Effect of isoflurane on somatosensory evoked potentials in a rat model

Jukka Kortelainen, *Member, IEEE*, Ashwati Vipin, *Member, IEEE*, Thow Xin Yuan, Hasan Mir, Nitish Thakor, *Fellow, IEEE*, Hasan Al-Nashash, *Senior Member, IEEE*, and Angelo All

***Abstract*—Somatosensory evoked potentials (SEPs) are widely used in the clinic as well as research to study the functional integrity of the different parts of sensory pathways. However, most general anesthetics, such as isoflurane, are known to suppress SEPs, which might affect the interpretation of the signals. In animal studies, the usage of anesthetics during SEP measurements is inevitable due to which detailed effect of these drugs on the recordings should be known. In this paper, the effect of isoflurane on SEPs was studied in a rat model. Both time and frequency properties of the cortical recordings generated by stimulating the tibial nerve of rat's hindlimb were investigated at three different isoflurane levels. While the anesthetic agent is shown to generally suppress the amplitude of the SEP, the effect was found to be nonlinear influencing more substantially the latter part of waveform. This finding will potentially help us in future work aiming at separating the effects of anesthetics on SEP from those due to injury in the ascending neural pathways.**

I. Introduction

SOMATOSENSORY evoked potentials (SEPs) are widely used to study the functional integrity of different parts of sensory pathways. In clinical diagnostics, they are applied, for example, to detect hypoxic ischemic encephalopathy after cardiac arrest [1] as well as to estimate the severity and prognosis of spinal cord injury [2],[3]. Today, SEPs are also routinely used for intraoperative monitoring of the central nervous system during high-risk surgeries [4]. For animal studies, the method provides a convenient way to quantitatively assess the functionality of somatosensory pathways with minimal invasiveness [5],[6]. In a SCI rat model, SEPs have been successfully utilized, for instance, in the estimation of the severity of injury [7] as well as in monitoring of the impact of therapeutic interventions [8].

Anesthetics are known to influence SEPs. While the subcortical and peripheral responses are less affected, substantial changes in the cortical responses have been observed with halogenated inhalational agents like isoflurane [9] as well as the most commonly used intravenous anesthetics like propofol [10]. Due to this, the usage of anesthetics before and during SEP measurement should be minimized. However, in the intensive care and operating room, for example, the presence of anesthetics cannot be avoided and the effect of these drugs has to be taken into account when interpreting signals.

In animal models, the usage of anesthetics during SEP measurement is inevitable. For maintaining a steady-state anesthesia, inhalational agents are more suitable compared to the drugs administered by, for example, injection. However, as mentioned above, inhalational anesthetics are known to strongly affect SEP features such as the amplitude of the signal. Since these features are generally used in animal models for assessing the functionality of the somatosensory pathway, anesthetics potentially compromise the reliability of the measurements. Understanding in detail the effects of anesthetics on SEPs could help us in separating them from those due to other factors such as an injury in the ascending neural pathways.

In this paper, the effect of isoflurane on SEPs is studied in a rat model. Both time and frequency properties of the cortical recordings generated by stimulating the tibial nerve are explored at different isoflurane levels. Special attention is given to the effect of the anesthetic on the signal characteristics at different time intervals after the stimulation. The structure of the paper is as follows. Section II explains the details related to the electrode implantation, SEP recording, and signal analysis. The results of the analysis are presented in Section III. In Section IV, a short discussion about the findings as well as the conclusion of the paper is given.

II. Materials and Methods

A. Electrode Implantation

The experimental procedure was approved by the Institutional Animal Care and Use Committee (IACUC) at the National University of Singapore. The animals were anesthetized for the electrode implantation with ketamine

This work was supported by 2013-MSCRFII-0109-00 from the Maryland Stem Cell Research Fund as well as R-175-000-121-133, R-175-000-121-733, R-175-000-122-112, and R-711-201-026-133 from the National University of Singapore.

J. Kortelainen was with the Singapore Institute for Neurotechnology (SINAPSE), National University of Singapore, Singapore. He is now with the Department of Computer Science and Engineering, BOX 4500, FIN-90014 University of Oulu, Finland (e-mail: jukortel@ee.oulu.fi).

