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Full paper

Simultaneous monitoring of mouse respiratory and cardiac rates through a single precordial electrode

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ABSTRACT

Normal respiratory and circulatory functions are crucial for survival. However, conventional methods of monitoring respiration, some of which use sensors inserted into the nasal cavity, may interfere with naïve respiratory rates. In this study, we conducted a single-point measurement of electrocardiograms (ECGs) from the pectoral muscles of anesthetized and waking mice and found low-frequency oscillations in the ECG baseline. Using the fast Fourier transform of simultaneously recorded respiratory signals, we demonstrated that the low-frequency oscillations corresponded to respiratory rhythms. Moreover, the baseline oscillations changed in parallel with the respiratory rhythm when the latter was altered by pharmacological manipulation. We also demonstrated that this method could be combined with *in vivo* whole-cell patch-clamp recordings from the hippocampus. Thus, we developed a non-invasive form of respirometry in mice. Our recording method using a simple derivation algorithm is applicable to a variety of physiological and pharmacological experiments, providing an experimental platform in studying the mechanisms underlying the interaction of the central nervous system and the peripheral functions.

1. Introduction

Normal function of the respiratory and circulatory systems is indispensable for survival. Catastrophic failure of either system leads to fatal diseases. Accordingly, electrocardiograms (ECGs) are widely used to quantify cardiac function.[1,2] Likewise, CO_2 sensors and thermally sensitive resistors in the nasal cavity, which detect respiration-related changes in CO_2 concentration and intranasal temperature, respectively,[3,4] are often used to measure respiratory rates. However, these measurements *per se* occasionally affect respiratory rates,[5] making it difficult to obtain intact respiratory information. This matter is particularly the case when small experimental animals are tested. Therefore, more reliable methods are required to measure intact respiratory rhythms.

In humans, a somewhat simple and non-invasive method in which the respiratory rhythms are mathematically isolated from the ECG signal has been clinically applied for monitoring the states of patients with sleep apnea syndrome or evaluating the exercise intensity.[6,7] Although the mathematical algorithm has allowed to derive the respiratory component from human ECGs, it remains unknown whether intact respiratory information can be derived from ECGs of small animals using simple statistical methods.

In the present study, we employed a single-point, direct measurement of ECGs from the pectoral muscles of anesthetized and waking mice. We found periodical slow oscillations in the ECG baseline. The periodic low-frequency (*i.e.*, approximately 3–4 Hz) oscillations reflected a respiratory component, which was assessed by two parameters of respiratory rhythms, *i.e.*, abdominal movement recorded through an isotonic transducer and local field potentials (LFPs) from the olfactory bulb. Moreover, we pharmacologically manipulated the respiratory rhythms of mice to examine whether the ECG low-frequency component changes in parallel with respiratory patterns. Finally, we applied this simple and reliable technique in combination with whole-cell patch-clamp

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recordings from hippocampal CA1 neurons and investigated the correlations between respiratory/circulatory functions and the central nervous systems.

2. Materials and methods

2.1. Animal ethics

All animal experiments were performed with the approval of the animal experiment ethics committee at the University of Tokyo (approval numbers: 29-9, 29-12, 29-14 and 29-15) and in accordance with the University of Tokyo guidelines for the care and use of laboratory animals. These experimental protocols were conducted in accordance with the Fundamental Guidelines for the Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions (Ministry of Education, Culture, Sports, Science and Technology, Notice No. 71 of 2006), the Standards for Breeding and Housing of and Pain Alleviation for Experimental Animals (Ministry of the Environment, Notice No. 88 of 2006) and the Guidelines on the Method of Animal Disposal (Prime Minister's Office, Notice No. 40 of 1995). All animals were housed under a 12-h/12-h dark–light cycle (*i.e.*, lights on from 07:00 to 19:00 for Figs. 1–3, 5; lights off from 07:00 to 19:00 for Fig. 4) at 22 ± 1 °C with *ad libitum* food and water.

2.2. Surgery

For physiological recordings from anesthetized mice, except for whole-cell recordings (*i.e.*, Figs. 1–3), 21- to 39-day-old male ICR mice (Japan SLC, Shizuoka, Japan) were used. The animals were anesthetized with 2.25 g/kg intraperitoneal urethane. Anesthesia was confirmed by the lack of paw withdrawal, whisker movement, and eye blink reflexes. A 1.0-cm incision was made in the precordial skin at a distance of 2.0 mm away from the midline, and a wire electrode (stainless steel wires; AS633, Cooner Wire Company, 15 cm long, 0.147 mm in diameter) was implanted in the pectoral muscle to record the ECG signals. The scalp was removed, and a metal head-holding plate was mounted on the skull. The plate was fixed firmly with dental cement. Then, a hole was made in the back of the head with a drill, and a stainless screw was implanted in the bone above the cerebellum to serve as ground. The screw was attached to a conductive lead wire (UEW, Oyaide Elec, Japan, Tokyo, 15 cm long, 0.14 mm in diameter). Then, a craniotomy was performed using a high-speed drill to create a hole (0.5 mm in diameter) centered at 1.5 mm anterior and 1.0 mm lateral to the bregma, and an electrode was inserted into the olfactory bulb to record LFPs. A suture thread was hooked to the subcutis of the side of the abdomen and was tied to an isotonic transducer, which converted the tension of the thread into electrical potentials.

