Ischemic Stroke Detection by Analyzing Heart Rate Variability in Rat Middle Cerebral Artery Occlusion Model

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***Abstract*— Although early reperfusion therapy is effective for acute ischemic stroke, limited therapeutic time-window resulted in only 10% of patients receiving reperfusion therapy. A fast and reliable stroke detection method is desired so that patients can receive early reperfusion therapy. It has been reported that ischemic stroke affects heart rate variability (HRV), which reflects activities of the autonomic nervous function. Thus, ischemic stroke may be detected at an acute stage through monitoring HRV. This paper proposes an HRV-based ischemic stroke detection algorithm by using multivariate statistical process control (MSPC), which is a well-known anomaly detection algorithm. As a feasibility study before collecting a large amount of clinical data from human patients, this paper used the middle cerebral artery occlusion (MCAO) model in rats for collecting HRV data shortly after ischemic stroke onsets. The 11 MCAO-operated rats and 11 sham-operated rats were prepared, and HRV data of three sham-operated rats were used for model construction. The data on the other 19 rats were used for its validation. The experimental result showed that sensitivity and specificity of the proposed algorithm were 82% and 75%, respectively. Thus, the present work shows the possibility of realizing an HRV-based ischemic stroke detection system for human patients.**

***Index Terms*— Stroke detection, middle cerebral artery occlusion model, heart rate variability, multivariate statistical process control, anomaly detection.**

I. Introduction

CEREBROVASCULAR disease (CVD) is a group of brain dysfunctions related to disease occurring in brain blood vessels, including cerebral ischemic stroke, cerebral hemorrhage, and subarachnoid hemorrhage [1]. They are better known as stroke [2]. CVD ranks fourth among all causes of death in the United States and Japan, and is recognized as a leading cause of serious long-term physical and cognitive disabilities [3]. In particular, ischemic stroke accounts for about 80% of CVD [4].

It is important for patients with acute ischemic stroke to receive treatment as early as possible in order to prevent permanent disability or death. Thrombolysis with a tissue plasminogen activator (tPA) that dissolves blood clots can be applied to acute ischemic stroke. However, it is only effective within 4.5 hours from a symptom onset [5]. The main symptoms usually depend on the blood vessel affected, which may lead to consciousness disorder, painless numbness or paralysis on one side of the body, and sudden problems with communication. It is difficult for the patients to be transferred to a hospital in a timely fashion. Delay of diagnosis is the most serious problem, and tPA has been administered to less than 10% of patients [6]. Thus, a fast and reliable stroke detection system is desired so that patients have the opportunity for early stroke treatment. Ideally, such diagnoses should be made in the ambulance system. Thus, development of an ischemic stroke detection system would enable patients to have the opportunity for early treatment.

The present study investigates the possibility of realizing an ischemic stroke detection system using a wearable RR interval (RRI) measurement device. RRI fluctuation in an electrocardiogram (ECG), called heart rate variability (HRV), is a well-known phenomenon which reflects the autonomic nervous function [7]. Ischemic stroke frequently entails cardiovascular complications such as cardiac arrhythmias, ECG changes, ischemic heart damages, and disturbances of blood pressure regulation [8], [9]. It has been reported that ischemic stroke affects HRV [10]–[15], and prognosis of ischemic stroke patients may be predicted through analyzing HRV [16], [17]. Thus, ischemic stroke onsets may be detected through monitoring HRV.

This study proposes an ischemic stroke detection algorithm based on HRV analysis. In order to develop the ischemic stroke detection algorithm, a large amount of HRV data before and after ischemic stroke onsets is needed, although collecting sufficient amount of clinical HRV data from patients requires much time. As a feasibility study of HRV-based ischemic stroke detection for humans, this study collected HRV data through an animal experiment using a middle cerebral artery

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occlusion (MCAO) model in rats [18] and evaluated the stroke detection performance of the proposed algorithm through its application to the experimental data.

This paper is organized as follows. Related works are introduced in Sec. II. Sections III and IV explain the MCAO model and HRV analysis in rats. An HRV-based stroke detection method using MSPC is proposed in Sec. V, and Sec. VI reports application results of the proposed method to the experimental data collected through animal experiments using rats. Section VII discusses the results. The conclusion and future work are described in Sec. VIII. Although a preliminary version of this work has been reported in [19], this paper also discusses the relationship between changes in HRV and ischemic stroke sizes and areas in the brain based on brain staining results.

II. Related Works

Very few researchers have investigated the possibility of wearable stroke detection systems. Mobashsher *et al.* [20] and Mohammed *et al.* [21] developed microwave-based stroke detection systems; however the use of microwaves for a wearable device has problems with safety and portability [20]. On the other hand, the possibility of stroke detection in the acute stage from blood samples has been shown [22], [23]; however, such methods need blood sampling.

HRV analysis is a safe and non-invasive diagnostic tool used in various medical fields [7]. For example, the relationship between changes in HRV and cardiovascular events has been reported [24]–[26]. In addition, psychiatric disorders greatly affect HRV [27], [28].

Recently, an application area of HRV-based analysis has been expanded due to advances of a wearable sensing technology and machine learning algorithms. Fujiwara *et al.* [29] proposed an HRV-based epileptic seizure prediction algorithm based on the fact that excessive neuronal activities in the preictal period of epilepsy affect the autonomic nervous system. Multivariate statistical process control (MSPC) was used for monitoring HRV in Fujiwara et al.'s algorithm [29], which is a well-known anomaly detection method [30]–[32]. Instead of MSPC, Pavei *et al.* [33] used support vector machine (SVM) for predicting epileptic seizures from HRV. In addition, various HRV-based drowsy driving detection algorithms have been proposed [34], [35]. In other words, health monitoring systems based on HRV have attracted much attention.

