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Compact Data Logger Carried on a Crow to Measure RR Intervals during Flight

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***Abstract*— Birds control respiration through air sacs, and the skeletal muscles that control them are always tense. Electrocardiograms (ECGs) with electrodes placed on the chest wall contain high-amplitude electromyograms. To measure the PR and RP intervals of an ECG accurately in such a physiological noise environment, which cannot be seen in mammals, it is necessary to lower the signal-to-noise ratio physically and reduce fluctuations in the peak R and P waves on the time axis. Therefore, we developed a catheter that can capture an ECG from the esophagus near the center of the thoracic cavity. As a field experiment, we attached the catheter to several carrion crows and obtained data before, during, and after flight. The changes in the PR and RP intervals before and after flight revealed that the sympathetic nerves were dominant during flight. To substantiate this, we also measured temporal fluctuations in the RR interval in chickens that had been administered drugs that act on the autonomic nervous system intravenously. Furthermore, we compared three correlations in regard to autonomic nerve control in chickens: RR interval variation, systolic arterial pressure variation, and cardiac output variation associated with respiration. Regarding the variation in the RR interval in birds, we were able to confirm three types of control with different cycles. In particular, the amplitude was small, and short-period fluctuations are not seen in humans. Therefore, future analysis is needed.**

Keywords—PR interval, RP interval, autonomic nerve fluctuations, angular velocity

I. Background

1.1.Purpose

We developed a small data logger and an esophageal catheter equipped with an electrocardiogram (ECG) electrode and angular velocity sensor to clarify the circulatory physiology of birds before and after flight. These devices were attached to carrion crows, which were then made to fly, and the data were recorded and analyzed. The same type of esophageal catheter was inserted under anesthesia in chickens and carefully observed. We expect to examine whether this small data logger and avian esophageal catheters can accurately record and analyze RR intervals and reflect various physiological conditions involving the autonomic nervous system.

1.2. Peculiarities of birds

Birds, unlike mammals, do not have a diaphragm. The lungs are rigid, and they have two types of bronchial tubes leading to the lungs: one in the forward direction from the oral cavity to the lungs, and the other a returning bronchus from the abdomen to the lungs. Birds have long necks, so they have a large dead space, and by reusing air currents, they increase the efficiency of gas exchange. This air sac-like mechanism is essentially controlled by pressure changes in the contraction and relaxation of the skeletal muscles of the chest wall and abdomen. In such a physiological noise environment, which is not seen in mammals, a high signal-to-noise ratio (SNR) is required to measure the peak ECG R and P waves along the time axis accurately.

1.3. Related research

In humans, which are mammals, the heart rate fluctuates as a result of the regulation of the autonomic nerves[1], but various conditions, such as diabetes and orthostatic dysregulation, can weaken the regulation function of the autonomic nerves, which makes a fluctuating heart rate difficult. By recording the heart rate variability in resting and deep breathing states, it is possible to evaluate whether and to what degree the control function of the autonomic nervous system is weakened; this can be used as a parameter to assess issues such as the progression of diabetes. Regarding birds, a study of RR intervals in chickens was reported in 1999, but here, the ECG obtained from 100 beats is analogously traced and compared using a graphical analog procedure[2]. Furthermore, in 2000, Japanese researcher recorded the RR interval in a cormorant using a data logger[3].

By contrast, the present study aimed to obtain over 50Hz high-frequency components of ECG signal using the filter function after performing a fast Fourier transform (FFT), and compare the noise level of esophageal catheter and of thoracic wall. The reason why the frequency is set to 50Hz or more is that the source component is coming from EMG, and fluctuation of base line due to partial contractions of atrium is 50Hz or less.

In 2018, Nakata et al.'s study of RR intervals in crows and chickens used an esophageal catheter to elicit trends in RR intervals and conducted a mathematical analysis for the first time. Here, the correlation between blood flow and the angular velocity sensor at the tip of the esophageal catheter was obtained using a statistical method. Thus, in this study, referring to the research by Nakata et al[4]. ECGs were measured with a similar esophageal catheter to obtain the RR interval.

