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Estimating Transition Point of Anesthetic-induced Loss of Consciousness in Mice by Detecting Motion in Response to Forced Movement

Eunjin Hwang, Seunghwan kim, and Jee Hyun Choi, *Member, IEEE*

Abstract—**To characterize transition periods of entrance to and emergence from anesthetic-induced unconsciousness in terms of thalamocortical neural activity, we devised a new method estimating a transition point of anesthetic-induced loss of consciousness. The method continuously monitors an animal's head motion in response to forced movement on treadmill and uses the motion signals as a criterion of the transition. Anesthetics were administered via previously secured intraperitoneal injection route in order not to disturb the animal's spontaneous movement. Resulting signals from the motion detector could discriminate the points of entrance into and emergence from the anesthetic-induced unconsciousness with resolution corresponding to the sampling frequency. This method makes it possible to track the anesthetic transition period continuously without contaminating EEGs and LFPs.**

I. Introduction

THE most dramatic change of conscious states one can experience during a life time might be a general anesthesia. Even with the structural diversity and non-identical targets that various anesthetic agents have, they seem to induce a similar behavioral end point of loss of consciousness [1]. Based on the fact, we can cast questions that whether there is a common anesthetic pathway and, if exists, what contribution this pathway exerts on the anesthetic-related alteration of conscious states.

One of the circuits critically involved in such transition between conscious and unconscious states is the thalamocortical network. Primarily it has been known to play an important role in the regulation of sleep and wakefulness [1, 2]. Because of behavioral similarities and physiological and pharmacological interactions that sleep and anesthesia have, researchers think that the anesthetics may act partly by activating, or mimicking the activity of, this endogenous sleep pathway [3].

The purpose of our study is to characterize transition periods of entrance to and emergence from anesthetic-induced unconsciousness in terms of thalamocortical neural activity, using various anesthetic agents. For the analysis of multi-channel relationship between anatomically connected regions of the thalamus and cortex, somatosensory-related (VPM and S1) and motor-related (VL and M1) pairs were selected as recording targets [4].

To begin with, we had to design an appropriate anesthetic experiment and identify the transition points between conscious and anesthetic-induced unconscious states as accurate as possible. In this paper we are mainly concerned in that point.

In animal experiments, stimulus-response-based methods like tail pinching and loss of righting reflex (LORR) have been used to assess the anesthetic depth [5, 6]. Such methods, however, have limited temporal resolution to pick out the transition point since a trial of pinching animal's tail or placing animal on its back needs certain time interval for monitoring the animal's response. Furthermore, examining righting reflex may increase the chance of artifact contamination in EEG signals due to the animal's movement of changing position.

To overcome the limitation of previous methods and pinpoint the transition, we devised a new method that detects animal's motion in response to continuous stimulus of forced walk and uses it as a criterion of the transition. Treadmill made the animal walk during experimental session and an accelerometer-based motion detector recorded the animal's movement concurrently with EEG and LFP signals. Anesthetic agent was smoothly administered via ready-secured intraperitoneal (IP) injection route. The continuous monitoring of the animal's movement could clearly point out its behavioral change caused by the anesthetic agent, which must be closely related to the point of anesthetic-induced loss of consciousness.

II. METHODS

A. Electrodes Implantation & Intraperitoneal Tubing

Mice of 8-10 weeks old (body weight 20~25g) were anesthetized with Ketamine-Xylazine cocktail (dose of 120mg/kg and 6mg/kg, respectively) and fixed in stereotaxic apparatus (Kofp instrument model 902). Two tungsten electrodes with impedance ranging 10-20 kilo-ohm were implanted to ventral lateral (VL) and ventral posteromedial (VPM) nuclei of the thalamus for LFPs, and two screw electrodes with impedance ranging 5-10 kilo-ohm were implanted to the interconnected cortical regions of M1 (to VL) and S1bf (to VPM) for EEGs. Ground and reference were chosen to be same and located on the cerebellar region.

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E. Hwang is with the Physics Department, Pohang University of Science and Technology, Pohang 790-784 Korea (e-mail: bluemoon@postech.ac.kr).

S. Kim is with with the Physics Department, Pohang University of Science and Technology, Pohang 790-784 Korea (e-mail: swan@postech.ac.kr).

J. H. Choi is with the Center for Neural Science, Korea Institute of Science and Technology, Seoul 136-791 Korea and the Department of Neuroscience, University of Science & Technology, Daejon 305-333 Korea. (corresponding author to provide phone: +82-2-958-6952; fax: +82-2-958-6937; e-mail: jeechoi@kist.re.kr).