A. Vipin and X. Thow are with the Singapore Institute for Neurotechnology (SINAPSE), National University of Singapore, Singapore.

H. Mir and H. Al-Nashash are with the Department of Electrical Engineering, American University of Sharjah, United Arab Emirates.

N. Thakor is with the Singapore Institute for Neurotechnology (SINAPSE), National University of Singapore, Singapore and the Department of Biomedical Engineering, Johns Hopkins University, USA.

A. All is with the Department of Orthopedic Surgery and Biomedical Engineering, National University of Singapore, Singapore and the Department of Neurology and Biomedical Engineering, Johns Hopkins University, USA.

978-1-4244-7929-0/14/$26.00 ©2014 IEEE

(75mg/kg) and xylazine (10 mg/kg) cocktail administered through intraperitoneal injection (0.4 mL). Four epidural screw electrodes (Plastics One) were implanted to the cranium of five 200 g female Sprague-Dawley rats. The electrodes were positioned above the somatosensory cortex of different limbs the forelimb recording sites located 0.2 mm posterior and 3.8 mm lateral to the bregma and the hindlimb recording sites 2.5 mm posterior and 2.8 mm lateral to the bregma. A fifth electrode was positioned in the right parietal area 3 mm lateral to lambda to serve as a reference. Carboxylate dental cement (Lang Dental 3M ESPE) was applied to fix the electrodes to the cranium.

B. SEP Recording

The SEP recordings were carried out 7-10 days after the electrode implantation using a Tucker Davis Technologies (TDT) workstation. The measurement system was composed of an isolated stimulator (Digitimer DS3), a head-stage amplifier (RA4LI), a pre-amplifier (RA4PA), and a Bioamp processor (RZ5). The processor was controlled by a computer and designed to perform two functions simultaneously: the triggering of the stimulator and the acquisition of SEP data in real-time. The SEPs were generated by stimulating the tibial nerves of the hindlimbs and median nerves of the forelimbs, one limb at a time, with a pair of stainless steel subdermal needle electrodes (Safelead F-E3-48, Grass Technologies). The stimulation of each limb was carried out using 150 consecutive current pulses with 3.5 mA amplitude and 200 μsec duration delivered at a frequency of 0.5 Hz. During the stimulation, the SEPs were recorded from the epidural screw electrodes using a sampling frequency of 4882 Hz.

For the SEP recordings, the animals were anaesthetized with a mixture of isoflurane and 100% oxygen given at a flow rate of 1.3L/min. They were initially introduced to 4.0% isoflurane mixed with room air in a closed chamber, after which they were moved to a measurement table within a Faraday cage where the stimulation electrodes and measurement wire were connected. Drug administration was then continued through a rodent-size anesthesia mask connected to a diaphragm with a C-Pram circuit designed to deliver and evacuate the gas. The effect of isoflurane on the SEPs was investigated by increasing the anesthetic dosage in a step-like manner from 1.5% to 2.5% using 0.5% increments. The evoked potentials were recorded at each step after keeping the dosage fixed for 5 min to guarantee the equilibrium.

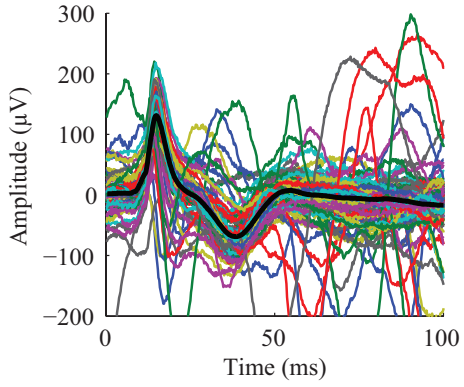

Fig. 1. Determination of the average somatosensory evoked potential (SEP) waveform. The average SEP waveform (bold black curve) is determined by calculating the average over 150 single sweeps (colored curves). The signals represent the recordings of one rat at 1.5% isoflurane anesthesia during left hindlimb stimulation. The stimulation happens at time 0.

