Laser Doppler flowmetry signals to quantify effects of isoflurane on the peripheral cardiovascular system of healthy rats

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The optical Doppler effect resulting from interactions between laser light photons and red blood cells of the microcirculation is used to characterize the influence of isoflurane, an halogenated volatile anesthetic, on the peripheral cardiovascular system. After having recorded laser Doppler flowmetry blood perfusion signals on isoflurane-induced anesthetized healthy rats, wavelet analyses show a significant decrease of the myogenic and neurogenic activities when isoflurane dose increases from 1.5% to 3%. Moreover, the approximate entropy shows a weak decrease of signal irregularity when dose of isoflurane increases. These findings demonstrate the usefulness of the optical Doppler effect in physiological and pharmacological applications. © *2007 American Institute of Physics*. [DOI: 10.1063/1.2825585]

Knowledge of the modifications brought by pharmacological agents on the cardiovascular system has become an active area of research. The applications include monitoring the depth of anesthesia, an ongoing problem in clinical routines. The recent notable approaches for monitoring the depth of anesthesia rely on the analysis of auditory evoked potential, electroencephalogram (EEG), facial electromyogram, heart rate variability (HRV), or HRV respiratory sinus arrhythmia.[1] However, although these methods allow the evaluation of anesthetic drug effects, they do not provide information on the influence of the pharmacological agents over the *peripheral* cardiovascular system. Our work, therefore, aims at bringing information on this area.

The peripheral cardiovascular system can be analyzed through the microvascular blood perfusion. The latter can be monitored with a laser Doppler flowmeter. In the latter case, the laser Doppler effect comes from the interactions between photons from a laser light and red blood cells of the microcirculation.[2] It has been shown that blood perfusion signals recorded in rats contain five oscillatory processes: the cardiac activity (near to 3.3 Hz), the respiration (near to 1.3 Hz), and the myogenic, neurogenic and endothelial-related metabolic activities.

In this letter, we demonstrate that the optical Doppler effect can characterize the influence of isoflurane on the peripheral cardiovascular system. Isoflurane is an halogenated volatile anesthetic commonly used in clinical practice and for which pharmacology[5] and mode of action have been reviewed.[6] To characterize isoflurane effects, we test two methods applied on laser Doppler flowmetry (LDF) blood perfusion signals recorded on isoflurane-induced anesthetized rats.

Thirty two Sprague Dawley rats were analyzed. Procedures for the maintenance and use of the experimental animals were carried out in accordance with Ref. 26. Isoflurane was administered to split the group of rats into two. The first group (population $n=16$) corresponds to rats with a light anesthesia: dose of isoflurane between 1.5% and 1.9%. The second group (population $n=16$) corresponds to rats with a dose of isoflurane between 2.4% and 3% (deep anesthesia).[7] For the blood perfusion signal acquisition, the LDF probe was connected to a laser Doppler flowmeter (PF5000 Master,

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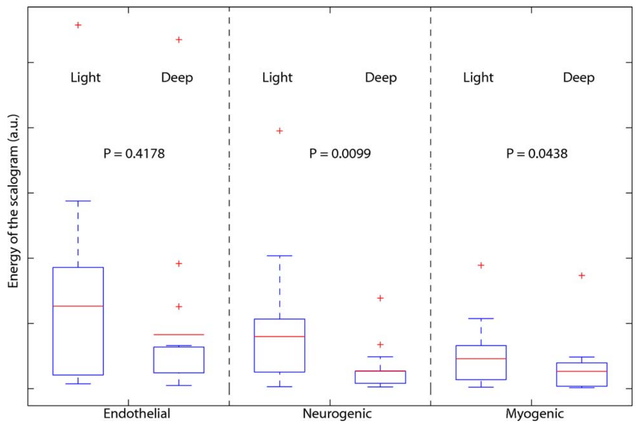

FIG. 1. (Color online) Energy of the scalogram computed in each frequency band for deep and light isoflurane-induced anesthesia.

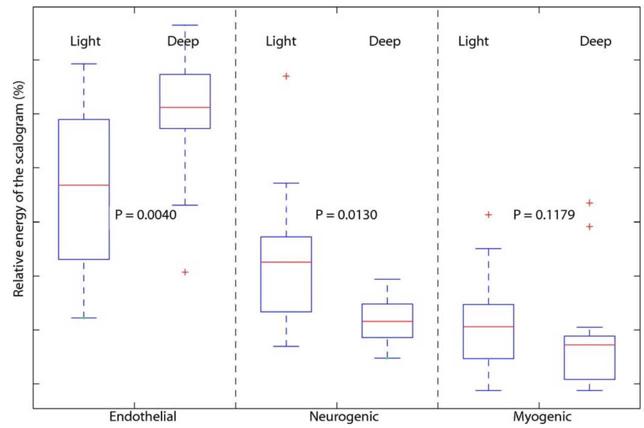

FIG. 2. (Color online) Relative energy of the scalogram computed in each frequency band for deep and light isoflurane-induced anesthesia.