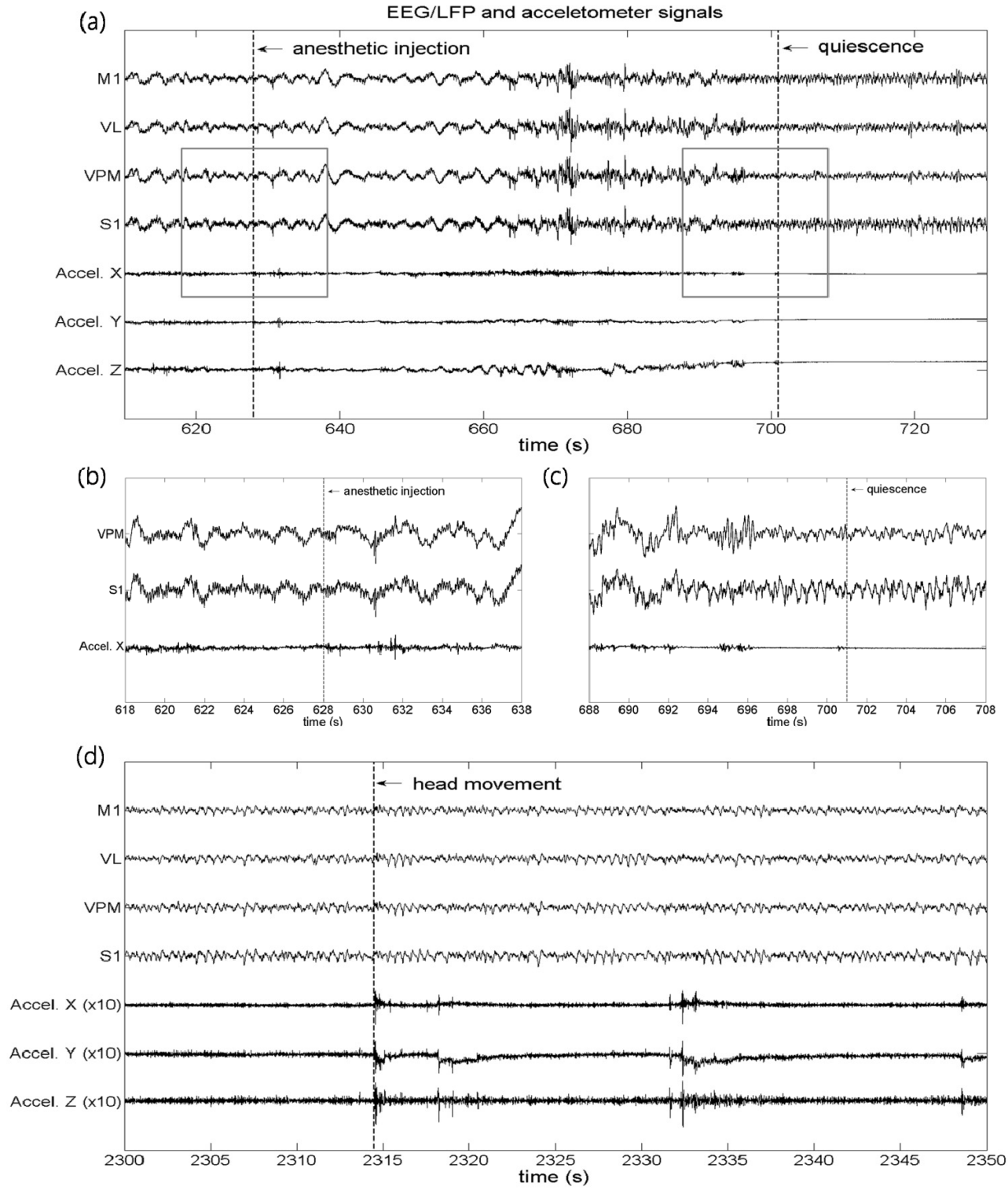

Fig. 1 Performance of the motion detector. (a) First four signals represent EEG and LFP recordings and last three signals represent the animal's motion detected by the three-axis accelerometer (y-axis: normalized arbitrary unit). Vertical lines indicate the moment of anesthetic agent injection (left) and quiescence of mouse movement (right). (b) Left box of upper panel (20 s window around the injection) is enlarged. (c) Right box of upper panel (20 s window around the quiescence) is enlarged. (d) The motion signals are magnified by 10 times to clearly show that the mouse regains its movement at 2314.5 s.

Coordinates of the electrodes followed the mouse brain atlas of Paxinos and Franklin [7].

In addition to the electrode implantation, a polyethylene tubing (PE 190, ID 1.19mm, OD 1.70mm) was inserted into the abdominal cavity in order to secure IP injection route allowing smooth administration of the anesthetics during EEG/LFP recording. Animals were cared with appropriate treatment after the surgery and given more than 5 days of recovery period before the experiment.

B. Experimental Setting & Recording

A treadmill (Panlab, LE8708) was used to force the animal to walk during the EEG/LFP recording and the motion of the animal was simultaneously recorded by three-axis accelerometer (Freescale semiconductor, MMA7260Q) attached to the EEG/LFP connector located on the animal's head. Video recording of the experiment was also prepared to backup the performance of the motion detector.

Before starting recording, the animal was given 15 minutes of adaptation period on the treadmill in stopped condition and 5 minutes in running. Speed of the lane was kept 5cm/s throughout the recording session. After the baseline recording of 10 minutes, anesthetic agent was administered through the previously secured IP injection route. Dose for Ketamine-Xylazine cocktail was 120 mg/kg and 6mg/kg, respectively. Recording went on about an hour until the animal regained the movement in response to the treadmill stimulus.