1.4. Arterial baroreflex (ABR)

In the case of a bipedal person, fluctuations in arterial pressure (relatively short cycle) associated with respiration impair cerebral circulation, so baroreceptors (pressure sensors) are located in the heart and great arteries (aorta) and in the carotid artery pathway, the parasympathetic system lowers blood pressure and heart rate via Vagus nerve, and increases heart rate and blood pressure via the sympathetic nerve. Purpose of this control is to stabilize blood flow autonomously and provide stable continuous flow to the brain. Each autonomic nerve has a different conduction velocity, which causes fluctuations associated with control.

Furthermore, there are reflexes that control pressure fluctuations in the thoracic cavity with respiration in the pulmonary vessels, vena cava, and atrium. This is called cardiopulmonary baroreflex (CPBR). In case of a bipedal person, we have another spinal reflex that stabilizes blood flow to the brain is called ABR, In fact, in humans, the control of cerebral blood flow is complicated, and multiple nerve pathways are intricately coordinated by the CPBR and ABR.

In birds, another vertebrate that also walks on two legs, some research has reported specific physiological data speculating the existence of the CPBR, but not ABR. If it were possible to display data that leveled the fluctuations in blood flow associated with respiratory fluctuations, it would be possible to suggest the existence of an ABR control system in birds.

1.5. What are RR intervals?

In an ECG, the RR interval is the time between an R wave that excites the ventricle and the next R wave. Generally, in mammals, the cardiac rhythm is governed by the sinus node, and if the atrioventricular conduction time (conduction time from the atrium to the ventricle) is constant, the sinus cycle becomes the ventricular excitation cycle, that is, the RR interval.

Measuring the RR interval and examining its cyclic fluctuations means observing the autonomic nerves that govern the sinus cycle. The RR interval is calculated by detecting the time difference between the peaks of the R wave. Furthermore, the interval from the P wave to the R wave is called the PR interval, and the interval from the R wave to the P wave is called the RP interval.

1.6. Calculation method for the RR, PR, and RP intervals

For measurement evaluation, we examined whether the coefficient of variation of the ECG R-R interval (CVRR) agreed with physiological evidence, even under noise. Similarly, the PR and RP were also determined. The following equations were used:

CVRR = RR standard deviation / {ΣRR interval (1–101) / 100}

CVPR = PR standard deviation / {ΣPR interval (1–101) / 100}

CVRP = RP standard deviation / {ΣRP interval (1–101) / 100}

II. Developments

2.1. Esophageal catheter

Development of a spatial noise reduction effect

In birds, the skeletal muscles of the chest and abdomen act like a diaphragm to control the air sacs. In general, breathing movements are involuntary. However, voluntary movements can be performed as needed, so in many cases, the skeletal muscles are considered to be controlled by nerves from the brain stem. In the central part of the thoracic cavity, electromyograms (EMGs) from the skeletal muscles are circumferential (0–360°)(Figure 1). Therefore, the expected mathematical value of the noise is zero.

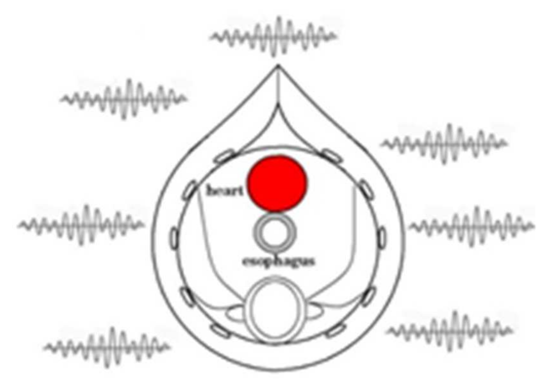

Fig. 1. Principle of the spatial denoising effect

As shown in Figure 2, the esophageal catheter is composed of an angular velocity sensor, a heart sound microphone, and an ECG electrode at the tip. The ECG is a bipolar ECG, which consists of Channel 1 for ventricular and Channel 2 for atrial electrodes (negative and neutral electrodes are common, total four electrodes). The depth of the catheter can be gauged by the shape of the P and R waves of the two ECGs.