The ischemic stroke detection algorithm proposed in the work utilized HRV analysis and MSPC, which is the same framework as the epileptic seizure prediction algorithm [29]. Since HRV can be easily monitored by using a wearable device [36], a wearable stroke detection system becomes available if the detection algorithm can be implemented in such a device.

III. Rat Middle Cerebral Artery Occlusion (MCAO) Model

Among experimental ischemic stroke models, an MCAO model is the most frequently used because of its minimal invasiveness [18]. In the MCAO model, a silicon filament is inserted, under anesthesia, into the internal carotid artery (ICA) from the external carotid artery (ECA), and advanced until it blocks the blood flow to the MCA. Since the insular cortex, which is an important cortical area for cardiovascular regulation, lies within the MCA territory [8], it is assumed that an MCAO operation affects HRV.

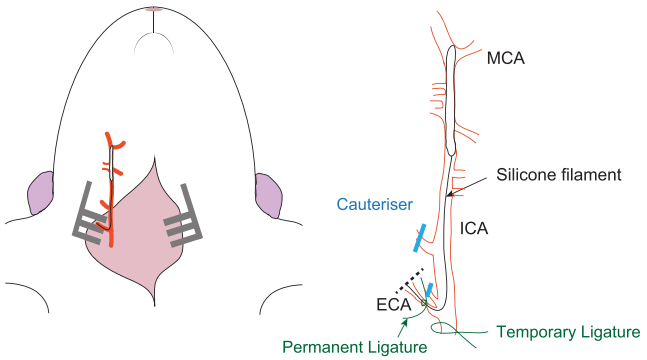


Fig. 1. MCAO model.

Among a variety of animals used in stroke studies, rats are the most commonly used animals because of their resemblance to humans in cerebrovascular anatomy and the ease of conducting reproducible studies therewith. A schematic illustration of a rat MCAO model is shown in Fig. 1. A midline skin incision is made in the anterior neck. The superficial fascia and muscle layers are separated with blunt dissection until the right carotid artery can be observed. Temporary ligature is applied to the common carotid artery (CCA) and a nylon monofilament coated with silicon resin (5-0 large MCAO suture B3 L23 PK10, Doccol Corporation) is inserted through ECA to occlude the right MCA. It is important to investigate the area and volume of ischemic stroke in the brain because they affect the severity of stroke. In experiments using the MCAO model, the area and volume of ischemic stroke are measured by staining of the brain.

In order to develop an ischemic stroke detection algorithm that does not generate an alert during normal conditions, we prepared MCAO-operated rats (MCAO rats) and sham-operated rats (sham rats) in which the neck is incised and only the ECA is tied up.

IV. Heart Rate Variability Analysis for Rat

The present work analyzes HRV data recorded from rats instead of human patients. Since collecting RRI data of rats is much easier than RRI data collection from patients, and rats' mechanism of HRV resembles that of humans [37], a feasibility study of the proposed algorithm through animal experiments with rats is reasonable.

A schematic ECG trace (standard lead II) obtained from a rat is shown in Fig. 2 (a). The ECG signal consists of several types of waves, in which the highest peak is called an R wave. The RR interval (RRI) is defined as an interval between the peaks of an R wave and the next R wave. Raw RRI data collected from a rat for one minute are shown in Fig. 2 (b). The HRV analysis method must be modified for rats since their heart rate (about 200-300 bpm) is much faster than that of a human (about 60-80 bpm).

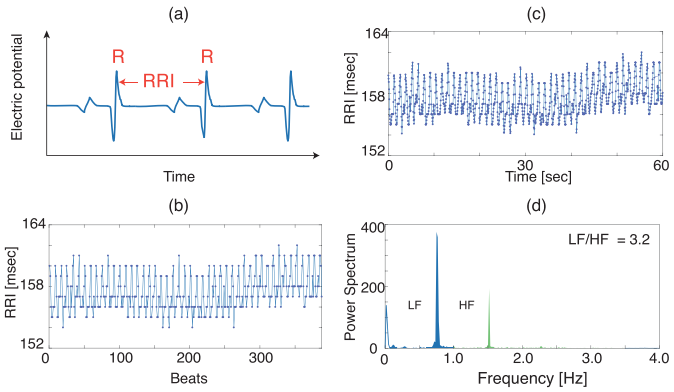


Fig. 2. Example of HRV analysis for rats: (a) rat's ECG trace, (b) the raw RRI data, (c) the resampled RRI data, and (d) the PSD and its LF/HF.

The HRV features used in this work are classified into time domain features and frequency domain features.

A. Time Domain Features

The following time domain features are calculated from the raw RRI data directly.

- **meanNN**: Mean of RRI.
- **SDNN**: Standard deviation of RRI.
- **RMSSD**: Root mean square of difference of adjacent RRI.

B. Frequency Domain Features

The following frequency domain features are defined as powers of a specific frequency range in a power spectrum density (PSD) of the RRI data. Although the powers in 0.04 Hz - 0.15 Hz and 0.15 Hz - 0.4 Hz are usually used as LF and HF for humans [7], these frequency range definitions are modified for rats. The following four frequency domain features [37] are used in this work.

- **Total Power (TP)**: The power in frequency range (0.04Hz -) in the PSD.
- **LF**: The power in low frequency range (0.04Hz - 1.00Hz) in the PSD. LF reflects sympathetic nervous activity and parasympathetic nervous activity.
- **HF**: The power in high frequency range (1.00Hz - 3.00Hz) in the PSD. HF reflects parasympathetic nervous activity.
- **LF/HF**: Ratio of LF to HF. LF/HF expresses the balance between the sympathetic and parasympathetic nervous activities.