C. SEP Analysis

SEP analysis was carried out using the Matlab technical computing language (The MathWorks Inc., Natick, MA). Only the signals related to the stimulation of hindlimbs were used in the analysis. Firstly, the part of the signals corresponding to the time window of 0 to 100 ms after the stimulation was extracted. An average SEP waveform was then determined by calculating the ensemble mean over the 150 single sweeps (see Fig. 1). To explore the time and frequency characteristics of the signals, spectrograms of the average SEP waveforms were calculated. The spectrograms, providing the power spectral density of the signal as a function of time, were calculated using the Short-Time Fourier Transform with a 10-ms Hamming window and 9.8-ms overlap.

From the spectrograms, two power parameters were defined: $P_{5–25\ \mathrm{ms}}$ and $P_{25–100\ \mathrm{ms}}$. The first one was calculated by summing the total power in the spectrum within the time window of 5–25 ms and the second one similarly using a window of 25–100 ms. These time windows were chosen based on the preliminary visual inspection of the data according to which the first wave of the SEP generally occurred before 25 ms from the stimulation. The preliminary analysis also suggested isoflurane as a having different effect on the two parts of the signal which we wanted to capture. The determination of the two power parameters is illustrated in Fig. 2.

III. Results

The average SEP waveforms resulting from the left hindlimb stimulation are given in Fig. 3 for all five rats at the three different isoflurane levels. As mentioned above, the

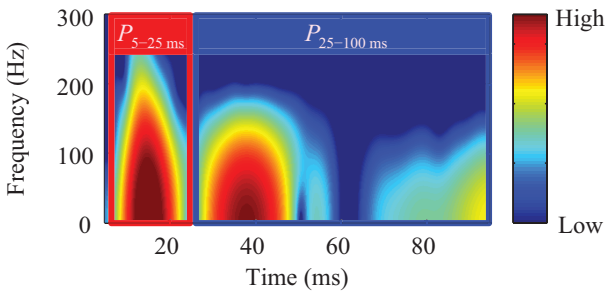

Fig. 2. Determination of the two power parameters from the spectrogram of the average somatosensory evoked potential (SEP) waveform. $P_{5–25\ \mathrm{ms}}$ and $P_{25–100\ \mathrm{ms}}$ are calculated by summing the total power in the spectrum within the time window of 5–25 ms and 25–100 ms, respectively, after the stimulation. The spectrogram given here corresponds to the average SEP waveform presented in Fig. 1 and the values are given in logarithmic scale.