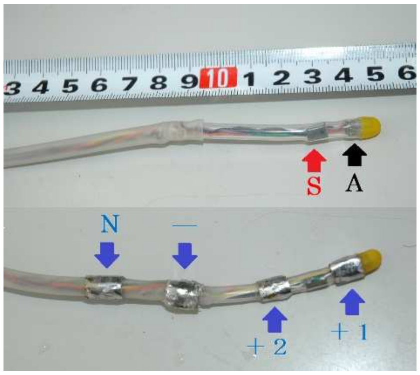

Fig 2. Esophageal catheter. A:Anglar velocity sensor, S:Sound microphone,+1: Ch1 ECG anode, +2: Ch2 ECG anode, --: ECG negative, N: ECG neutral electrode

Because crows are born with a missing esophageal crop (Ingluvies) , their mouths can be opened and catheters can be inserted into the lower esophagus without resistance. For this reason, crows have become the target birds for esophageal catheterization. However, because chickens have a crop, it is difficult to insert a catheter into the lower esophagus (in the thoracic cavity) without a radiographic screen. Under the environment of inhalational anesthesia with Isoflurane, we pulled the crop at the lower neck strongly forward with the fingers and blindly guided the catheter toward the dorsum of the crop. Nearly 100% catheterization into the lower esophagus is achieved by pulling the crop forward without any X-ray equipment.

2.2. Data logger

A lightweight data logger was developed for the purpose of measuring the RR interval of crows. The weight, including the battery, is 24 g, which is 5% payload for a crow weighing 480 g. The upper limit of the payload is generally said to be 8%, so it is still within the range where it can fly freely. The data logger can record 16 minutes and 40 seconds at 1000 samples per second, on the other hand, the remaining battery power allows for more than 3 hours of measurement time with full CPU activities. It was only depended on the Excel's file management limit of 10^6 (16min.40sec. is 6x60+40 = 1000 =10^3) seconds, sampling 1000 /s = 10^3, total number of data = 10^3x10^3 =10^6) In Excel, the upper limit is 1,048,576 rows (CSV file), and in general processing it is 1 million rows. Sure, there are formats that break that cap. However, our selection is simple CSV file format in order to share our results for all participants' PCs. The specifications of the data logger are summarized in Table I.

Table I. Specifications of data logger

Items	Contents
CPU	ARM 1768
A/D channel	7 channels
A/D conversion	1000/sec./ch, 16-bit
Amplifiers x:gain	2 ECG channels: ×300 (bipolar amplification) Angler velocity: ×50
	3-channels amplification for air sac pressure ×30
Battery	weight: 2.8 g, Voltage: 3.7 V, 150mAh
Weight	24g
Size	L:5 cm, W:4 cm, H:3cm

In addition, crows can fly freely in cages with 6-m-high poles set up around a large tennis court and nets stretched around them, but single flight time is only about 10 seconds. The reason for this is that when the crow hits the net, it stops and takes a rest. Crows are very clever, so after flying just a few times, they get a grasp of the net, and when they realize that they cannot escape, they do not try to fly away. Therefore, the assistant in the experiment needs to chase the crows and make them fly. If this is done for 16 minutes, both the assistant and the crow become fatigued, so we considered 16 minutes of measurement to be sufficient. A block diagram and a photograph of the data logger are shown in Figure 3.

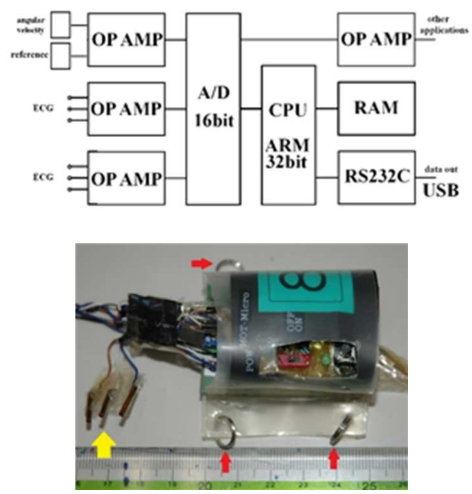

Fig. 3. Block diagram and data logger. For the red arrow, through four rings and attach it to the back of the crow. Yellow is ECG connector CH1.