All EEGs and LFPs were acquired monopolarly by Grass electroencephalograph 8-16C model and digitized by Digidata 1400A (Axon instruments) at sampling frequency of 10000Hz. Motion signals were also acquired by Digidata 1400A at the same sampling frequency.

C. Spectrogram Analysis

To characterize the single channel EEG or LFP signal, we used spectrogram analysis. As preprocessing, the signal was resampled to 200Hz and normalized by the average power of 90-100Hz. The spectrogram spect(t,w) is given by the squared magnitude of short-time Fourier transform (STFT) of the signal:

$$\text{spect}(t, w) = |\text{STFT of signal}|^2.$$

Short time moving window of 30 seconds was taken to compute STFT along time.

III. RESULTS & DISCUSSION

Our method of detecting the animal's motion during the anesthetic experiment could distinctly identify behavioral changes due to administration of anesthetic agent. In Fig. 1 (a), upper four channels represent EEG and LFP signals and lower three channels show the animal's movement recorded by the motion detector. Dashed vertical lines indicate the injection of anesthetic agent (left) and quiescence of the animal (right). The moment of injection was identified by the video recorded simultaneously. 73 seconds after the injection, the animal stopped movement and the motion signals became flat. Gray boxes around the anesthetic injection point and the quiescence of movement are enlarged in Fig. 1 (b) and (c), respectively. The emergence from anesthetized state also could be discriminated with the motion detector. In Fig. 1 (d), the motion signals show that the mouse regains movement at 2314.5 s.

Since the motion signals have temporal resolution as high as EEGs and LFPs, we can identify the point of quiescence in hundreds of millisecond level. Time intervals between the injection and quiescence and quiescence and re-movement were determined for several mice and tabulated in Table 1. It took 74.9 s on average for C57BL/6 x 129 F1 hybrid mice to be quiescent after the administration of Ketamine-Xylazine cocktail (120 mg/kg and 6mg/kg, respectively) and 1916.7 s to regain the head movement. Such a quantitative description on the timing of anesthetic effects can be achieved only by the continuous monitoring of the animal behavior like the one proposed in our method.

TABLE 1
Time Intervals between the Transitions

Subjects C57BL/6 x 129 F1 hybrid	From Injection To Quiescence (unit: s)	From Quiescence To Re-movement (unit: s)
A	70	2127
B	85	930
C	54	1910
D	83	1589
E	73	1519
F	87	2400
G	72	2942
Average	74.9	1916.7
Standard deviation	11.5	654.2

In addition, as seen in Fig. 2, we could get clear signals during transition period between injection (leftmost vertical line) and quiescence (middle vertical line) points without particular noise reduction. We also found that behavioral changes identified by the motion detector (vertical lines) occurred in accordance with the change of EEG characteristics in frequency domain.

Since the IP injection route was prepared in advance, the anesthetic agent could be administered without disturbing

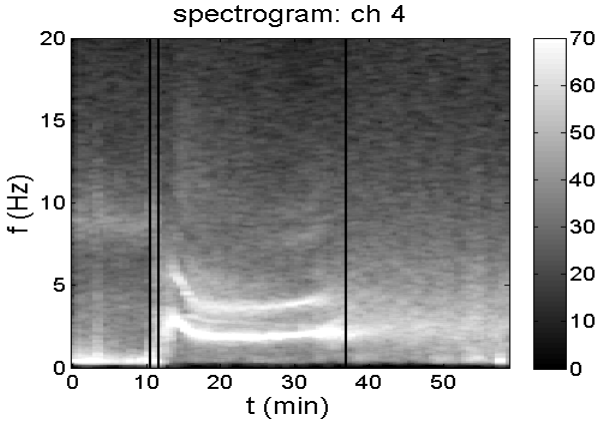

Fig. 2 Spectrogram of EEG signal recorded from S1bf. Three vertical black lines indicate injection of anesthetic agent, quiescence of movement and restoration of movement from left to right.

spontaneous movement of the animal, making less movement artifact in EEGs and LFPs. This method of estimating a transition point of anesthetic-induced loss of consciousness also can be applied with volatile anesthetics much easily.

IV. CONCLUSION

A new method for estimating transition points of anesthetic-induced loss of consciousness and regaining consciousness in animals during EEG/LFP recoding is proposed. The method identifies the transition points based on the animal's motion changes in response to forced movement. During the experiment, the animal is put on the treadmill which forces it to walk and the accelerometer records the animals head motion concurrently with EEGs and LFPs. Anesthetic agent is smoothly administered through the ready-secured IP injection route to prevent disturbing the animal's movement. Resulting signals from the motion detector pinpoint behavioral changes induced by the administration of anesthetic agent with temporal resolution corresponding to its sampling frequency while EEG and LFP signals are not much affected by the movement artifact, otherwise exists. This method makes it possible to track the anesthetic transition period continuously without contaminating EEGs and LFPs.

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