In order to calculate the PSD, the raw RRI data recorded from rats need to be interpolated because they are not sampled at equal intervals. Although a sampling interval of HRV analysis for humans is usually about 200 ms, 50 ms is adopted for rats [37] in which a linear interpolation method is used. Figure 2 (c) and (d) show the resampled RRI data, the PSD, and its LF/HF.

V. HRV-Based Ischemic Stroke Detection Algorithm

Ischemic stroke detection can be formulated as an anomaly detection problem because stroke is a rare event in one's lifetime. This motivates us to adopt multivariate statistical process control (MSPC) for a stroke detection algorithm, which is a useful statistical technique for detecting abnormalities in manufacturing processes [30]–[32]. In general, anomaly detection methods construct models from only data representing normal conditions, which is suitable for an HRV-based ischemic stroke detection problem because it is difficult to collect large amounts of HRV data shortly after ischemic stroke onsets. In this section, the proposed ischemic stroke detection algorithm utilizing MSPC is explained.

A. Data Preprocessing

The proposed ischemic stroke detection algorithm uses seven HRV features described in Sec. IV as input variables. For HRV feature calculation, a rectangular moving window is used and the window size is one minute. Before extracting HRV features, an outlier in RRI data is replaced by the Hampel identifier [38] whose window size is 251 samples. In the following sections, the nth sample of HRV features is denoted by $\boldsymbol{x}(n) = [x_1(n), x_2(n), \cdots, x_M(n)]^T$ where $x_m(n)$ is the nth sample of the mth HRV feature, where $M = 7$ in this study. In addition, $\boldsymbol{X} \in \Re^{N\times M}$ is a matrix whose nth row is $\boldsymbol{x}(n)^T$.

B. Multivariate Statistical Process Control (MSPC)

MSPC is an anomaly detection method based on principal component analysis (PCA) which is used for finding major trends in the data and reducing dimensionality of the data. In MSPC, two fault detection indices, i.e., the T^2 and Q statistics, are monitored [39].

It is assumed that a normal data matrix $\boldsymbol{X} \in \Re^{N\times M}$ is given. All variables in $\boldsymbol{X}$ are mean-centered and appropriately scaled. The singular value decomposition of $\boldsymbol{X}$ is described as

$$\begin{aligned}\boldsymbol{X} &= \boldsymbol{U}\boldsymbol{\Sigma}\boldsymbol{V}^T \\ &= \begin{bmatrix}\boldsymbol{U}_R & \boldsymbol{U}_0\end{bmatrix}\begin{bmatrix}\boldsymbol{\Sigma}_R & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{\Sigma}_0\end{bmatrix}\begin{bmatrix}\boldsymbol{V}_R & \boldsymbol{V}_0\end{bmatrix}\end{aligned} \tag{1}$$

where $\boldsymbol{U}$ is the left singular matrix, Σ is the matrix whose diagonal elements are singular values, and $\boldsymbol{V}$ is the right singular matrix. The loading matrix $\boldsymbol{V}_M \in \Re^{M\times R}$ is derived from the right singular matrix $\boldsymbol{V}$ and the column space of $\boldsymbol{V}_R$ is the subspace spanned by principal components. Here, $\boldsymbol{R}(\geq M)$ denotes the number of principal components retained in the PCA model. $\boldsymbol{X}$ can be reconstructed from T_R with linear transportation V_R in the PCA model.

$$\hat{\boldsymbol{X}} = \boldsymbol{T}_R\boldsymbol{V}_R^{\mathrm{T}} = \boldsymbol{X}\boldsymbol{V}_R\boldsymbol{V}_R^{\mathrm{T}}. \tag{2}$$

Using this reconstruction, the Q statistic is defined as

$$Q = \sum_{m=1}^{M}(x_m - \hat{x}_m)^2 = \boldsymbol{x}^T(\boldsymbol{I} - \boldsymbol{V}_R\boldsymbol{V}_R^T)\boldsymbol{x} \tag{3}$$

where $\boldsymbol{x} \in \Re^M$ is a newly measured sample. The Q statistic is a measure of dissimilarity between the sample and the modeling data from the viewpoint of the correlation among variables. Hotelling's T^2 statistic is used to monitor anomaly on the subspace spanned by principal components.

$$T^2 = \sum_{r=1}^{R}\frac{t_r^2}{\sigma_{t_r}^2} = \boldsymbol{x}^T\boldsymbol{V}_R\boldsymbol{\Sigma}_R^{-2}\boldsymbol{V}_R^T\boldsymbol{x} \tag{4}$$

where σ_{t_r} denotes the standard deviation of the rth score t_r. When the T^2 statistic is small, the sample is close to the mean of the modeling data.

In MSPC, an anomaly is detected when either the T^2 or Q statistic exceeds the corresponding control limit. The control limits, for example, are determined as $\alpha\%$ confidence limits, which means they are set so that $\alpha\%$ of samples representing the normal condition are below the control limits.

