METHODICAL PAPERS

A Method for Chronic Registration of Brain Cortical Electrical Activity in Rats

Yu. I. Sysoev^{a,b,c,d}, V. A. Prikhodko^{a,d,*}, R. D. Idiyatullin^a, R. T. Chernyakov^a, V. E. Karev^e, and S. V. Okovityi^{a,d}

^aSaint Petersburg State Chemical and Pharmaceutical University, St. Petersburg, Russia
 ^bInstitute of Translational Biomedicine, Saint Petersburg State University, St. Petersburg, Russia
 ^cPavlov Institute of Physiology of the Russian Academy of Sciences, St. Petersburg, Russia
 ^dBechtereva Institute of the Human Brain, St. Petersburg, Russia

^ePediatric Research and Clinical Center for Infectious Diseases of the Federal Medical Biological Agency, St. Petersburg, Russia
*e-mail: veronika.prihodko@pharminnotech.com

Received November 21, 2021 Revised December 24, 2021 Accepted December 24, 2021

Abstract—Electrocorticogram registration and analysis (electrocorticography, ECoG) is widely used in small-animal biomedical research. To date, a considerable number of techniques for ECoG electrode manufacture and implantation have been proposed. Many commercial manufacturers of neurophysiological equipment offer a wide variety of such electrodes, ranging from the simple needle or concentric ones to flexible microelectrode arrays. However, due to several reasons, neurophysiological methods (including ECoG) are much more rarely used in practical research than, for example, behavioural testing or molecular genetics. In this paper, we propose a simple and straightforward method for rat ECoG electrode assembly and implantation. Brain electrical activity registration in laboratory animals can be used to evaluate the functional state of the central nervous system, the effects of pharmacological interventions, and sensory system function by recording visual and somatosensory evoked potentials. The method we propose can be employed in neuropharmacological as well as neurophysiological experiments and may prove a valuable addition to the more conventional biomedical research techniques.

DOI: 10.1134/S0022093022010252

Keywords: electrocorticography, electroencephalography, brain electrical activity, visual evoked potentials, somatosensory evoked potentials, electrophysiology

INTRODUCTION

Neurophysiological research methods such as electroencephalography (EEG) or electrocorti-

cography (ECoG) allow for a wide range of tasks in neurobiology. For example, registration of cortical activity in small laboratory animals can be used to assess the functional state of both individ-



Fig. 1. A single-row, 8-pin, 2.54 mm pitch, straight female BLS-8 header connector.

ual structures and cerebral function as a whole, which can be important in modelling various pathological conditions and searching for new therapeutic approaches to their correction [1, 2]. Separate studies are aimed at finding correlates of ECoG activity and emotional aspects of animal behaviour [3, 4]. The data obtained may be useful for studying the physiology of behaviour, or activity of new psychoactive drugs. Recording and analysing cortical responses to various stimuli (e.g. cutaneous electrical stimulation, photostimulation) is often used by neurophysiologists to study sensory systems or conductive pathways [5–7]. The above examples only partially demonstrate how diverse the purposes of ECoG recording in small laboratory animals can be.

Despite the many advantages of EEG and ECoG methods in neurobiological research, only a small number of research groups use them in their work, increasingly preferring behavioural tests, if possible supplemented by molecular genetic methods (Western blotting, polymerase chain reaction method, etc.). The reason for this may be the difficulties in making and/or implanting EEG/ECoG electrodes. There are several tutorials [8, 9] that describe the approaches to microelectrode fabrication in sufficient detail. However, despite this, when getting acquainted with the ECoG method, the question often arises as to which electrodes should be used for solving specific experimental problems. In addition, the fabrication of several electrodes requires special skills. Commercially available electrodes (simple needle or concentric electrodes, or more complex electrodes such as flexible microelectrode arrays) produced by manufacturers of neurophysiological equipment, as a rule, are made abroad, on order, and therefore have a rather high cost. In addition, the precise location of recording electrodes determines the need to use a stereotactic device, which also makes such studies expensive and inaccessible to many laboratories. Given the above, the aim of the present work was to develop a simple method of manufacturing and implantation of ECoG electrodes in rats, allowing to register spontaneous bioelectrical activity of the brain, as well as cortical responses to photo- and somatosensory stimulation.