III. Experiment and Results

3.1. Spatial gain of the esophageal catheter

Echocardiogram acquisition in the esophagus has a noise reduction effect because all 360° phases of the electrical potential emitted from the chest wall muscle can be summed. A previous study by Nakata et al. reported that ECG measurements in the esophagus reduced the SNR by about 8 dB compared with chest wall ECGs [4].

However, we considered that a higher frequency EMG had a higher SNR because it counted contractions at the segmental level of the atria with low frequencies as noise.

As we wanted to identify the time axis that takes the peak value of the ECG R wave (q wave), we focused on a slightly higher noise frequency. When there is movement, EMGs of other than respiratory muscles are also picked up, so we compared the ECG electrode on the body surface and the esophageal electrode under isoflurane inhalation anesthesia (spontaneous breathing) (Figure 4).

Fast Fourier Transform (FFT) was performed on both ECG waveforms, and the potentials obtained by Inverse Fast Fourier Transform(IFFT) with frequency components of 50 Hz or higher (high pass filter) were further differentiated (Figs. 5 and 6), and the respective absolute values were compared. The results showed that the chest wall electrode was 4.42 times higher in terms of the voltage ratio than the esophageal catheter, and that the esophageal catheter had noise of 12.9 dB for frequencies above 50 Hz (mainly EMGs of the chest wall). It can therefore be said that the esophageal catheter has a damping effect.

Fig. 4. Comparison of the esophageal catheter and chest electrodes in an isoflurane inhalation anesthesia environment

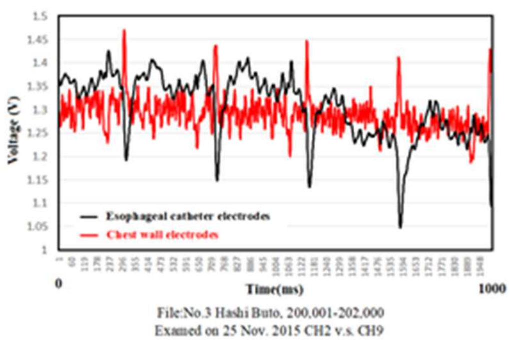

Fig. 5. Comparison of an ECG with an electrode and an esophageal electrode

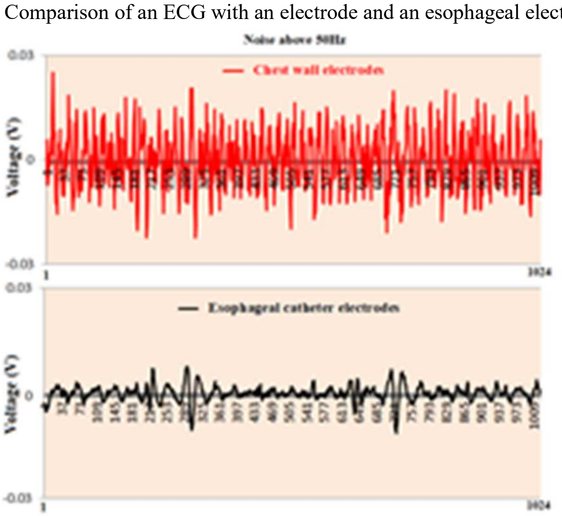

Fig. 6. Comparison of IFFT results above 50 Hz between ECG and esophageal electrodes. The ECG electrode of the esophageal catheter had a spatial noise reduction effect of about 12.9 dB comparison from body surface electrodes of chest wall. The upper figure is the waveform with large noise after IFFT of the chest wall, and the lower figure is the waveform after IFFT of the esophageal electrode;(method: after FFT, IFFT is performed at 50 Hz or more, and the waveform is differentiated to eliminate the DC component)

3.2. Fluctuations in PR and RP intervals during flight

Carrying a data logger on its back, a carrion crow took flight (Fig. 7), and the PR and RP intervals were measured (Figs. 8 and 9). The results indicated that the CVRP = 2.0 [%] CVPR = 0.5 “%” This result means that the PR interval, that is, the duration from P (atrial excitation) to R (ventricular excitation), has little fluctuation, while the RP interval, in which the SA(Sino-Atrial) node's time adjustment is involved, has large fluctuations. The RP interval was found to be the main source of fluctuations in the RR interval. In addition, the CVRR at this time was 7.6% immediately before flight and 1.2% during flight. These findings indicate that the sympathetic nerves are overwhelmingly dominant during flight.