C. Stroke Detection Model Construction

In this study, HRV data obtained from MCAO rats and sham rats are defined as anomalous data and normal data, respectively. Algorithm 1 describes a model construction procedure. $\tilde{\boldsymbol{X}}^{\{i\}}$ denotes the raw HRV features of the ith rat ($i = 1, \cdots, I$) and I is the number of sham rats used for model construction. HRV features collected from rats $\tilde{\boldsymbol{X}}^{\{i\}}$ ($i = 1, \cdots, I$) are merged into one matrix $\tilde{\boldsymbol{X}}$ and preprocessed in steps 4 and 5. As a preprocessing, the HRV features except for meanNN are logarithmically transformed for considering distribution skewness [40], [41]. The merged and transformed data $\boldsymbol{X}$ are normalized with the mean and each HRV feature. In step 6, the singular value matrix $\boldsymbol{\Sigma}_R$ and the loading matrix $\boldsymbol{V}_R$ are obtained from $\boldsymbol{X}$. At this time, the number of principal components R have to be determined appropriately. Finally, the control limits of the T^2 and Q statistics $\bar{T}^2$ and $\bar{Q}$ are defined in step 7.

Algorithm 1 Model Construction

1: **for all** i such that $1 \leq i \leq I$ **do**
2: Extract the HRV features of the ith rat $\tilde{\boldsymbol{X}}^{\{i\}}$.
3: **end for**
4: Merge matrixes $\tilde{\boldsymbol{X}}^{\{1\}}, \cdots, \tilde{\boldsymbol{X}}^{\{I\}}$ into one matrix $\tilde{\boldsymbol{X}}$.
5: Preprocess $\tilde{\boldsymbol{X}}$ appropriately, which is referred to as $\boldsymbol{X}$.
6: Derive $\boldsymbol{\Sigma}_R$ and $\boldsymbol{V}_R$ by singular value decomposition of the preprocessed data matrix $\boldsymbol{X}$.
7: Define the control limits of the T^2 and Q statistics, $\bar{T}^2$ and $\bar{Q}$.

D. Stroke Detection

The proposed ischemic stroke detection algorithm detects an ischemic stroke when an anomaly is detected from HRV data by following Algorithm 2. $\tilde{\boldsymbol{x}}$ and $\boldsymbol{x}$ denote the raw and preprocessed HRV features, respectively. In step 3, the HRV features are preprocessed in the same way as in Algorithm 1. Since the HRV features can fluctuate due to arrhythmia or ECG artifacts, which leads to excessive fluctuation of the T^2 and Q statistics, the rat is discriminated as having ischemic stroke only when either the T^2 or Q statistic continuously exceeds its control limit for $\bar{T}^2$ and $\bar{Q}$ more than the predefined period γ in steps 5-7.

VI. Experiment

This section describes experiment protocols and application results of the proposed algorithm.

Algorithm 2 Stroke Detection

1: **while do**
2: Extract the HRV features of the tth $\tilde{\boldsymbol{x}}(t)$.
3: Preprocess $\tilde{\boldsymbol{x}}(t)$, which is referred to as $\boldsymbol{x}(t)$.
4: Calculate the tth T^2 and Q statistics, $T^2(t)$ and $Q(t)$, from $\boldsymbol{x}(t)$ by using Eq. (3) and (4).
5: **if** Either the T^2 or Q statistic continuously exceeds its control limit for more than γ **then**
6: Diagnose the development of ischemic stroke.
7: **end if**
8: **end while**

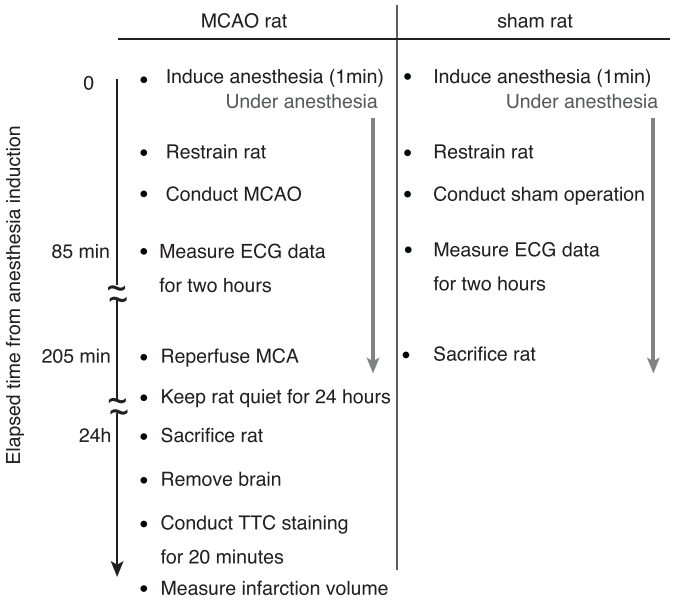
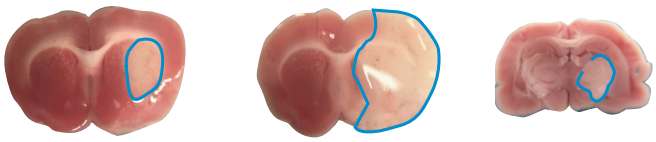


Fig. 3. Experimental protocol.



a) MCAO 5 (80.6 mm^2) b) MCAO 8 (382.2 mm^2) c) MCAO 11 (52.2 mm^2)

Fig. 4. Stained results and ischemic stroke volumes: a) MCAO 5, b) MCAO 8, and c) MCAO 11.

A. Experiment Material and Method

This study used seven to eight-week old adult male SD rats (weighing 211g - 287g) which were obtained from Sankyo Labo Service Corporation. All procedures were reviewed by the Committee for Animal Experimentation at the Jikei University School of Medicine, and performed according to its policies. In this study, eleven MCAO rats and eleven sham rats were prepared. The kth MCAO rat and the lth sham rat are referred to as MCAO k and sham l, respectively.