MATERIALS AND METHODS

The study was performed by the principles of the Basel Declaration, the Order of the Ministry of Health of the Russian Federation from 01.04.16 No. 199n "On approval of the rules of good laboratory practice" and the recommendations of the Bioethical Commission of the Federal State Budgetary Educational Institution of Higher Professional Education of the Ministry of Health of Russia. Experiments were performed on male white outbred rats weighing 250-300 g obtained from FGUP PLZ "Rappolovo" (Russia, Leningrad region, Vsevolozhsky district, Rappolovo village). All animals were quarantined for 14 days before the study. Rats were kept under standard vivarium conditions on the usual food ration, with free access to water.

Corticographic electrodes were made from 0.5 mm diameter nichrome wire (for the active and reference electrodes) and 0.16 mm diameter for the grounding electrode. Isolation was performed with shrink tubing (1.5/0.5 mm); the length of the recording (uninsulated) part was ≈ 1 mm. All electrodes were connected in a BLS-8

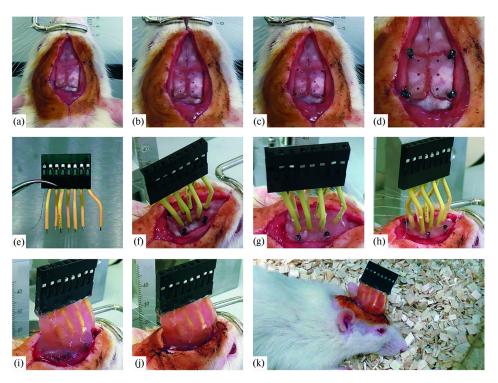


Fig. 2. Electrocorticographic electrode implantation steps. (a) Animal head fixation in the stereotaxic frame and cranial surface preparation. (b) Burr hole drilling for the active and reference electrodes. (c) Burr hole drilling for the fastening screws. (d) Fastening screw implantation. (e) Electrode assembly in the connector. (f) Active and reference electrode implantation. (g) Acrylic resin foundation casting. (h) Ground electrode implantation under neck skin. (i) Electrode assembly coating with acrylic resin. (j) Incision suturing. (k) Implanted animal housing in an individual cage.

header connector (Connfly Electronic Co. Ltd., PRC) with a pitch of 2.54 mm (Fig. 1).

For surgical manipulations, animals were preanaesthetized with chloral hydrate (2,2,2-Trichloroethane-1,1-diol) (MilliporeSigma, USA; 400 mg/kg, intraperitoneal) or tiletamine (2-Ethylamino-2-(2-thienyl)cyclohexanone)/zolazepam (4-(2-fluorophenyl)-1,3,8-trimethyl-6H-pyrazolo[3,4-e][1,4]diazepin-7-one) 50 (Zoletil®, Virbac, France; 10 mg/kg, intramuscular). The choice of a particular anaesthetic depended on the duration of the planned experiment. Due to its high toxicity, chloral hydrate is not recommended for long-term studies, since test animals may develop adverse reactions over time, such as severe atonic ileus [10] and gastric ulceration [11]. According to our observations, disruption of intestinal peristalsis in rats may occur one to two weeks after a single anaesthetic dose of chloral hydrate. Therefore, for long-term experiments, we recommend using tiletamine/zolazepam in the above dose, or the inhaled anaesthetic isoflurane ((RS)-2-Chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane).