Fig. 7. A carrion crow in flight with a data logger on its back

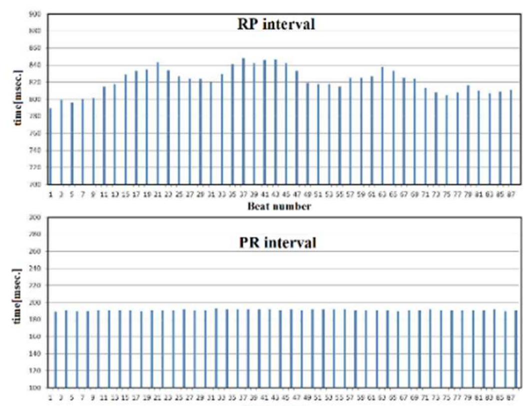

Fig. 8. RR interval before and after flight with a varying PR interval and fixed RP interval (carrion crow)

3.3. Pre-, mid-, post-flight, and sustained RP and PR interval variations

The assistant held the carrion crow, let it fly from his hand, and it flew through a large net. In this state, we measured the changes in the RP and PR intervals during repeated movements before, during, and after flight (Fig. 9). The PR interval is always constant, and the PR is shortened during flight, and two types of waveforms (short cycle: respiratory fluctuations, long cycle: autonomic nerve-derived fluctuations) appear in the time period between flights.

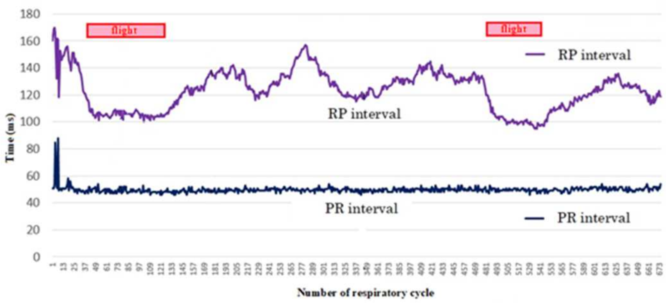

Fig. 9. Fluctuations in the RP and PR intervals during repeated actions before, during, and after flight

3.4. Verification experiment with chickens

In flight experiments with crows, continuous monitoring of invasive arterial pressure and estimation of cardiac output using an angular velocity sensor are physically difficult. Here, validation experiments were performed using similar catheters in chickens under anesthesia (spontaneous breathing).

We measured invasive systolic arterial pressure in chickens and its variation with each beat and recorded the amplitude potential of the angular velocity sensor (proportional to cardiac output) at the tip of the esophageal catheter and its variation with each beat. In addition, we determined the correlation between RR interval variation and invasive systolic blood pressure when a beta-blocker (propranolol) was administered intravenously. Also in this experiment, fluctuations in respiration and those due to sympathetic/parasympathetic control were observed (Fig. 10).

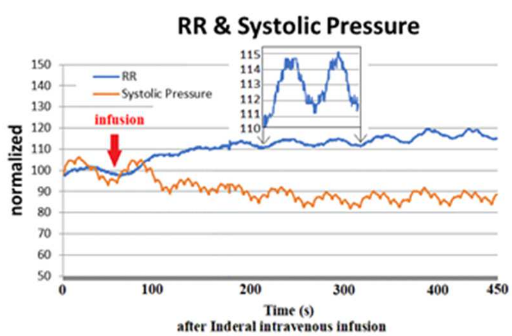

Fig.10. Relationship between RR interval fluctuations and systolic blood pressure (invasive) when a beta-blocker (propranolol) was administered. The Y-axis has been normalized for ease of comparison. Propranolol was slowly administered intravenously for 60 seconds after the start of measurement (female chicken: 1.2 kg; propranolol: 0.1 mg).

Figure 11 shows that an esophageal catheter was inserted into a chicken, multiple air sac pressures were measured simultaneously, and magnets and magnetic field sensors were attached to the left and right sides of the chest wall to measure the distance (to view respiratory movements of thoracic wall).

