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The forced walking test: A novel test for pinpointing the anesthetic-induced transition in consciousness in mouse

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ABSTRACT

In consciousness or anesthesia studies, pinpointing the precise moment of consciousness or anesthetic transition has been challenging because of the variable lag time between a treatment and its induced response. Here, we describe a novel behavioral method, a forced walking test, which pinpoints the moment of the anesthetic-induced loss of motion (LOM) without handling the animals manually. The mouse is forced to walk on a treadmill, and an anesthetic drug is administered into the peritoneum via a previously secured injection route. The physical activity and the angle of head posture are tracked using a motion sensor preinstalled on the animal's head. The moments of LOM and recovery of motion (ROM) are identified from the physical activity parameters obtained by the sensor. Comparison of our method with the conventional loss-of-righting-reflex assay showed that the time point of LOM was not significantly different between the two methods when examined with two different types of anesthetic agents, propofol and ketamine/xylazine cocktail. In addition, the electrophysiological signals simultaneously acquired in the cortex and the thalamus of the mouse during the forced walking test showed that the brain rhythms induced by ketamine/xylazine anesthesia were generated and terminated in a time-locked manner with respect to LOM and ROM, respectively. In conclusion, the forced walking test allows an objective and precise detection of anesthetic-induced LOM, as well as ROM during awakening from anesthesia, in test animals.

1. Introduction

One of the most challenging tasks in science is understanding the neurobiological basis of consciousness. Anesthetic agents serve as a useful tool, particularly when used with genetically modified laboratory animals. A prevailing hypothesis on the transition to loss of consciousness (LOC) involves the disruption of corticocortical or thalamocortical integration, as evidenced by reduced functional or effective connectivity (Alkire et al., 2008; Crick and Koch, 1990). A stereotypic activity of neuronal populations preceding LOC is disrupted interplay among distant areas involved in neural responses, such as impaired anterior–posterior synchronization in light-induced event-related potentials (Imas et al., 2006) and suppressed frontoparietal feedback processing during propofol-induced anesthesia (Lee et al., 2009a). Reduced connectivity between brain regions observed during sleep (Massimini et al., 2005) and anesthesia (Peltier et al., 2005), and decreased capacity for information integration during anesthesia (Lee et al., 2009b) also support the hypothesis.

Many of the studies about consciousness focus on human subjects and usually take approaches comparing the characteristics of two distinct stationary states. In human study, commercial developments in clinical monitoring of anesthetic depth have been recently made, and some of them are available in clinical environments (Bruhn et al., 2006) and can be used as reference of anesthetic-induced change of consciousness. Current technologies incorporate auditory-evoked potentials (Mantzaridis and Kenny, 1997; Thornton et al., 1989) or electroencephalogram-derived indices, such as bispectral index (Rampil, 1998; Sigl and Chamoun, 1994), spectral entropy (Rezek and Roberts, 1998; Viertio-Oja et al., 2004), and spectral edge frequency (Kuizenga et al., 2001), along with traditional clinical signs such as respiratory depression and hypertension. It is important to note that no single parameter represents anesthetic transition, and these mathematically derived variables do not have a direct physiological equivalent, nor can they be easily confirmed by other measures. In addition, using positron emission tomography, various patterns of cerebral metabolic changes have been observed for different kinds of anesthetics

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(Alkire et al., 2000; Fiset et al., 1999; Veselis et al., 2004), implying that the physiological derivations accompanied by LOC are variable and depend on the mechanisms of action of the anesthetics.

In studying such various mechanisms of action of the anesthetic agents, animal studies play an important and complementary part, especially at molecular level. However, pinpointing the moment of LOC is a main problem for such animal studies. LOC in lab animals is often identified by abolition of reflexes or analgesia given noxious stimuli. The inability to verbally communicate with animals requires assessment of the depth of anesthesia via either physiological or behavioral analysis, as described most inclusively in the works of Field et al. (1993). Among these assessment methods, righting reflex and withdrawal reflex tests have been the most commonly used tools in animal studies (Flecknell, 1996). Both tests objectively assess unresponsiveness, but the intermittent monitoring of the animals fails to detect the precise moment of loss of responsiveness or reflex. Furthermore, interruption by the investigators is disadvantageous, especially when the cognitive or neurological processes during the anesthetic-induced transition are of particular interest in the study. To understand the underlying mechanisms of phase transition between conscious and unconscious states, a continuous tracing of brain activities with an undisturbed decisive reference point for the transition is critical since it makes possible to identify the brain structures first affected by action of anesthetic agents and relate it with spontaneous behavior changes. Conventional reflex test hardly meet this demand, and different kinds of stimulus–response tests which can grasp the moment of anesthetic-induced transition of consciousness becomes necessary.