Upon reaching the stage of deep anaesthesia, the rats were shaved and treated with iodine solution on the head surface, after which they were fixed in a stereotactic apparatus (RWD Life Science Inc., USA). Before further manipulations, the eyes were covered with carbomer 974P (Oftagel®, Santen Oy, Finland) to prevent corneal drying. The incision of the scalp was made in the rostrocaudal direction from the point between the eyes to the beginning of the cervical region. After preparation of the skull surface (removal of the muscular-fascial layer, periosteum, coagulation of bleeding areas (Fig. 2a)), holes of appropriate diameters were drilled for electrodes (Fig. 2b) and fixation screws (Fig. 2c) (drill immersion depth up to 1 mm). To prevent heating of the brain, drilling was performed at short intervals. Then, the fixation screws (Fig. 2d) and corticographic electrodes (Fig. 2e) were implanted.

The coordinates of electrode locations were determined using a stereotactic atlas of the rat

brain by Paxinos and Watson [12]. The recording electrodes were evenly and symmetrically distributed on the surface of the cerebral hemispheres. Electrodes FP1 and FP2 were placed in the primary motor cortex (AP = 0.0, ML = 2.0, DV = 1.0), C3 and C4 in the primary somatosensorv cortex (AP = -4.0, ML = 2.0, DV = 1.0), and O1 and O2 in the secondary visual cortex (AP = -7.0, ML = 2.0, DV = 1.0). The reference electrode was placed ≈5 mm rostral to electrodes FP1 and FP2 (Fig. 2f). The obtained structure was poured with a liquid solution of Villacryl S dental plastic (Zhermack SpA, Italy) to form a firm base (Fig. 2g). After the first layer of plastic hardened, the ground electrode was implanted under the skin in the neck area (Fig. 2h). Next, the electrodes were covered with plastic using a syringe and spatula (Fig. 2i). The skin incision was then sutured and the sutures and adjacent area were antiseptically treated (Fig. 2j).

The operated rats were kept in individual cages (type III for BIO. A.S.® IVC systems, Bioscape/ Zoonlab GmbH, Germany) (Fig. 2k) with free access to water and food during the entire study period. The condition of the animals was monitored immediately after coming out of anaesthesia and then daily in the morning and evening; the sutures were treated with iodine solution if necessary. To prevent infection, immediately after surgery the animals were given benzathine benzylpenicillin/benzylpenicillin potassium/ sodium, novocaine salts (Bicillin®-3, Sintez OJSC, Russia; 5000 units/kg, subcutaneously), to relieve postoperative pain, ketoprofen ((RS)-2-(3-benzoylphenyl)propanoic acid) (Ketoprofen®, Velfarm LLC, Russia; 2.5 mg/kg, subcutaneously once a day for 3 days). To avoid dehydration, 0.9% sodium chloride solution (Grotex LLC, Russia; 5 mL once a day) was injected subcutaneously into the rats during the first 3 days after surgery.

Recording of spontaneous cortical bioelectrical activity in animals was performed using an 8-channel Neuron-Spektrum-1 EEG system (Neurosoft LLC, Russia) with a bandwidth of 0.5–35 Hz and a sampling rate of 500 Hz. The signal recording was performed simultaneously with a video recording of the behaviour in the conditions of a home cage under artificial light.

Registration of visual evoked potentials (VEPs) was performed in a dark room. To evoke cortical responses to photostimulation, the animals, reliably fixed in the hands of the experimenter, were placed in front of a PhS-1 photic stimulator (Neurosoft LLC, Russia) with white glow LEDs. Stimulation frequency was 3 Hz, stimulation duration was 30 s, stimulus duration was 50 ms. The choice of optimal parameters of photostimulation was based on the data from the publications of other authors [13, 14], as well as our observations.

Before registration of somatosensory evoked potentials (SSEPs), the animals were anaesthetized by intraperitoneal injection of chloral hydrate (400 mg/kg). Current stimulation of *n. sciaticus* was performed alternately on the right and left sides using a Neuro-MEP electrical stimulator (Neurosoft LLC, Russia) (current strength 2 mA, rectangular wave, stimulus duration 0.1 ms, frequency 1 Hz). As with VEP registration, the choice of stimulation parameters sufficient to elicit a pronounced motor response was based on data from the literature [15] with modifications after our preliminary studies.