The three graphs below show the RR interval, invasive systolic blood pressure, and peak value of the angular velocity sensor attached to the end of the esophageal catheter (equivalent to systolic pulsation = proportional to blood flow) for 0–32 seconds.

As shown in the figure, the RR interval, systolic blood pressure, and angular velocity peak values were all subject to respiratory fluctuations. Its movement is synchronized with the posterior thoracic air sac pressure and chest wall distance. Fluctuations in systolic blood pressure are shown in units of 1 mmHg. Figure 12 depicts the correlation between the RR interval and systolic blood pressure, the so-called Oxford gain. Compared with humans, the correlation coefficient R2 does not approach 1. This is because wave 1 is riding on the chicken's RR interval.

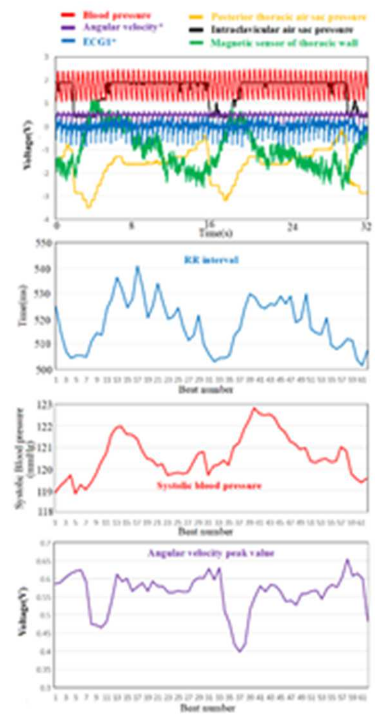

Fig. 11. RR interval obtained by esophageal catheter, peak value of the angular velocity sensor, and invasive blood pressure (systolic blood pressure), and their correlations with respiratory waveforms

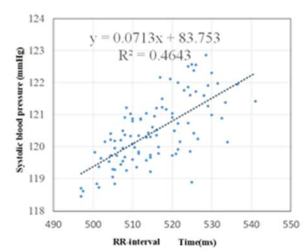

Fig. 12. Correlations between the RR interval and systolic blood pressure, R^2=0.46.

IV. CONSIDERATIONS

4.1. The RR interval during flight is overwhelmingly sympathetic-dominant

From the experimental results in Figure 9, there is a period of RR intervals (about 40–50 seconds) during the non-flying time of carrion crows. This is thought to involve fluctuations in sympathetic and parasympathetic control. On the other hand, when the crow flew, the fluctuation completely disappeared, becoming tachycardia. In other words, for carrion crows, flight is a state in which the sympathetic nerves are overwhelmingly dominant.

As shown in Figure 11, under isoflurane inhalation anesthesia, there were two types of RR intervals: 1) respiratory fluctuations with a period of about 5 seconds, and 2) fluctuations with a period of about 40–50 seconds due to the control of the sympathetic and parasympathetic nerves. The peak values (absolute values) of the RR interval, systolic blood pressure, and angular velocity of the tip of the esophageal catheter are each subject to respiratory variability. These fluctuations are synchronized with the posterior thoracic air sac pressure and the distance of the chest wall (more precisely, the square of the distance).

Table II. Types of RR interval fluctuations

Period (converted to heart rate)	Waveform	Amplitude	Fluctuation factors
5–7 (heartbeats) Rapid wave Autonomous control	(1)	Very small	Observing the phase difference of the control signal that keeps the venous return due to respiration constant
Respiratory rate cycle, synchronized with the respiratory rate	(2)	Small	Physical fluctuations of respiration
Almost 100 (heartbeats, crow) Almost 100 (heartbeats, chicken) 40–50 seconds	(3) Slow wave	Large	Control of the sympathetic and parasympathetic nerves (CPBR)