The animal experiment protocol of this study is shown in Fig 3. Anesthesia was induced by 4% Isoflurane, and rats were restrained on a stage. During the experiment, rats were

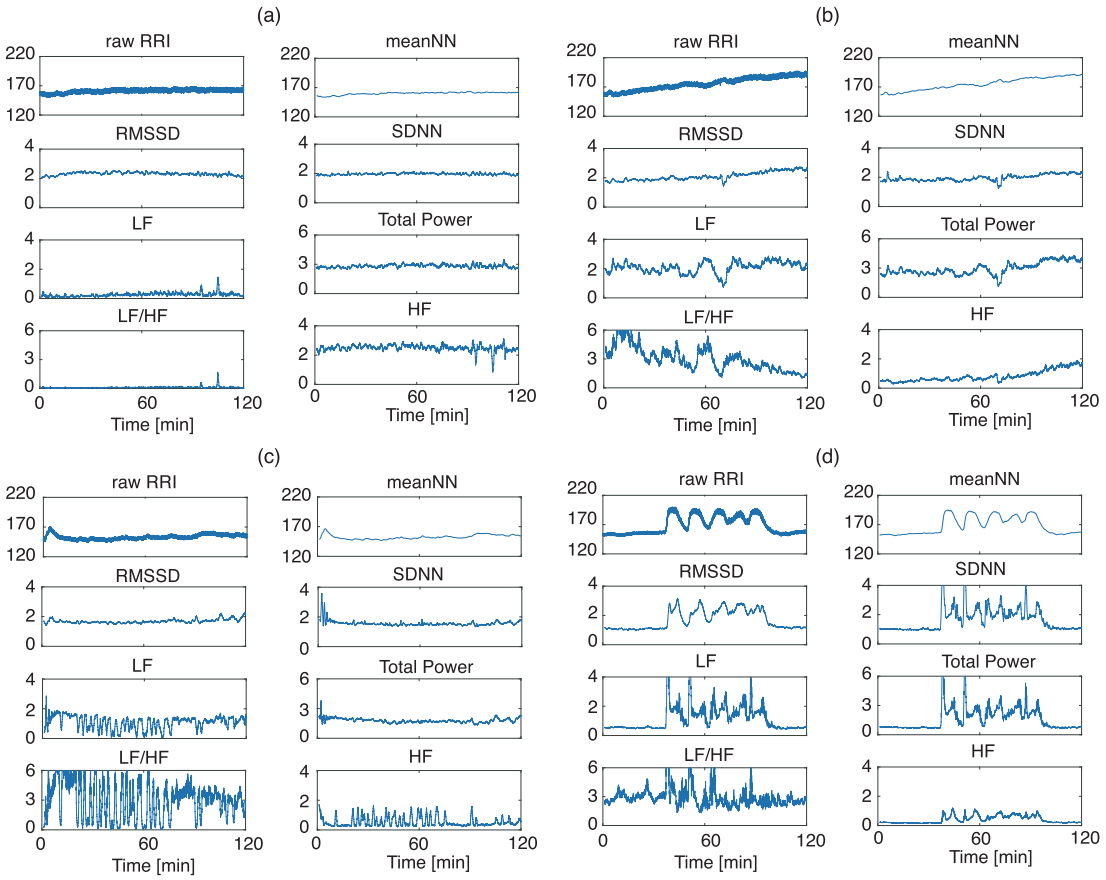


Fig. 5. Obtained HRV features: (a) sham 6, (b) sham 9, (c) MCAO 8, and (d) MCAO 11.

maintained with 2% Isoflurane at O2 200 mL per minute by using an anesthesia machine (MODEL-AP50, MERA). In MCAO rats, a nylon monofilament coated with silicon resin (5-0 large MCAO suture B3 L23 PK10, Doccol Corporation) was used to occlude the right MCA. In sham rats, the neck was incised and the ECA was tied up. In both MCAO rats and sham rats, ECG signals were recorded for two hours from 85 minutes after anesthesia induction start by using a self-made ECG measurement device that was tuned for rat ECG. The recorded ECG signals were digitized by an analog-digital converter (cDAQ-9171, National Instruments) at 1000 Hz and were stored on a hard disk.

After finishing ECG recording, sham rats were sacrificed. In MCAO rats, the MCA was reperfused. The neck incision was closed and the rats were kept quiet without anesthesia for 24 hours. Then, they were sacrificed, and their brains were carefully removed in order to examine the ischemic stroke area and volume through staining. The removed brain was sliced into 2 mm thick coronal sections and they were placed in a 2% triphenyltetrazolium chloride (TTC) solution (2, 3, 5-Triphenyltetrazolium chloride, Sigma), which indicates mitochondrial dehydrogenase activity, at 37 °C for 20 minutes. The volume and area of ischemic stroke were determined from the stained sections. Since the TTC solution cannot be used if rats die within 24 hours after reperfusion, stroke volumes of MCAOs 4 and 10 could not be measured. Figure 4 shows stained sections of MCAOs 5, 8, and 11 and their ischemic stroke volumes. MCAO 8 had the largest stroke volume among all of MCAO rats and the ischemic stroke area of MCAO 11 was in the brain stem. On the other hand, the stroke volume of MCAO 5 was almost the average of all stained MCAO rats (87.5 mm^2) and its area was not close to the brain stem.

B. HRV Analysis

The R waves in the collected ECG datasets were detected by using a first derivative-based peak detection algorithm and each RRI was calculated. Then, seven HRV features described in Sec. IV were extracted.

Figure 5 shows the RRI data and the HRV features obtained from shams 6 and 9, and MCAOs 8 and 11, respectively. The horizontal axis denotes the elapsed time from the end of the MCAO or sham operation (0 min). The HRV features of MCAOs 8 and 11 fluctuated more greatly than those of sham 6. On the other hand, the frequency features of sham 9 also fluctuated as with MCAOs. These results indicate that it is difficult to detect ischemic stroke by monitoring changes in respective HRV features and multiple HRV features should be monitored simultaneously.