The present study, therefore, aimed to pinpoint the anesthetic transition on a neuronal temporal scale without disturbing the animals during anesthetic administration and monitoring. The system used a highly sensitive accelerometer, forced walking of a mouse on a treadmill, non-interruptive administration of anesthetics, and electroencephalography (EEG) recording. The results indicated that our system could yield reliable information on the precise time points when the animal enters and recovers from the anesthetic state. The experimental setup was designed for mice, which is technically challenging because of the size of the animals but is beneficial for studying the molecular and neuronal correlates of consciousness using genetically modified mice. The precise moments of the anesthetic and awaking transitions detected in our system could be used for identifying the fundamental component triggering the transitions referred to as “dynamic core” (Tononi and Edelman, 1998) by analyzing the transitional dynamics of neuronal populations. Besides, this novel paradigm of the forced walking test could also be used for characterizing the dose–response relation of drugs in high temporal resolution.

2. Materials and methods

2.1. Animals and surgery

Seven male C57BL/6 × 129 F_1 hybrid mice (8–12 weeks; body weight 20–25 g) were used in our study including electrophysiological field potential recording, and thirty-five male ICR mice (8–12 weeks; body weight 25–30 g) were used in comparison experiments between forced walking and righting reflex tests. The animals were housed in a colony maintained on a 12-h light/dark schedule at 22 °C with 55% humidity and were allowed ad libitum access to food and water. The animals were born and raised in this specific-pathogen-free environment. All surgical, handling, and experimental procedures were conducted in accordance with the guidelines for the Institutional Animal Care and Use Committee, following Act 1992 of the Korea Lab Animal Care Regulations and associated guidelines.

Electrode implantation and recording procedures were adopted and customized from the procedure used to record local field potentials (LFPs) and EEGs from mice in our laboratories (Shin et al., 2005). Mice were anesthetized intraperitoneally (IP) with a ketamine–xylazine cocktail (120 and 6 mg/kg, respectively) and fixed in a stereotaxic apparatus (David Kopf Instruments, Model 902, Tujunga, CA, USA) for implantation surgery. Two tungsten electrodes with impedance ranging from 10 to 20 kΩ at 10 kHz were implanted into the ventral lateral (anterioposterior, −1.06 mm; mediolateral, 1.1 mm; dorsoventral, 3.5 mm) and ventral posteromedial (anterioposterior, −1.82 mm; mediolateral, 1.5 mm; dorsoventral, 3.7 mm) nuclei of the thalamus for LFPs, and two screw electrodes with impedance ranging from 5 to 10 kΩ at 10 kHz were implanted into primary motor cortex (anterioposterior, 0.74 mm; mediolateral, 1.5 mm; dorsoventral, 0 mm) and primary somatosensory cortex (anterioposterior, −1.82 mm; mediolateral, 3.0 mm; dorsoventral, 0 mm) for EEG recordings. A ground electrode was implanted on the interparietal bone. All the coordinates of the electrodes followed the mouse brain atlas (Paxinos and Franklin, 1997).

For remote administration of the anesthetic agent during the experiment, polyethylene tubing (10 cm in length, 1520/86 μm in outer/inner diameter; PE 100, Intramedic polyethylene tubing, Clay Adams, Sparks, MD, USA) was inserted into the abdominal cavity and knotted in the lateral abdomen for positioning. The other end of the tube was attached to a lump of dental cement, which was used to fixate the implanted electrodes as depicted in Fig. 1. The exposed length of the tube was approximately 3 cm.

2.2. Experimental design

Prior to experiments, each mouse was placed on a treadmill (LE8708, Panlab, Spain) in a suspended condition for 15 min and in a walking condition for 5 min to habituate to the treadmill. Depending on the reaction, some mice had a longer habituation period. After the habituation, baseline signals were acquired for 10 min, followed by remote administration of the ketamine–xylazine cocktail (120 and 6 mg/kg, respectively) through the previously secured IP injection route. The speed of the lane was kept at 5 cm/s throughout the whole measurement, which was never too fast for the animals and induced minimal walking behavior equivalent to a spontaneously behaving condition. The running lane also served as a continuous somatosensory stimulus throughout the experimental session in our study. The signals of the motion sensor and field potentials were recorded until the mouse resumed the forced walking.

2.3. Motion sensor system

A motion sensor for mouse head movement was constructed with a three-axis accelerometer (MMA7260Q, Freescale Semiconductor Inc., Austin, TX, USA). A sensor board was fabricated for the accelerometer according to the manufacturer-recommended circuit design and glued to the electrode connector. The wires from the accelerometer were connected to a lab-built electric board for pre-amplification and power supply. The amplified accelerometer signals were transmitted to the analog-digital converter (Digidata 1440A, Molecular Devices, Sunnyvale, CA, USA) and sampled at 10 kHz (Fig. 1). The measurement range of the accelerometer was ±1.5 G with a sensitivity of 800 mV/G, where G means acceleration due to the gravity at the Earth’s surface. The *x*-, *y*-, and *z*-axes signals correspond to rostral-caudal, dorsal-ventral, and lateral-medial motion, respectively. The measurement range was sensitive enough to detect sniffing behavior but was not saturated for quick motion of the mouse. The total weight of the headstage was 1.65 g, and the dimensions were 10 mm × 9.5 mm × 1.65 mm.