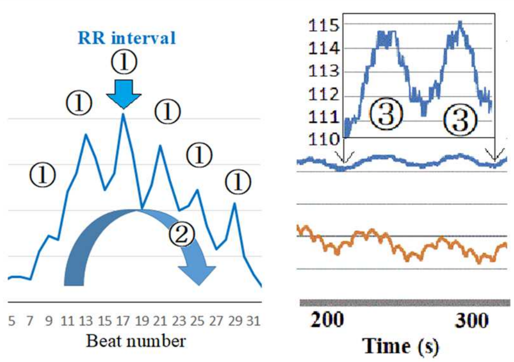

Fig. 13. Three types of fluctuations in the RR interval. The RR fluctuations in birds reflect the control signal of the circulatory system, and as shown in Figure 13, there are roughly three frequencies, the characteristics of which are summarized in Table 2. Waveforms (2) and (3) in Figure 13 can be observed in humans, cats, and other animals, but waveform (1) has not been observed in human RR intervals.

4.2. Does waveform (1) correct the venous return associated with respiration with ABR control?

In humans, the conduction velocity of the Vagus nerve, which is a thin myelinated fiber, is 10 m/s, while the sympathetic nerve, which is an unmyelinated fiber, is 10 times slower, at 1 m/s. The delay time from acetylcholine release to the heart rate beginning to decrease is about 300 (ms), while the delay time from norepinephrine release to the heart rate beginning to increase takes about 2 seconds.

As a result, in humans, the time required for heart rate changes via the Vagus nerve shows a maximum effect within 2 seconds after baroreceptor stimulation, whereas heart rate changes via the sympathetic nerve show a maximum effect within 10 seconds.

The difference in the control time between the sympathetic and parasympathetic nerves is what causes fluctuations in the RR interval (waveform 3). To eliminate this delay, humans give autonomy to the atrial node (or sinus node). On the other hand, considering the bird's RR control, it can be seen that there are three control frequencies, as opposed to the two in humans (Table II).

The angular velocity of the esophageal catheter is proportional to the cardiac output, and the respiratory fluctuations of the angular velocity are stable and flat compared with the fluctuations in the RR interval and systolic blood pressure (Fig. 14). This suggests that blood pressure changes associated with intrathoracic pressure changes due to respiration are instantly fed back from baroreceptors in blood vessels, tensing the walls of the blood vessels, including central veins, and stabilizing the circulatory system. Waveform 1 looks at the phase difference of control, so it may be that the fluctuation of venous return due to respiration is controlled, and constant blood flow is provided to the left ventricle at all times.

The heart rate during flight is about 500/min for carrion crows and about 1000/min for hummingbirds, and RR control is controlled only by the efferent Vagus nerve (the only parasympathetic nerve that enters the heart) and the sympathetic nerve from the spinal cord (Fig. 15). There might be some basic control, such as spinal cord reflex. In other words, the control mechanism that subtly controls the heartbeat of birds, which is 10 times that of humans, and keeps the stroke volume constant must be a more primitive spinal or medullary reflex. We therefore wonder whether the autonomic reaction is not rapid enough to control the output flow from the left ventricle, which the ABR should be able to handle. We believe that the triangle waves; waveform (1) of the RR intervals indicate that ABR regulation is strongly present in birds. Additional research on this issue is needed in the future.

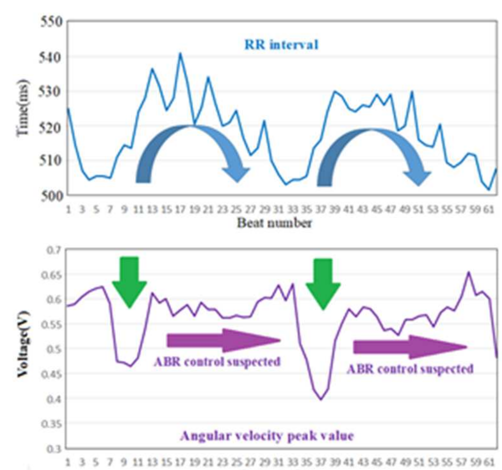

Fig. 14. Waveform that appears to indicate ABR control of blood flow. It should be noted that the depression of respiration, which seems to be uncorrected, is probably due to the angular velocity of the movement (external force) of the

esophagus itself, which is depressed due to respiration (green arrow).