C. Stroke Detection Preparation

Ischemic stroke detection was prepared by following Algorithm 1. In a study on epileptic seizure prediction, Fujiwara *et al.* [29] used about one third of the clinical data

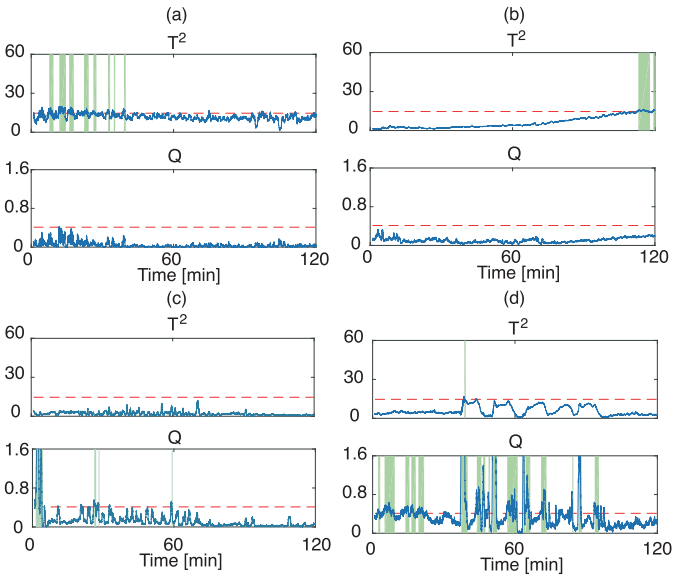


Fig. 6. Stroke detection results by MSPC: (a) sham 6, (b) sham 9, (c) MCAO 8, and (d) MCAO 11.

TABLE I
ISCHEMIC STROKE DETECTION TIME AND ISCHEMIC STROKE VOLUME

rat		Ischemic stroke detection time [min]		Ischemic stroke volume [mm^2]
		T^2	Q	
sham	4	1.3	-	
	5	-	-	
	6	7.6	-	
	7	-	-	
	8	-	-	
	9	113.0	-	
	10	-	11.5	
	11	-	5.2	
MCAO	1	1.3	17.8	124.1
	2	-	49.5	16.7
	3	-	46.0	11.4
	4	29.1	2.4	"Not Examined"
	5	-	21.7	80.6
	6	59.0	-	22.5
	7	-	-	48.2
	8	-	1.4	382.2
	9	-	18.9	50.0
	10	118.8	99.8	"Not Examined"
	11	38.7	2.3	52.2

for modeling. Thus, the HRV data of shams 1, 2, and 3 out of eleven sham rats were used for model construction. The other 19 types of HRV data were used for validation.

In MSPC, the number of principal components R was determined so that the cumulative proportion reached more than 90% and $R = 3$, and the control limits of the T^2 and Q statistics were defined so that they represent 99% confidence limits, which are common settings in MSPC. The parameter γ was determined as 20 seconds in consideration of an effect of arrhythmia.

D. Stroke Detection Result

Application results of the proposed ischemic stroke detection algorithm to shams 6 and 9 and MCAOs 8 and 11 are shown in Fig. 6. The horizontal red dashed lines indicate the control limits of the T^2 and Q statistics, and colored bands indicate periods during which the T^2 or Q statistic exceeded their control limits for more than 20 seconds. Although the T^2 statistic did not discriminate sham 6 and sham 9 as normal, the Q statistic discriminated shams 6 and 9 as normal and detected ischemic stroke of MCAOs 10 and 11 correctly.

Table I summarizes application results of the proposed algorithm to all rats except for shams 1 - 3 and staining results of MCAO rats. The table shows the ischemic stroke detection time elapsed from the end of the MCAO or sham operation and ischemic stroke volume examined by the TTC solution. "Not Examined" in the right column in Table I means that ischemic stroke volume was not measured.

The experimental result showed that the proposed HRV-based ischemic stroke detection algorithm detected ischemic stroke in nine out of eleven MCAO rats correctly and two out of eight sham rats incorrectly, when the Q statistic was used for monitoring. Thus, the sensitivity and the specificity of the proposed algorithm were 82% and 75%, respectively. It is indicated that HRV-based ischemic stroke detection for human patients is promising because rats resemble humans in HRV as well as cerebrovascular anatomy.

VII. DISCUSSION

In our experiment, shams 10 and 11 were incorrectly discriminated as stroke by the Q statistic. Figure 7 (a) and (b) and Fig. 8 (a) and (b) show their HRV features and stroke detection results. Total Power (TP) of sham 11 was the lowest among the validated data and its mean was 0.54, although the mean of TP in all of the validation rats was 2.40 ± 1.95. Anesthesia might have affected false detection in sham 11 because it has been reported that TP decreases after induction of Isoflurane [42]. Regarding sham 10, the autonomic nervous system might have been abnormal due to anesthesia, and LF/HF fluctuated significantly during the 60 minutes after the end of sham operation. Such an effect of anesthesia was also observed in Sham 9. Figure 5 shows that HF of sham 9 increased gradually from 60 minutes after the end of sham operation. Thus, sham 9 was incorrectly discriminated as stroke by the T^2 statistic at 113 min.