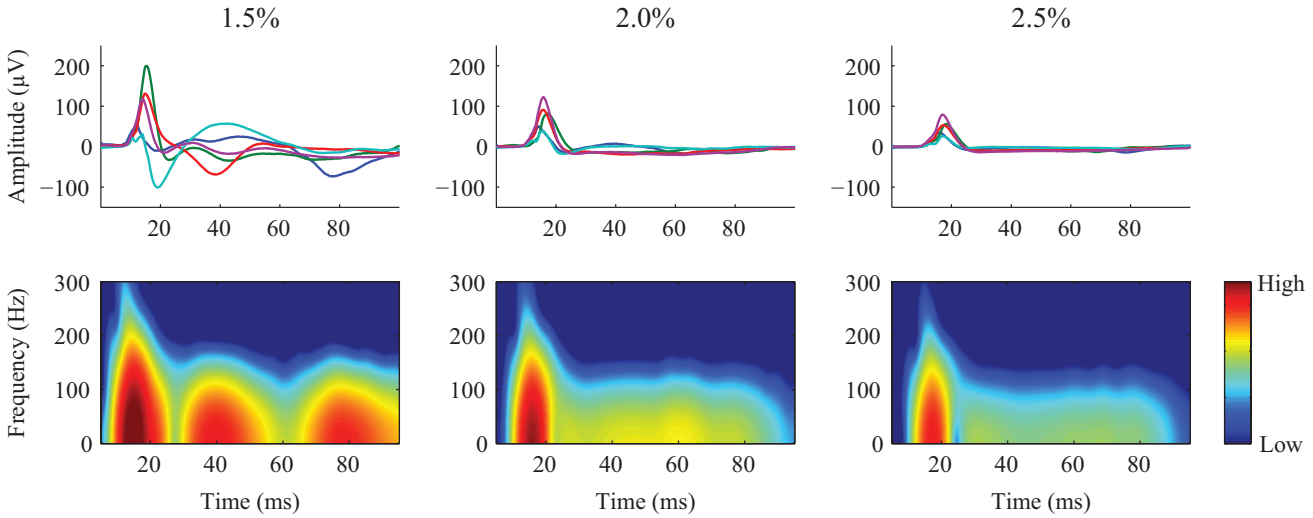

Fig. 3. The effect of isoflurane on time and frequency characteristics of the somatosensory evoked potentials (SEPs). In the upper row, the average SEP waveforms are given for the five rats at 1.5%, 2.0%, and 2.5% isoflurane anesthesia. The signals represent the recordings related to left hindlimb stimulation happening at time 0. In the lower row, mean spectrograms calculated from the average SEP waveforms are given. The spectrogram values, representing the power spectral densities as a function of time, are given in logarithmic scale.

signals can be roughly divided into two parts: the first sharp wave occurring approximately between 10 and 25 ms after the stimulation, and the rest of the SEP comprised of one or more smoother waves with lower amplitude. The SEPs related to the right hindlimb stimulation were comparable to the left side recordings. Fig. 3 also shows the mean spectrograms calculated by averaging the five individual spectrograms derived from the SEP waveforms. Due to the spike-like morphology of the first wave, its power is spread more to the higher frequencies compared to the waves following it. The spectrograms also clearly show the power difference between the first wave and the rest of the SEP.

The effect of isoflurane on the SEPs is illustrated in Fig. 3, Fig 4, and Fig. 5. Fig. 3 shows that the amplitude of the average SEPs is suppressed by higher concentration of anesthetic. While the phenomenon is seen in the entire signal, the effect also seems to be nonlinear influencing more substantially the latter part of the waveform. In Fig. 4, an illustrative example of this nonlinear effect is given. Even though the amplitude of the first spike-like wave is decreased, its morphology remains unchanged at all levels of anesthesia. However, this is not the case with the latter part of signal, where the waves occurring in lighter anesthesia are entirely erased as the effect of isoflurane gets stronger. The phenomenon is expressed quantitatively in Fig. 5, in which the effect of isoflurane on $P_{5\text{–}25\ \mathrm{ms}}$ and $P_{25\text{–}100\ \mathrm{ms}}$ is given. Again, the higher concentration of anesthetic is shown to more substantially and consistently suppress the latter part of the signal.

IV. Discussion and Conclusions

The suppressive effect of isoflurane on SEPs found in this study is in line with the literature. The decrease of signal amplitude due to this anesthetic agent has been reported earlier in both humans [11] as well as rats [12]. In humans, there is also some evidence that the late cortical responses are more sensitive to the anesthetics [13]. However, according to our knowledge, the difference between the effects of anesthetics on the early and late cortical SEPs has not been reported in a rat model until now.