On day 7 after implantation, the animals were euthanized with carbon dioxide (Bioscape/Zoonlab GmbH euthanasia box, Germany). The extracted brain was fixed with 10% neutral formalin for 24 h, then it was opened so that the vertical incision went through the zone of electrode implantation, and tissue samples were cut out in the form of 0.2-0.3 cm thick plates. The obtained specimens were subjected to tissue processing, infiltration and embedding in paraffin according to the generally accepted technique. Tissue sections were made of paraffin blocks using a rotary microtome, placed on slides, stained with haematoxylin and eosin, then dehydrated and placed under coverslips. Histological preparations were subjected to total scanning using a scanning system for laboratory research with a set of programs for image processing Pannoramic **MIDI** (3DHISTECH Kft., Hungary).

RESULTS

Registration of spontaneous bioelectrical activity In the awake resting state, delta rhythms prevailed in the test animals, the index of which

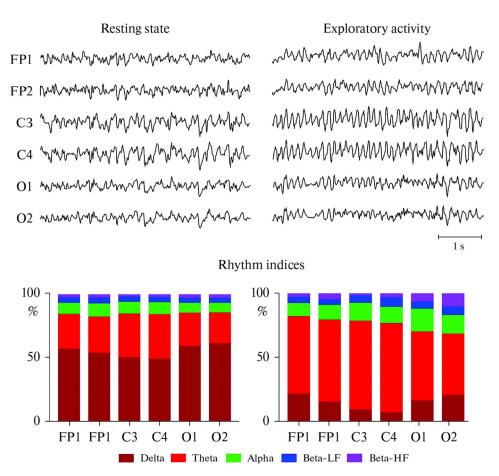


Fig. 3. Rat electrocorticogram examples in the resting state and during exploratory activity. The rhythm index diagrams represent the percent ratios of delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–14 Hz), low (beta-LF, 14–20 Hz), and high (beta-HF, 20–35 Hz) beta frequency bands.

could reach 50% and higher. About 20–30% were occupied by theta waves, and the remaining time was divided between alpha and low- and high-frequency beta rhythms. In the case of exploratory activity in rats (e.g., while sniffing the cage wall or looking out from behind it), the theta rhythms began to dominate in all leads and, in particular, in the parietal cortex above the hippocampus (electrodes C3 and C4) (Fig. 3).

Registration of spontaneous bioelectrical activity after pharmacological treatment

The ECoG signal is sensitive not only to the functional state of the nervous system (rest/research activity) but also to the action of psychoactive drugs. Thus, upon intraperitoneal injection of the alpha-2-adrenoreceptor agonist dexmedetomidine, used in clinical practice as a

sedative, a rise in delta rhythm activity in all leads was observed in the test rats already after several minutes. These changes were most pronounced in the frontal and parietal electrodes (FP1/FP and C3/C4, respectively) and were manifested as characteristic slow, high-amplitude waves (Fig. 4).

Recording the VEP

During photostimulation with a frequency of 3 Hz, we observed responses in the form of peaks N1 (\approx 20 ms), P2 (\approx 40 ms), N2 (\approx 60 ms), P3 (\approx 80 ms), and N3 (\approx 110 ms) in all leads in rats. The amplitudes of the recorded waves decreased in the caudal-rostral direction (as the distance from the occipital area with electrodes O1 and O2). It should be noted that in some animals a particular peak could be absent in some leads (Fig. 5).

