Journal of Neuroscience Methods 286 (2017) 22–30

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Regular paper

A novel device for continuous long-term electroencephalogram recording and drug administration in mice with a nice, powerful and sophisticated wired system

Shigeru Watanabe[a], Masanori Saito[a], Masaki Soma[b], Hitoshi Miyaoka[a], Masami Takahashi[c,*]

[a] Department of Psychiatry, Kitasato University School of Medicine, 2-1-1 Asamizodai, Minami-ku, Sagamihara-shi, Kanagawa 252-0380, Japan
[b] Department of Research & Development Center, Kitasato University School of Medicine, 1-15-1 Kitasato, Minami-ku, Sagamihara-shi, Kanagawa 252-0374, Japan
[c] Department of Biochemistry, Kitasato University School of Medicine, 1-15-1 Kitasato, Minami-ku, Sagamihara-shi, Kanagawa 252-0374, Japan

HIGHLIGHTS

- We constructed a sophisticated device for long term EEG recording and drug infusion in moving mice.
- Continuous EEG recording was recorded for up to 63 days.
- Continuous drug infusion with EEG recording was succeeded for up to 22 days.
- The device will be useful to develop new antiepileptic drugs.

ARTICLE INFO

Article history:
Received 14 January 2017
Received in revised form 10 April 2017
Accepted 11 April 2017
Available online 20 April 2017

Keywords:
Epilepsy
Antiepileptic drug
Epileptogenesis
Epileptic maturation
Long-term drug administration
Electroencephalogram
Free-moving mouse

ABSTRACT

Background: To elucidate mechanisms of epileptogenesis and epileptic maturation, and to develop new AEDs, it is indispensable to administer various drugs and to examine their effects on EEG over a long period of observation.
New Method: We constructed a device for the continuous measurement of electroencephalography (EEG) and the infusion of anti-epileptic drugs over a prolonged period of time in moving mice. The system includes a slip ring and a swivel to prevent twisting of the recording cable and infusion tube, respectively. We introduced three arms, ball bearing, and stabilizing frame to rotate the slip ring and swivel with only a small applied force, and to facilitate the start of rotation of the slip ring and the swivel.
Results: Continuous EEG recording was successfully performed for up to 63 days in 99 mice, for a total of 1872 days of EEG data. Continuous drug infusion with continuous EEG recording was successfully performed for up to 22 days.
Comparison with Existing Method(s): Our system is superior to current system in continuous drug delivery during long-term EEG recording in moving mouse.
Conclusions: Our device will be quite useful for long-term EEG recording and drug application in moving mice.

1. Introduction

Epilepsy is a chronic neurological disorder that is characterized by recurrent spontaneous seizures due to neuronal hyperactivity in the brain. Epilepsy is the most common serious neurological condition, affecting more than 50 million people worldwide. Although

Abbreviations: AED, antiepileptic drug; CCD, charge-coupled device; EEG, electroencephalogram; ESM, ethosuximide; GS, generalized seizure; ID, inside diameter; i.p., intraperitoneal; OD, outside diameter; s.c., subcutaneous; SNAP-25, synaptosomal-associated protein of 25 kDa; SWD, spike and slow-wave discharge.

* Corresponding author at: Kitasato University School of Medicine, 1-15-1 Kitasato, Minami-ku Sagamihara-shi, Kanagawa, 252-0374, Japan. Tel.: +(+81) 42-748-9111, ext.2716 (office), (+81) 42-748-9111, ext.2546 (lab); fax: +(+81) 42-765-3570.
E-mail addresses: nabesige@kitasato-u.ac.jp (S. Watanabe), 7n2ecx@mtf.biglobe.ne.jp (M. Saito), msoma@kitasato-u.ac.jp (M. Soma), miyaoka@med.kitasato-u.ac.jp (H. Miyaoka), masami@med.kitasato-u.ac.jp (M. Takahashi).

http://dx.doi.org/10.1016/j.jneumeth.2017.04.005
0165-0270/

70–80% of people with epilepsy achieve remission, the remaining patients will continue to have seizures, eventually developing resistance to antiepileptic drugs (AEDs). Effective management of these patients requires a comprehensive understanding of the processes of epileptogenesis and epileptic maturation to support the development of new AEDs and new methods of treatment. To study the processes of epileptogenesis and epileptic maturation, continuous electroencephalogram (EEG) recording is inevitably necessary. However, obtaining continuous EEG records from human patients, from the time of brain insult to the onset of clinical seizures, is difficult, as the timeline of epilepsy development is often protracted, ranging from several months to many years. Furthermore, many human patients receive AEDs to suppress symptomatic or "early" seizures that are directly associated with the brain injury, with most patients being treated with AEDs after one or a few "late" epileptic clinical seizures. Therefore, quantitative analysis of the temporal features of acquired epileptogenesis in humans, independent of the effects of AEDs, is nearly impossible. Thus, adequate animal models of epilepsy are necessary to understand the neuronal basis of epileptogenesis and epileptic maturation.

To date, many mutant mice with spontaneous epileptic seizures have been established using either gene engineering techniques or by isolating spontaneous mutants (Steinlein and Noebels, 2000; Meisler et al., 2001; Crunelli and Leresche, 2002; Upton and Stratton, 2003; Baraban, 2007; Zhu et al., 2008; Watanabe et al., 2015). Animal models of acquired epilepsy have also been developed based on status epilepticus that is induced with administration of a chemo-convulsant drug (Coulter et al., 2002; Morimoto et al., 2004; Curia et al., 2008; Scorza et al., 2009; Otsuka et al., 2016), electrical stimulation (Kalynchuk, 2000; Löscher, 2002; Morimoto et al., 2004; Sloviter and Bumanglag, 2013) or hypoxic-ischemic brain injury (Williams et al., 2004; Kadam et al., 2010).