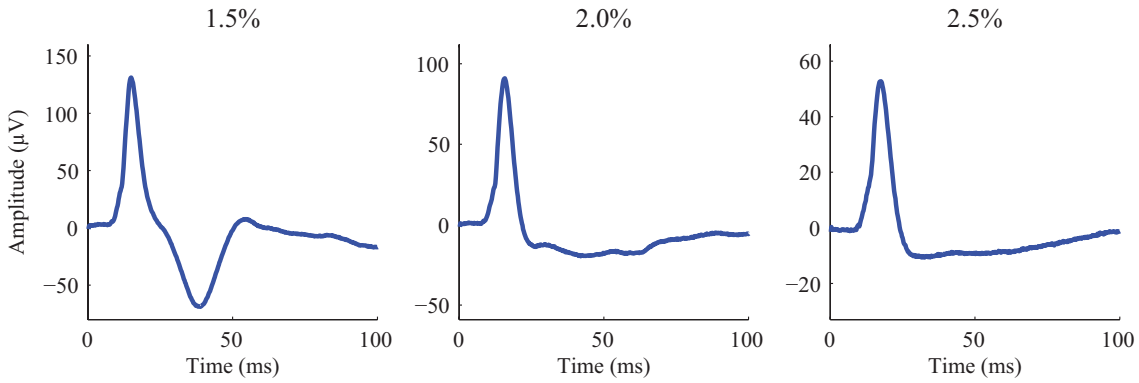

Fig. 4. The nonlinear effect of isoflurane on the somatosensory evoked potential (SEP) waveform. The average SEP waveform is given for one rat at 1.5%, 2.0%, and 2.5% isoflurane anesthesia. The amplitude of the SEP is scaled to illustrate the nonlinear effects of the anesthetic on the signal morphology. The signals represent the recordings related to left hindlimb stimulation happening at time 0.

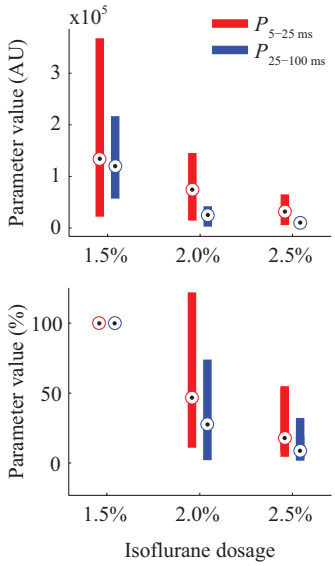

Fig. 5. The effect of isoflurane on the two power parameters derived from the spectrograms of the average somatosensory evoked potential (SEP) waveform. Each bar represents the median and range of the values of five rats. The parameters are derived from the recordings corresponding to left hindlimb stimulation at 1.5%, 2.0%, and 2.5% isoflurane anesthesia. The upper plot shows the absolute values of the parameters given in arbitrary units (AU). In the lower plot, the parameters are given relative to values at 1.5% isoflurane anesthesia.

Even though our findings about the more substantial effect of isoflurane on the latter part of SEP seem to be novel, they are indirectly supported by previous studies. Isoflurane is known to cause gradual changes in the spontaneous EEG activity leading to burst suppression pattern and eventually to total suppression of the signal [14]-[17]. The anesthetic effect on cortical electrophysiology is assumed to reflect mainly the loss of cortical responsiveness while the input from subcortical structures is less affected [18]. It can therefore be expected that the anesthetics influence more substantially the latter part of the SEP waveform representing the late cortical responses to the stimulation.

In future research, the results of this study will be utilized for improved estimation of the severity of injury in the ascending neural pathways. At the moment, significant scientific effort has been directed at developing new therapeutic approaches for the treatment of such injuries [19],[20]. Refined measures are needed for reliable assessment of these therapies. As the treatment most likely will take place in the early phase of recovery, trauma patients, for example, might be sedated during the measurements. Separation of the effect of anesthetics on SEP from those due to the neural injury would thus be needed.

As a conclusion, the effect of isoflurane on SEPs was studied in a rat model. Both time and frequency properties of the cortical recordings generated by stimulating the tibial nerve of rat's hindlimb were explored at three different isoflurane levels in five healthy rats. While the anesthetic was shown to generally suppress the amplitude of the SEP, this effect was found to be nonlinear influencing more substantially the latter part of the waveform. In future, the finding potentially provides us the possibility to separate the effects of anesthetics on SEP from those due to an injury in the ascending neural pathways.

REFERENCES

[1] G. Young, "Clinical practice. Neurologic prognosis after cardiac arrest," *N. Engl. J. Med.*, vol. 361, pp. 605–611, 2009.

[2] S. Kirshblum, K. O'Connor, "Predicting neurologic recovery in traumatic cervical spinal cord injury," *Arch. Phys. Med. Rehabil.*, vol. 79, pp. 1456–1466, 1998.

[3] A. Curt, P. Ellaway, "Clinical neurophysiology in the prognosis and monitoring of traumatic spinal cord injury," *Handb. Clin. Neurol.*, vol. 109, pp. 63–75, 2012.

[4] N. Malhotra, C. Shaffrey, "Intraoperative electrophysiological monitoring in spine surgery," *Spine*, vol. 35, pp. 2167–2179, 2010.