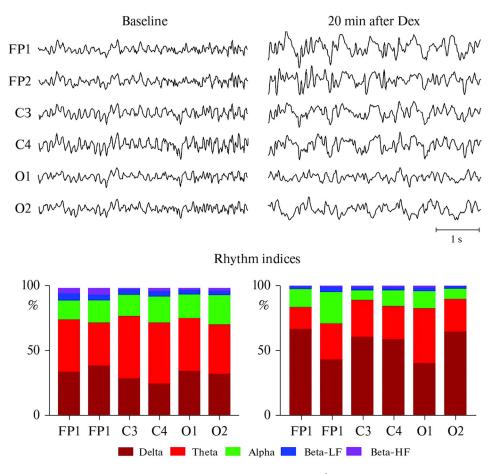


Fig. 4. Rat brain electrical activity at baseline and 20 min after $100 \text{ mg} \times \text{kg}^{-1}$ dexmedetomidine (Dex) administration. The rhythm index diagrams represent the percent ratios of delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–14 Hz), low (beta-LF, 14–20 Hz), and high (beta-HF, 20–35 Hz) beta frequency bands.

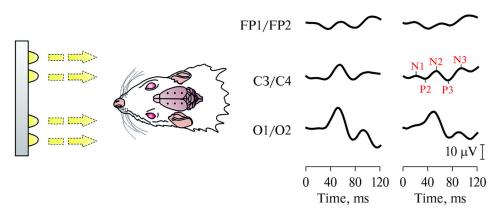


Fig. 5. Examples of frontal (FP1/FP2), parietal (C3/C4), and occipital (O1/O2) cortical responses to rhythmic photic stimulation.

Recording the SSEP

During electrical stimulation of *n. sciaticus* in rats, SSEP curves of the motor cortex of the con-

tralateral hemisphere included peaks N1 (\approx 30 ms), P2 (\approx 50 ms), N2 (\approx 70 ms), P3 (\approx 80 ms), and N3 (\approx 100 ms). The ipsilateral responses had

JOURNAL OF EVOLUTIONARY BIOCHEMISTRY AND PHYSIOLOGY Vol. 58 No. 1 2022

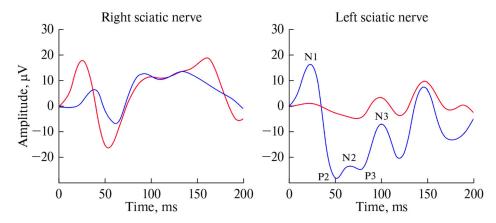


Fig. 6. Examples of primary motor (M1) cortical responses to electrical stimulation of the right and left sciatic nerves. Responses at electrode C3 (left hemisphere) are shown in red, and at electrode C4 (right hemisphere), in blue.

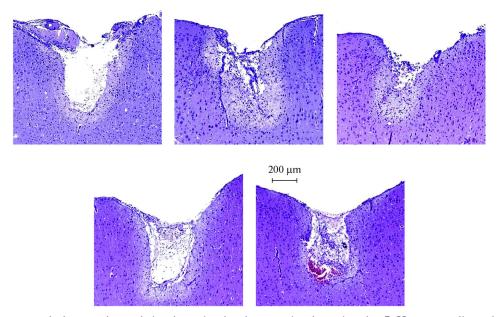


Fig. 7. Brain tissue morphology at electrode implantation level at post-implantation day 7. Haematoxylin and eosin staining.

similar components and, as a rule, their early N1 and P2 peaks had lower amplitude and greater latency compared with the contralateral ones. The latencies and amplitudes of the late N2, P3, and N3 waves did not generally differ between hemispheres. As in the case of VEPs, some components could be absent in individual rats (Fig. 6).

DISCUSSION

The method of ECoG electrode fabrication and implantation proposed in this article is easy to use and does not require any special skills or equipment. In spite of the fact that in our work we use a stereotactic unit for more precise and symmetrical electrode localization, its use does not always give pronounced advantages. First of all, this is because the thickness of the recording electrodes is quite large (0.5 mm), hence, the area of the cerebral cortex from which the signal is recorded is also quite large. Another problem is the necessity of reliable fixation of an animal in a stereotactic unit, which, on the one hand, increases the operation time and, on the other hand, imposes special requirements for the depth and duration of anaesthesia. Therefore, depending on the goals

and objectives of the study, implantation of electrodes can be performed without using a stereotactic unit.

