the animals by creating a single interrupting suture on the skin using a needle for plastic surgery (1/2 circle, 11 mm in diameter; Cat No. C-24-540-00, Natsume Seisakusho Co.). The CNT-Y were placed in five positions, i.e., the (A) left apex of the auricular surface, (B) cervical region, (C) interscapular region, (D) bregma, and (E) base of the right auricular surface. An overview of CNT-Y placement and electrode configuration are shown in Fig. 1.

The electrode placement for ECG recording comprised a modified lead II electrode configuration as follows: the positive electrode on the left apex auricular surface (Fig. 1, A), the negative electrode on the interscapular region (Fig. 1, C), and the body-earth electrode on the cervical region (Fig. 1, B), i.e., between the positive and negative electrodes. The EEG recording was obtained using a bipolar lead, with the exploring/negative electrode on the bregma (Fig. 1, D) and the reference/positive electrode on the base of the right auricular surface (Fig. 1, E). The state of spontaneous brain activity was recorded without stimulation under isoflurane anesthesia conditions.

Because CNT-Y is a nonmetal material, it cannot be soldered. Therefore, to connect it to a conventional electronic circuit, it was crimped and fixed through a hollow electrode. Except for the section connected to the skin, the CNT-Y was covered with a silicone rubber tube (outer diameter: 1.2 mm; inner diameter: 0.6 mm) to prevent short-circuiting. A whole image of the CNT-Y electrode is shown in Fig. 2, and an enlarged view of the part of the hol-

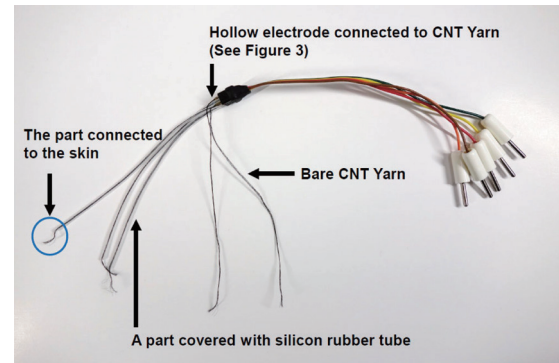


Fig. 2. Whole image of a CNT-Y electrode.

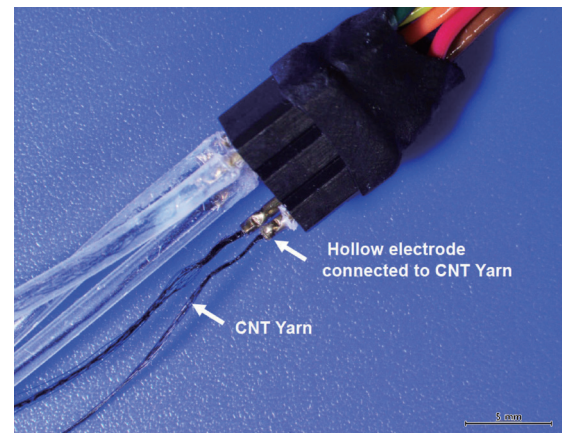


Fig. 3. An enlarged view of the part of the hollow electrode connected to the CNT-Y.

low electrode connected to the CNT-Y is shown in Fig. 3.

The CNT-Y electrodes were sequentially connected to a biological signal amplification unit (BAS-3012, Biotex, Kyoto, Japan) and a DC-DC converter including a power supply (IF-2, Biotex). The characteristics of the biological signal amplification unit were as follows: input impedance: $> 10 \text{ M}\Omega$; amplification factor: 2,000 times; frequency characteristics: 1–250 Hz for ECG and 0.5–250 Hz for EEG; input conversion noise: $< 10 \text{ }\mu\text{Vp-p}$; output voltage: $> \pm 5 \text{ V}$. Finally, the biopotential signals were collected into a personal computer using data acquisition and analysis software (AcqKnowledge; BIOPAC Systems, Inc. Goleta, CA, USA) with an analog-to-digital converter (MP150; BIOPAC Systems, Inc.) and a sample rate of 2 kHz.