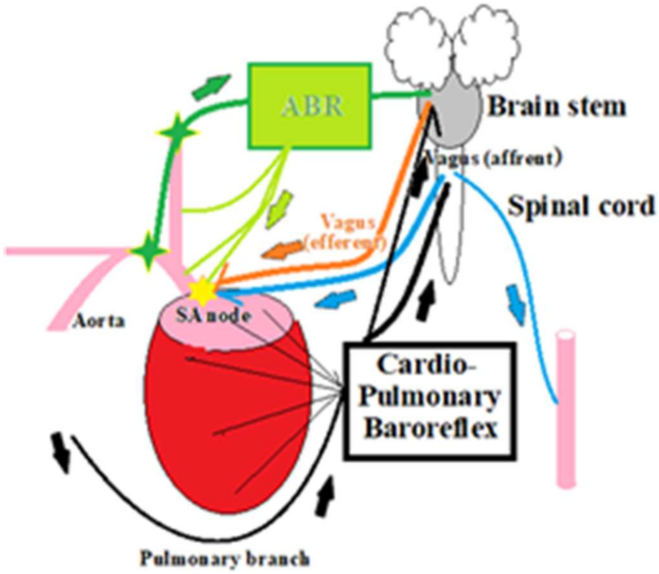

Fig. 15. ABR and new anatomical sympathetic/parasympathetic nerve pathways

4.3. Avian influenza infection and cytokine storms

The avian influenza virus proliferates in type II alveolar epithelium, and infected individuals become shallow breathing and increase in number of respiration. In virus-infected cells, macrophages and dendritic cells release large amounts of chemical mediators to help permeabilize the blood vessels of T cells (a type of lymphocyte). When this becomes excessive, it becomes a so-called cytokine storm, in which the leakage of water from the blood vessels does not stop and the sympathetic nervous system becomes overwhelmingly dominant in an attempt to raise blood pressure. That is, the RR interval becomes shorter. If the RR interval of chickens can be monitored without restraint or contact, it may be possible to screen infected animals early[6.7].

We have also developed a device that measures the RR interval of chickens using transmission microwaves, which we believe will make it possible to compare the RR intervals obtained with an esophageal catheter and those obtained with microwaves accurately.

V. Conclusion

We developed an esophageal catheter for birds, collected an ECG that was almost unaffected by the EMG of the skeletal muscles, and developed a small data logger that can store it.

As a field experiment, we attached this device to several carrion crows and obtained data before, during, and after flight. The results demonstrated that the PR intervals before and after flight did not fluctuate, but the RP interval did, indicating that the sympathetic nerves were overwhelmingly dominant during flight. Furthermore, to substantiate this finding, we also measured changes in the RR interval and invasive systolic blood pressure simultaneously in chickens intravenously administered with a beta-blocker that acts on the autonomic nervous system.

We believe that understanding such autonomic activities may help detect the early hypotension of cytokine storms that affect individuals with highly pathogenic avian influenza viruses.

Acknowledgment

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From 2011 to 2023, the animal experiment protocol using crows and chickens passed review by the Tokai University Experimental Animal Ethics Committee. Regarding the animal experiments with chickens, we received technical assistance from Ms. Sachie Tanaka and Mr. Shuho Hori (Life Science Support, Medical Science Collage Office, Tokai University). In addition, we would like to thank Ms. Miyoshi Tanaka and Ms. Noriko Numata from the Nakajima Laboratory of Tokai University School of Medicine for their support in conducting the crow flight experiments.

For the chicken experiment in Takaoka city, we would like to thank Mr. Masuhisa Ta, Tasada Works, Inc., for use of the laboratory, and Ms. Megumi Amano of Seisa University, who coordinated the experiments in Takaoka city. Carrion crows were supplied by Tsuruoka city free of charge, and large-billed crows were captured by Dr. Masanori Hamada (veterinarian, Hiratsuka city) and received free of charge.

Regarding Nakajima Lab’s original data logger, application boards such as the angular velocity and pressure sensor amplifier circuits were manufactured independently, but the CPU board was outsourced to ROHRM RIKEN in Kosei city, Shizuoka Prefecture. The Excel program (VBA) for RR analysis was instructed by Dr. Hiroshi Juzoji (EFL Inc., Takaoka city).

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