A fundamental issue in any neurophysiological study involving chronic implantation is the safety and duration of fixation of the electrodes or electrode arrays used. In our studies, we did not observe signs of a pain response, which in rodents can be expressed as a decrease in general motor and exploratory activity, increased aggressiveness, tremor, absence of grooming, respiratory disturbances or changes in posture [16]. Also, no external pathological neurological signs—impaired support of a normal head position, spasticity, seizures, stereotypies, paresis or limb paralysis—were observed in the animals [16]. The latter is especially important because the electrodes were implanted, among other things, in the motor cortex area. The histomorphological picture of the implanted area on the 7th day after surgery was characterized by the formation of a small zone of necrosis around the electrode localization site, a weak exudative cellular reaction, and a widespread glial reaction of the brain tissue around the implanted area (Fig. 7). Because the electrodes used had no adverse effect on the condition of the test rats, the structure fixed on the skull could last from several weeks to several months if used carefully.

Another important question is to what extent the electrodes used can register local (specific) changes in the bioelectrical activity of a particular part of the brain. The results presented in the present work demonstrate that during exploratory activity the most pronounced rise of thetarhythms is recorded in the area of C3/C4 electrodes located in the area of the primary sensory cortex above the hippocampus (Fig. 3). This peculiarity is natural due to the fact that it is the hippocampus in rats that is the main generator of theta activity, which is largely associated with exploratory activity and memory [17, 18]. The increase in the amplitude of VEP responses as one move in the rostrocaudal direction, as well as the greater amplitude of SSEPs of the contralateral hemisphere (as compared with the ipsilateral one) during sciatic nerve stimulation similarly speak in favour of the specificity of the registered bioelectrical activity in the area of a particular electrode.

We previously used the present ECoG tech-

nique to study characteristic changes in spontaneous bioelectrical activity, as well as VEPs and SSEPs occurring in rats during the acute (3rd day) and subacute (7th day) periods after traumatic brain injury. Changes in the amplitude-spectral characteristics of ECoG [19], disturbances in interhemispheric and intrahemispheric connections (through cross-correlation and coherence analysis) [20], and changes in latencies and amplitudes of early and late responses of VEPs [21] and SSEPs [22] were revealed. Subsequently, the neuroprotective activity of the alpha-2adrenoreceptor agonist mafedine was confirmed in a study using all the above analysis methods [2]. This demonstrates that the electrodes used to allow to register not only the effects of drugs that have a pronounced influence on the psychoemotional state of rats (such as dexmedetomidine presented in the present work) but also rather delicate changes that neuroprotective agents can introduce into the course of pathological processes in traumatic brain injury.

Thus, the present work proposes a technique for making and implanting ECoG electrodes that can be made available to most laboratories involved in neurobiological research. The experience of using these electrodes suggests that they are well tolerated by the test animals, causing no signs of pain reaction or neurological disorders, as well as pronounced changes in the histomorphological pattern of brain tissue in the implantation area.

AUTHORS' CONTRIBUTION

Idea of work and planning of (Y.I.S., S.V.O.) conducting experiments and data processing (Y.I.S., V.A.P., R.D.I., R.T.C., V.E.K.), preparing illustrations (Y.I.S., V.A.P., V.E.K.), preparing and editing the manuscript (Y.I.S., V.A.P., V.E.K., S.V.O.).

FUNDING

The work was performed using the equipment of the Analytical Center of the Russian Ministry of Health under Agreement no. 075-15-2021-685 of July 26, 2021, funded by the Russian Ministry of Education and Science. The work was performed within the framework of the project no. 73025408 of St. Petersburg State University.

CONFLICT OF INTEREST

The authors declare that they have neither evident nor potential conflict of interest related to the publication of this article.

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Translated by A.V. Dyomina