[5] R. Vialle, M. Loureiro, B. Ilharreborde, S. Liu, P. Lozeron, M Tadié, "The feasibility of detecting motor and sensory potentials in a sheep model," *Lab. Anim.*, vol. 40, pp. 469–473, 2006.

[6] J. Kuluz et al., "Pediatric spinal cord injury in infant piglets: description of a new large animal model and review of the literature," *J. Spinal Cord Med.*, vol. 33, pp. 43–57, 2010.

[7] G Agrawal, N. Thakor, A. All, "Evoked potential versus behavior to detect minor insult to spinal cord in rat model," *Journal of Clinical Neuroscience*, vol. 16, pp. 1052–1055, 2009.

[8] A. Maybhate, C. Hu, F. Bazley, Q. Yu, N. Thakor, C. Kerr, A. All, "Potential long-term benefits of acute hypothermia after spinal cord injury: Assessments with somatosensory-evoked potentials," *Crit. Care Med.*, vol. 40, pp. 573–579, 2012.

[9] T. Sloan, "Evoked potentials," in *Textbook of neuroanesthesia with neurosurgical and neuroscience perspectives*, M. Albin, Ed. New York: McGraw-Hill, 1997, pp. 221–276.

[10] A. Angel, F. LeBeau, "A comparison of the effects of propofol with other anaesthetic agents on the centripetal transmission of sensory information," *Gen. Pharmacol.*, vol. 23, pp. 945–963, 1992.

[11] T. Sloan, "Anesthetic effects on electrophysiologic recordings," *J. Clin. Neurophysiol.*, vol. 15, pp. 217–226, 1998.

[12] A. Angel, D. Gratton, "The effect of anaesthetic agents on cerebral cortical responses in the rat," *Br. J. Pharmacol.*, vol. 76, pp. 541–549, 1982.

[13] E. Freye, H. Dehnen-Seipel, D. Rohner, "Somatosensory evoked potentials (SEP) during isoflurane and enflurane anesthesia in heart surgery," *Anaesthesist*, vol. 34, pp. 670–674, 1985.

[14] J. Kortelainen, X. Jia, T. Seppänen, N. Thakor, "Increased electroencephalographic gamma activity reveals awakening from isoflurane anaesthesia in rats," *Br. J. Anaesth.*, vol. 109, pp. 782–789, 2012.

[15] J. Kortelainen, E. Väyrynen, X. Jia, T. Seppänen, N. Thakor, "EEG-based detection of awakening from isoflurane anesthesia in rats," in *Proc. 34th Annu. Int. Conf. IEEE EMBS*, San Diego, USA, 2012, pp. 4279–4282.

[16] J. Kortelainen, M. Koskinen, S. Mustola, T. Seppänen, "Effects of remifentanil on the spectrum and quantitative parameters of electroencephalogram in propofol anesthesia," *Anesthesiology*, vol. 111, pp. 574–583, 2009.

[17] J. Kortelainen, M. Koskinen, S. Mustola, T. Seppänen, "EEG frequency progression during induction of anesthesia: from start of infusion to onset of burst suppression pattern," in *Proc. 29th Annu. Int. Conf. IEEE EMBS*, Lyon, France, 2007, pp. 1570–1573.

[18] D. Liley, N. Sinclair, T. Lipping, B. Heyse, H. Vereecke, M. Struys, "Propofol and remifentanil differentially modulate frontal electroencephalographic activity," *Anesthesiology*, vol. 113, pp. 292–304, 2010.

[19] A. Curt, M. Schwab, V. Dietz, "Providing the clinical basis for new interventional therapies: refined diagnosis and assessment of recovery after spinal cord injury," *Spinal Cord*, vol. 42, pp. 1–6, 2004.

[20] A. All, F. Bazley, S. Gupta, N. Pashai, C. Hu, A. Pourmorteza, C. Kerr, "Human embryonic stem cell-derived oligodendrocyte progenitors aid in functional recovery of sensory pathways following contusive spinal cord injury," *PLoS One*, vol. 7, e47645, 2012.