Among MCAO rats, ischemic stroke of MCAOs 6 and 7 could not to be detected by the Q statistic. According to the staining results, ischemic stroke was certainly induced in their brains. Their HRV features and monitoring results are shown in Fig 7 (c) and (d) and Fig. 8 (c) and (d), in which the Q statistic increased gradually in MCAO 6, although it did not exceed its control limit within the experimental period. This indicates that ischemic stroke might have affected autonomic nerve activities gradually in MCAO 6. There is the possibility that stroke of MCAO 6 would have been detected over two hours after occlusion. On the other hand, it is not known why ischemic stroke in MCAO 7 was not detected within two hours.

The proposed ischemic stroke detection algorithm monitors changes in HRV features by using MSPC, which is the same framework as the epileptic seizure prediction proposed by

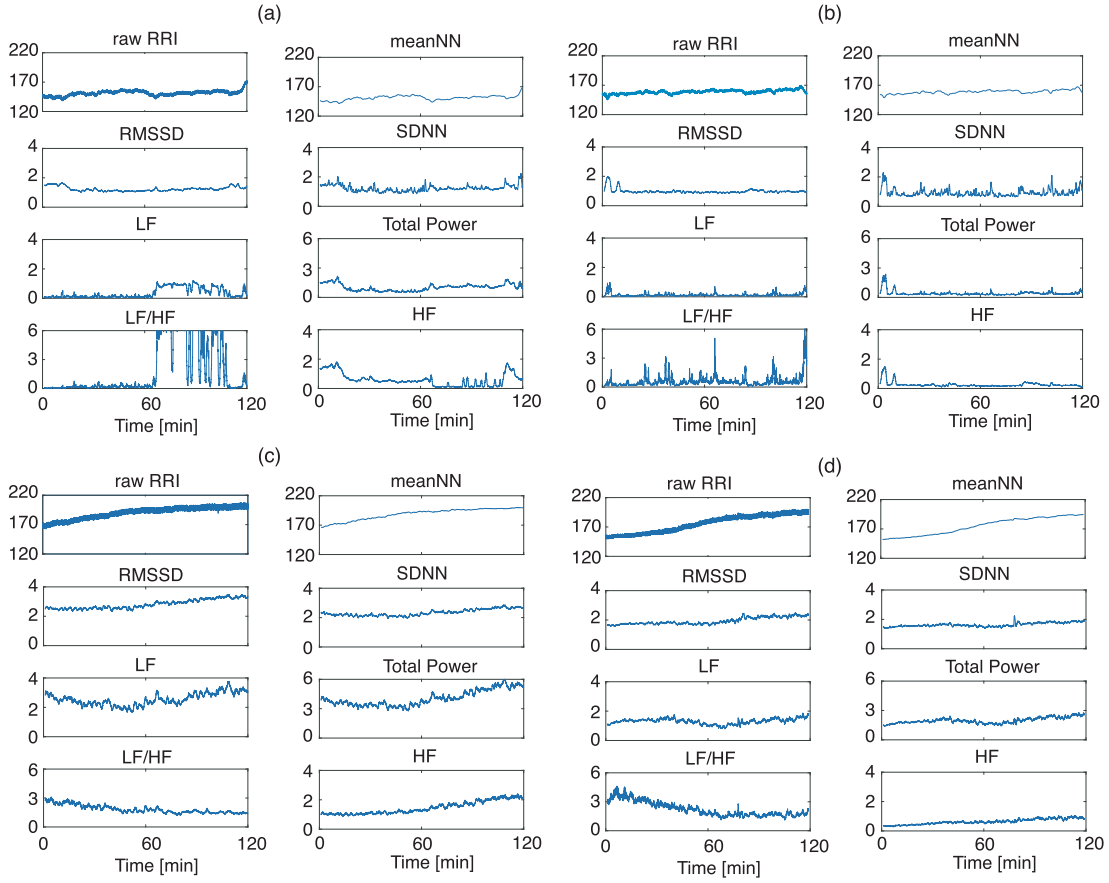


Fig. 7. Obtained HRV features: (a) sham 10, (b) sham 11, (c) MCAO 6, and (d) MCAO 7.

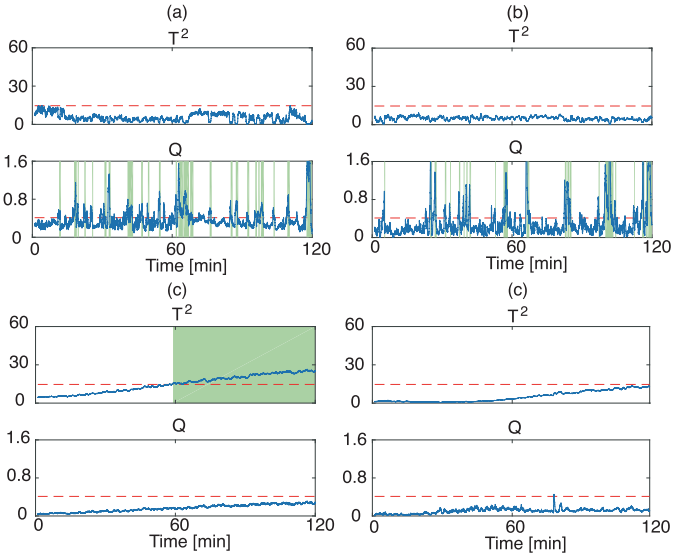


Fig. 8. Stroke detection results by MSPC: (a) sham 10, (b) sham 11, (c) MCAO 6, and (d) MCAO 7.