For chronic recordings of ECG signals and olfactory bulb LFPs from behaving mice (Fig. 4), a total of 2 male ICR mice (3–4 months old) with preoperative weights of 45–50 g was used in this study. The detailed surgical procedures have been described elsewhere[8,9]). Briefly, mice were anesthetized with 1–2% isoflurane gas in air, and an incision (~1 cm) was made on each side of the upper chest. Then, 2 ECG electrodes (AS633, Cooner Wire Company) were sutured to the tissue underneath the skin of the upper chest. Circular craniotomies (1 mm in diameter) were made and stainless steel screws were implanted at the following coordinates: 5.0 mm anterior and 0.5 mm unilateral to bregma for the olfactory bulb (as an indicator of respiration); 6.5 mm posterior and 1.5 mm bilateral to bregma for the cerebellum (as ground). After all surgical procedures were complete, anesthetic administration was stopped, and the mice were allowed to awaken from the anesthesia spontaneously. For postoperative recovery, each mouse was housed individually in a cage for more than one week until its body weight returned to the preoperative level.

For *in vivo* whole-cell recordings (Fig. 5), 28- to 40-day-old male ICR mice (Japan SLC, Shizuoka, Japan) were used as previously described.[10,11] Briefly, the mice were anesthetized with urethane (2.25 g/kg, intraperitoneal [i.p.]). The skin was subsequently removed from the head, and the animal was implanted with a metal head-holding plate. A craniotomy (2.5 × 2.0 mm^2) was then performed, centered at 2.0 mm posterior to the bregma and 2.5 mm ventrolateral to the sagittal suture, and the neocortex above the hippocampus was carefully aspirated.[12] The exposed hippocampal window was covered with 1.7% agar at a thickness of 1.5 mm.

2.3. Behavioral task

For chronic recordings (Fig. 4), mice were allowed to explore an elevated plus maze, which was made of acrylonitrile butadiene styrene resin and consisted of a central square (7.6 × 7.6 cm) and four arms (28 cm long × 7.6 cm wide, two open arms with no railing and two closed arms enclosed by vertical walls 15 cm in height). The maze was elevated 30 cm from the floor. In each recording session, a mouse was placed in the middle of the central square, facing one of the open arms, and explored the maze apparatus for 5 min. The floor of the apparatus was cleaned with water and 70% ethanol after every exploration period.

2.4. Data acquisition

Simultaneous recordings of ECG signals, abdominal movement signals and olfactory bulb LFPs were made at a sampling frequency of 2 kHz using a CerePlex Direct recording system (Blackrock Microsystems, Salt Lake City, UT, USA). The physical abdominal movement was first converted into a change in electrical potentials through an isotonic transducer. The signal was then transmitted to the CerePlex system. These three bio-signals were recorded for 5 min.

For chronic recordings, the animal's behavior in the maze was monitored at a frame rate of 60 Hz using a top-view video camera. The frame rate of the video was down-sampled to 3 Hz, and the instantaneous speed of the animal in each frame was calculated from the distance traveled within a frame (~333 ms). The ECG signals and the olfactory bulb LFPs were acquired using a CerePlex Direct recording system.

Whole-cell recordings were made from pyramidal neurons in the CA1 *stratum pyramidale* using borosilicate glass electrodes (4–7 MΩ). Pyramidal cells were identified by their regular spiking properties and by *post hoc* histological analysis.[12] For current-clamp recordings, the intra-pipette solution contained the following solutes (in mM): 120 potassium gluconate, 10 KCl, 10 HEPES, 10 creatine phosphate, 4 MgATP, 0.3 Na_2GTP, 0.2 EGTA (pH 7.3), and 0.2% biocytin. Cells were discarded when the series resistance exceeded 75 MΩ or the mean resting potential exceeded −55 mV. Moreover, recordings were rejected when the resting potential increased by more than 8 mV from its value at the onset of the recording. ECG signals were simultaneously acquired as described above. The neuronal and cardiac signals were amplified by a MultiClamp 700 B (Molecular Devices, Union City, CA, USA) and a DAM80 (World Precision Instruments, Sarasota, FL, USA), respectively. All the signals were digitized at 20 kHz by a Digidata 1440A (Molecular Devices) and analyzed with pCLAMP 10.3 (Molecular Devices).

All signals were acquired at a room temperature.

2.5. Drugs

Acetazolamide (0.4 mg/kg, i. v., MP Biomedicals, Solon, OH, USA)[13] and diazepam (0.2 mg/kg, i. v., Wako, Osaka, Japan)[14] were

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