To elucidate mechanisms of epileptogenesis and epileptic maturation, and to develop new AEDs, it is indispensable to administer various drugs and to examine their effects on EEG over a long period of observation. A cable system had been used for EEG recording, however, since it is moored with a cable, the behavior of the mouse is considerably restricted, and troubles such as cables becoming tangled often occur. In order to avoid such troubles, the improvement of the telemetry system has advanced in recent years, and long-term EEG recording under free movement has become possible (Kramer and Kinter, 2003; Kadam et al., 2010; Zayachkivsky et al., 2015; Lundt et al., 2016). As for the drug administration, intraperitoneal (i.p.) or subcutaneous (s.c.) injections of AEDs are widely used for drug administration. However, since epileptogenesis and epileptic maturation are long lasting, it is necessary to repeatedly perform drug administration. Capturing animals for i.p. and s.c. administration while recording EEG is difficult, and moreover, it is possible that trapping itself may affect the process of epileptogenesis and epileptic maturation. Therefore, repetitive i.p. and s.c. administration of AEDs should be avoided. As well, in order to act continuously, it is necessary to continue drug administration even at night when there is no experimenter. For these reasons, a continuous injection method using an infusion tube and syringe pump would be most appropriate; however, the problems of restraint of animal movement and twisting of infusion tube arise in this system. Osmotic pump is useful for drug administration to free-moving animals. However, in order to change the dosage or the type drug during continuous application using an osmotic pump, additional surgery to substitute the pump is necessary. Surgery is very stressful for mice, so it is not preferable to do it frequently.

For these purpose, we developed a novel apparatus for continuous EEG recording and AED infusion that uses a swivel and a slip ring to eliminate twisting of the infusion tube and EEG recording cable, respectively, caused by in situ rotations (spin) movements of animals during unrestrained free motion. Current swivel and slip ring systems attach the infusion tube and recording EEG cable directly to the shaft of the slip ring and swivel and, consequently, the shaft is heavy to rotate and both the infusion tube and recording cable can become twisted, making free movement difficult. A collar placed around the neck of the mice has been used in these cases to prevent twisting of the infusion tube and recording cable. However, the collar itself often becomes twisted, forcing the mice to adopt stressful and forced postures which, again, impedes free movement. Our new system provides solutions to these problems, allowing continuous long-term EEG recording and drug infusion with moving mice, in which restraint was minimize as much as possible.

The aim of our study was to evaluate the effectiveness of our device through an analysis of continuous EEG recording and AED drug infusion in SNAP-25 mutant mice (Kataoka et al., 2011; Watanabe et al., 2015) and mice treated with pilocarpine causing spontaneous epileptic seizures (Otsuka et al., 2016).

2. Materials and Methods

2.1. Animals

All procedures involving animals complied with the guidelines of the National Institutes of Health and were approved by the Animal Experimentation and Ethics Committees of the Kitasato University School of Medicine. All efforts were made to minimize animal suffering and to reduce the number of animals used. *Snap25*$^{S187A/S187A}$ mice were generated using previously described methods (Kataoka et al., 2011). Briefly, mice with a heterozygous Snap25^{S187A} locus were bred with C57BL/6 N mice and maintained using the standard husbandry procedure. After completion of 13-back-crossing cycles of the C57BL/6 N genetic mice, homozygous mice were routinely obtained by in vitro fertilization using ICR mice as foster mothers. Animals in the breeding house were maintained in a 14:10 h light–dark cycle from 6:00 a.m., under constant temperature ($25 \pm 1\,^{\circ}C$) in a room with a clean air conditioning system.

2.2. Outline of the Device

A schematic representation of our system for continuous EEG recording and drug infusion is shown in Fig. 1. The whole device was set on a housing case placed in a soundproof box. Mice were free to move within a donut-shaped observation cage, having an outside diameter (OD) of 30 cm and inside diameter (ID) of 18 cm. Paper chips were laid on the floor of the observation cage and the cages were cleaned every 3–4 days. A cable for EEG recording and an infusion tube for drug administration were connected to the mouse via a socket (R317-83; Tokiwa & Co, Tokyo) secured to the animal's head. The recording cable was connected to a biological amplifier (BA1008; DIGITEX LAB, Tokyo), and the infusion tube to a syringe pump (KDS-220; KD Scientific Inc., Holliston, MA), both installed outside of the soundproof box. Twisting of the recording cable and the infusion tube was prevented by using a slip ring (SPM-35-8P-03; HIKARI DENSHI KOGYO, Tokyo) and a swivel (TCS1-20; Eicom, Kyoto), respectively. Movement of the mice was supported by the use of three different arms, namely the swivel arm, the slip ring arm and the free arm. Horizontal motion was supported by a ball bearing (SSXC000ZZ; NANKAI SEIKO, Osaka) connection between the main shaft and the slip ring shaft, while vertical movement was supported by the free arm, and rotational movements by a spindle. The behavior of the mice was recorded using an infrared charge-coupled device (CCD) camera.

2.3. An i.p. Catheter

An i.p. catheter was made using a polyethylene tube (OD 0.61 mm, ID 0.28 mm), with two silicone balls attached at one end