Fujiwara *et al* [29]. In their article, the property difference between the T^2 and Q statistics in HRV-based epileptic seizure prediction algorithm was discussed. They reported that most of the false positives by the T^2 statistic occurred when epileptic patients moved violently or ate something and that the seizure prediction performance of the Q statistic was higher than that of the T^2 statistic. In addition, they speculated that most large fluctuations in HRV are covered by major principal components and are represented by the T^2 statistic and that the Q statistic expresses residual changes in HRV and reflects changes in autonomic nervous activities by epileptic seizures. Finally, it was concluded that the use of the Q statistic is appropriate for seizure prediction.

Nine out of eleven MCAO rats were correctly discriminated as experiencing ischemic stroke when the Q statistic was used for monitoring, while only five MCAO rats were detected by the T^2 statistic. That is, the Q statistic is more appropriate than the T^2 statistic also in stroke detection. However, it is difficult to discuss the property difference between the T^2 and Q statistics in our experiment, since anesthesia might have affected both the T^2 and Q statistics. In fact, false positives in sham rats occurred by both the T^2 and Q statistics according to Table I. In order to investigate the effect of stroke on HRV without anesthesia by animal experiments, stroke-prone animals like stroke-prone spontaneously hypertensive rats (SHRSP) should be used [43].

Stroke detection based on another anomaly detection algorithm, one class support vector machine (OCSVM), was evaluated. Support vector machine (SVM) is a well-known nonlinear classification technique, which uses a kernel function for nonlinear data mapping into a high dimensional space and constructs an optimal separation hyperplane in the high

dimensional space. OCSVM is an extension of SVM for anomaly detection [44], whose modeling process is formulated as SVM when all of the modeling data belong to the same class and the origin is regarded as another class. Since OCSVM also uses a kernel function, it can cope with nonlinearity. In our experiment, the Gaussian kernel function with parameter $\sigma = 5$ was used, which was determined by cross-validation. In addition, the OCSVM model was constructed so that 99% of the modeling data were classified as normal. The sensitivity and the specificity of stroke detection in OCSVM were 72% and 50%, respectively. This result shows that MSPC is appropriate for stroke detection because it achieved a higher detection performance than OCSVM. However, further performance improvement of OCSVM may be achieved when appropriate tuning parameters are found.

According to the staining results shown in Table I, the ischemic stroke size of MCAO 8 was the largest and its stroke detection time was the earliest among MCAO rats. Stroke of MCAO 11 was detected the second earliest and ischemic stroke occurred in the brain stem, which controls the autonomic nervous function. The ischemic stroke of MCAO 5 was close to the average volume and not in the brain stem, and its stroke detection time was the sixth earliest. These staining results indicate that the ischemic stroke sizes and areas affected stroke detection time.

In addition, this may explain the cause of the latest ischemic stroke detection of MCAO 10. According to the HRV features of MCAO 10, its frequency domain features were gradually altered from around 85 min after the end of MCAO operation. Although the ischemic stroke size and area of MCAO 10 could not be measure by staining, this delay may have been caused by its small stroke size or remote stroke area from the brain stem.

Although some studies have reported that ischemic stroke reduces HRV [10]–[15], such a trend was not found in this study. On the other hand, HRV features of some MCAO rats fluctuated and increased temporarily. For example, Fig. 5 (d) shows how HRV features recorded from MCAO 11 increased about 40 minutes after occlusion. The difference in reported changes in HRV by ischemic stroke between previous studies and the present study may have been caused by the measurement periods after ischemic stroke onsets. Previous studies analyzed HRV for twelve hours or more after ischemic stroke onsets, while this study analyzed HRV within two hours from occlusion. This indicates that HRV fluctuates greatly at the acute stage of ischemic stroke in which damage to the brain is progressing and that HRV decreases at the chronic stage of ischemic stroke in which the brain tissue has become necrotic.

In order to realize an ischemic stroke detection system for humans, an easy-to-use wearable heart rate sensor is needed. A wearable device, which can measure RRI without any medical skills, has been developed [36]. However, this device requires users to put electrodes on the skin, because precise RRI measurement based on ECG is needed for HRV analysis. Since it is burdensome to attach electrodes, a new type of electrode that is easy to use should be developed. Recently, Tsukada *et al.* [45] developed a new wearable textile electrode using a conductive fiber, and a smart shirt woven with textile electrodes has been developed for ECG measurement. Therefore, it will be easy to use the proposed HRV-based stroke detection system when the smart shirt becomes available.

It is concluded that the proposed HRV-based ischemic stroke detection algorithm is promising with respect to physiological mechanisms between HRV and acute ischemic stroke as well as practical use.

VIII. Conclusion and Future Work

This work proposed an ischemic stroke detection algorithm by integrating HRV analysis and MSPC so that patients have the opportunity to receive stroke treatment at the acute stage. As a feasibility study before clinical application to human patients, this study collected the HRV data shortly after occlusion through animal experiments using the MCAO model in rats, and applied the proposed algorithm to the experimental data. The application result showed that its sensitivity and specificity were 82% and 75%, respectively. Although this study used the MCAO model in rats for algorithm evaluation, our experimental results also showed the possibility of realizing an HRV-based ischemic stroke detection system for human patients, due to cerebrovascular anatomy resemblance with humans.

The limitations of this study include data collection, such as the limited number of animals used in the experiment, and, in particular, the amount of HRV data for modeling was not enough. In addition, the effect of anesthesia on HRV could not be excluded in our experiment.

Since the constructed stroke detection algorithm for rats cannot be directly applied to humans, we are collecting clinical HRV data from stroke patients in hospitals for future development of an ischemic stroke detection algorithm for humans. In addition, a smart shirt that measures ECG has been tested in a clinical environment for accuracy evaluation. Because our stroke detection system under development informs patients and people around them of stroke occurrences in the acute stage, the system will contribute to an improvement in the rate of patients that receive early stroke treatment in the future.

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