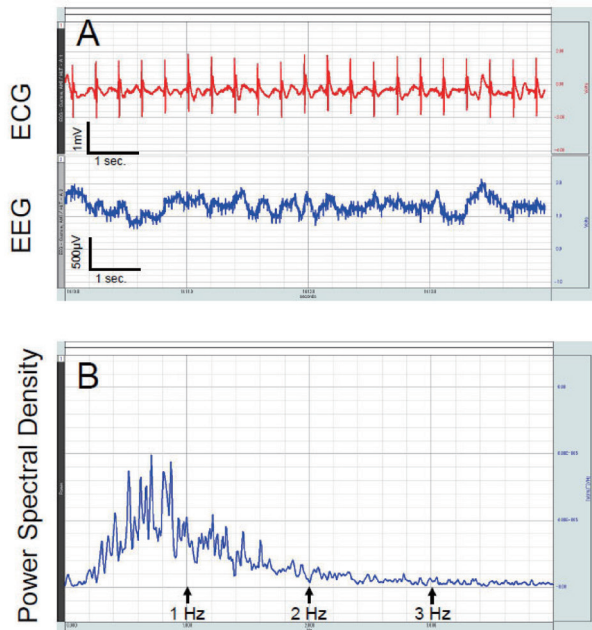


Fig. 4. Representative ECG and EEG traces and the power spectral density of EEG from hairless rats.

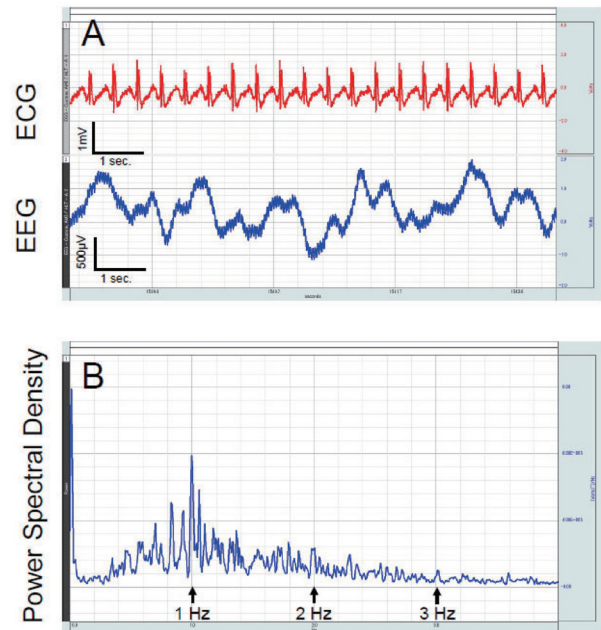


Fig. 5. Representative ECG and EEG traces and the power spectral density of EEG from a guinea pig.

RESULTS AND DISCUSSION

We obtained a clear ECG waveform with low noise levels from the rats and guinea pig (Figs. 4A and 5A). Because the amplitude of the QRS was ~ 2.8 V in the analysis software at 2,000-times amplification, the original QRS amplitude was calculated as ~ 1.4 mV.

In rats, we obtained an EEG waveform with an amplitude of ~ 1.5 V in the analysis software, i.e., an amplitude of ~ 150 μ V for the original waveform (Fig. 4A). Power spectral density analysis revealed the peak frequency to be 0.8 Hz with a frequency range of ~ 3 Hz (Fig. 4B). In the guinea pig, an EEG waveform with an amplitude of ~ 500 μ V for the original waveform was induced (Fig. 5A). According to power spectral density analysis, the first peak was 0.1 Hz, the second peak was 1 Hz, and the frequency range was ~ 3 Hz (Fig. 5B). In general, EEG of small experimental animals is conducted by implanting electrodes deep into the brain around the hippocampus or by implanting epidural screw electrodes (Kramer and Kinter, 2003; Lundt *et al.*, 2016; Madhok *et al.*, 2012; Mashour *et al.*, 2010). The EEG performed in this study was induced using bipolar leads from the bregma and the base of the right auricular surface; thus, the recording electrode was located further than the source of the neural activity. Moreover, the signals were recorded through tis-

suess with different impedances, such as the cerebral cortex, cerebrospinal fluid, skull, and skin. Therefore, the EEG waveform obtained in this experiment may have characteristics that differ from those of previously reported EEG waveforms. Further studies are needed to confirm these EEG characteristics; studies involving EEG recording while awake or asleep or auditory evoked potentials and visual evoked potentials will improve our understanding of the EEG waveforms induced using the developed method. To achieve biopotential measurements from conscious and unconstrained animals, we are currently developing a wireless device with a Bluetooth module suitable for the CNT-Y electrode.

Because suturing the CNT-Y to the skin is a simpler and less invasive method compared with implanting electrodes into the body of an animal, the developed method does not require a recovery period prior to measurements being taken. In addition, as the CNT-Y is not a metal electrode, it does not polarize and can be operated in a conductive gel-free manner. It has also been reported that ECG measurements can be achieved using CNT threads sewn into T-shirts in humans (Taylor *et al.*, 2021). In the future, it may be possible to obtain biopotential measurements using CNT-Y as a complete surface electrode by devising an appropriate shape. Overall, CNT-Y provides an extremely effective material for electrodes used to

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measure biopotential in small animals. Thus, it could contribute to easy and inexpensive acquisition of high-resolution biological signals in toxicology studies.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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