Periflux, Perimed, Sweden) and positioned on the thigh of the rat placed in the prone position. The wavelength of the laser Doppler flowmeter was 780 nm and the signals were recorded for 16 min and 40 s with a frequency sampling of 32 Hz.

The two methods chosen to evaluate the effects of isoflurane on the peripheral cardiovascular system of rats have already been applied on EEG signals recorded during anesthesia (see below). The first method is a wavelet-based analysis,[3,8–10] which has already been applied on EEG signals with the Morlet wavelet.[11] Therefore, wavelets are used herein to quantify the influence of isoflurane on the myogenic, neurogenic, and endothelial-related metabolic activities. For that purpose, the scalograms of the LDF blood perfusion signals (resampled by taking one point over three to decrease the scalogram computation time) are studied between 0.01 and 0.74 Hz, in order to analyze three characteristic frequencies: intervals of 0.2–0.74, 0.076–0.2, and 0.01–0.076 Hz for the myogenic, neurogenic, and endothelial-related metabolic activities, respectively.[3] In order to compare the behaviors of the myogenic, neurogenic, and endothelial activities for light and deep isoflurane-induced anesthesia, quantitative measures are calculated:[3,9,10] energy and relative energy of the scalogram on a given frequency band.

The second method used herein relies on the approximate entropy. Approximate entropy has already been used on EEG signals during anesthesia[14–18] and is, therefore, tested herein on peripheral cardiovascular signals (LDF blood perfusion signals).[19] Approximate entropy [ApEn(m,r,N)], which analyzes the regularity of time series, was introduced by Pincus.[12,13] It can be thought of as the negative natural logarithm of the probability that sequences that are close for m points remain close for an additional point. The choice of the values for m and r for ApEn is critical in determining the outcome of ApEn. On the basis of several works,[13,20–22] we choose $m=2$, a LDF sequence of $N=1000$ samples (running windows of 1000 points all along each recording), and a tolerance $r=0.2^{*}$SD (where SD is the standard deviation of the LDF sequence) since it is convenient to set the tolerance r proportional to SD, so as to allow measurements on data sets with different amplitudes to be compared.

For the two abovementioned processes, a Mann-Whitney test is used to evaluate the differences between the two groups of rats. Statistical significant differences are defined as $P < 0.05$. The results of the two signal processing methods are the following: the scalogram energy in each frequency band corresponding to a given process is lower for deep than for light isoflurane-induced anesthesia (see Fig. 1). The differences between the two groups are statistically significant for the myogenic and neurogenic activities. Moreover, the relative energies for the myogenic and neurogenic activities are lower for deep than for light anesthesia, whereas the relative energy for the endothelial-related metabolic activity is higher for deep than for light isoflurane-induced anesthesia (see Fig. 2). The differences between the two groups of rats are statistically significant for the relative energies of the neurogenic and endothelial-related metabolic activities. Furthermore, the values of the approximate entropy are presented in Table I for the two anesthetic states. The results show that the mean value of the approximate entropy decreases when the dose of isoflurane increases. However, the Mann-Whitney test shows that the differences are not significant.

A spectral analysis conducted on signals related to the *central* cardiovascular system (heart rate variability signals)[23] has shown that powers in frequency bands of the respiratory and myogenic activities are lower during isoflurane anesthesia on humans, with a concentration-dependent manner. Our work shows that isoflurane also affects the *peripheral* cardiovascular system. Moreover, two recent studies have shown, using wavelet analyses, that both local and general anesthesia affect laser Doppler flowmetry signals in humans.[24,25] However, these studies used a combination of several anesthetic drugs. Therefore, their results relate the total effect of the drugs and cannot predict the behavior for each one of them. Our work provides information on the effect of a single anesthetic, isoflurane, which is moreover commonly used in clinical practice.

In conclusion, the effects of isoflurane on the peripheral cardiovascular system of healthy rats are herein quantified:

TABLE I. Average values for the minimum, maximum, mean, and standard deviation of the approximate entropy (average values computed over 16 signals for each category).

Signal	Minimum value	Maximum value	Mean value	Standard deviation
Light anesthesia	1.43	1.53	1.50	0.02
Deep anesthesia	1.30	1.53	1.48	0.06

isoflurane leads to a weak loss of irregularity on the peripheral cardiovascular system signals and to modifications of the myogenic, neurogenic, and endothelial-related metabolic activities. After validations, this study could be applied to quantify other pharmacological agents' effects.

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