Basic Physiological Research on the Wing Flapping of the Sweet Potato Hawkmoth Using Multimedia

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Abstract

We have developed a device for recording biological data by inserting three electrodes and a needle with an angular velocity sensor into the moth for the purpose of measuring the electromyogram of the flapping and the corresponding lift force. With this measurement, it is possible to evaluate the moth-physiological function of moths, and the amount of pesticides that insects are exposed to (currently LD50-based standards), especially the amount of chronic low-concentration exposure, can be reduced the dose. We measured and recorded 2-channel electromyography (EMG) and angular velocity corresponding to pitch angle (pitch-like angle) associated with wing flapping for 100 sweet potato hawkmoths (50 females and 50 males) with the animals suspended and constrained in air. Overall, the angular velocity and amplitude of EMG signals demonstrated high correlation, with a correlation coefficient of R = 0.792. In contrast, the results of analysis performed on the peak-to-peak (PP) EMG intervals, which correspond to the RR intervals of ECG signals, indicated a correlation between ΔF fluctuation and angular velocity of R = 0.379. Thus, the accuracy of the regression curve was relatively poor. Using a DC amplification circuit without capacitive coupling as the EMG amplification circuit, we confirmed that the baseline changes at the gear change point of wing flapping. The following formula gives the lift provided by the wing: angular velocity × thoracic weight − air resistance – (eddy resistance due to turbulence). In future studies, we plan to attach a micro radio transmitter to the moths to gather data on potential energy, kinetic energy, and displacement during free flight for analysis. Such physiological functional evaluations of moths may alleviate damage to insect health due to repeated exposure to multiple agrochemicals and may lead to significant changes in the toxicity standards, which are currently based on LD50 values

Key Words: Chronic low dose exposure, Physiological functional evaluation, Electromyography, LD50-based standards.

I. BACKGROUND

Insects, particularly species of large moths, are in danger of disappearing from the natural world. A key factor underlying this peril may be the significant impact of repeated exposure to multiple chemicals. Our concern—an assumption at this point—is that the current practice of judging acute toxicity, as represented by the use of LD50 values, is disproportionately damaging biodiversity of large insect species. We launched this basic research with the goal of screening for subtle physiological anomalies [1], [2], [3], [4].

It is conceivable that the repeated use of multiple agrochemicals will eventually cause irreparable damage to mankind.

Large moths, which travel great distances in the natural environment, are highly likely to be repeatedly exposed to multiple types of agrochemicals. While LD50 values are standard indicators of acute exposure, chronic exposure is assumed as 1/100 of the LD50 value. Simple arithmetic indicates if an individual organism is exposed to 20 different types of agrochemicals, theoretical exposure to toxins polluting the environment is $1/100 \times 20 = 1/5$ of the LD50 dose. Our team has cast doubts on such a simplified method of calculating chronic toxicity levels based on LD50. Our study seeks to obtain physiological data of moth flight to screen basically healthy individual specimens for those exhibiting physiological anomalies in physiological function at low level exposures, with the goal of identifying an indicator that may replace 1/100 of the LD50 value.

1.1 Repeated exposure to multiple agrochemicals

1.2. Analysis of flapping motion

Many reports of studies of flapping wings have focused on the theme "why insects can fly." In recent years, military research has investigated insect flight mechanisms as potential propulsion mechanisms for micro air vehicles (MAVs). A mainstream trend in the fluid dynamics approach to the study of flight mechanisms applies numerical simulation techniques to analyze the lift provided by wings. These studies typically assume the steady-state wing theory, while taking into account the additional effects such as separation vortex motion [4]-[9].

Table 1. Repeated exposure to multiple agrochemicals.

1. Insecticides	Chemicals used to control insect pests detrimental to crops
2. Fungicides	Chemicals used to prevent diseases detrimental to crops
3. Insecticide-fungicide mixtures	Chemicals used to control insect pests and diseases simultaneously
4. Herbicides	Chemicals used to control weeds
5. Rodenticides	Chemicals used to control rodent pests, such as field rats, that are detrimental to crops
6. Plant growth regulator	Chemicals that promote or inhibit the growth of crops
7. Attractant	Chemicals that lure and trap mainly insect pests with smell, etc.
8. Spreading agent	Chemicals mixed with other agrochemicals to enhance adhesion to plant surfaces
9. Microbial pesticides	Chemicals used to control insect pests and diseases detrimental to crops using microbes

The following equation gives the lift L produced by the translational motion of wings required to achieve airspeed U:

$$L = 1/2 \, (\rho U^2 SC), \qquad (1)$$

where

C is the coefficient of lift;
ρ is air density; and
S is the surface area of the wing.

This equation describes only a relationship that exists at a momentary snapshot of wing motion meeting a specific criterion (steady-state wing condition). Accounts based on pioneering studies and experiments suggest that for continuous wing flapping, the lift created by the second and third flap differs from that of the first flap. For this study, we decided to attach a monoaxial (mainly pitch axis) angular velocity sensor to a moth, albeit under restrictive conditions wherein the moth was suspended and constrained by a string.

We focused on angular velocity because we thought that due to its correlation with lift, we could observe muscular contraction by measuring angular velocity. This is because the measurement of the angular velocity has been technically completed in the past by measuring the force emitted by the flight of the whooper swan and the heart of the chicken (proportional to blood pressure) [10]-[12].

II. METHODS

2.1. Target insect of experiment

We gathered and analyzed physiological data of wing flapping for 100 hawkmoth (*Agrius convolvuli*) specimens suspended in air using string (in a constrained state).

Number of moth specimens: 100 specimens of *Agrius convolvuli* (50 males and 50 females)
Mean body weight: 1.1 g
Mean dry weight: 0.28 g (approximately 75 % of the body weight is water)

Agrius convolvuli is the largest hawkmoth found in the Japanese islands. Its wingspan may reach 105 mm, often resulting in its being mistaken for a bird. Larvae feed on the leaves of plants belonging to the Convolvulaceae family, such as sweet potatoes, making them despised pests among farmers. They are bred for research purposes at the University of Tokyo and other institutions [13]-[15].

We selected the hawkmoth for our study due to its maximum payload, which is regarded to be around 0.3 g. This allows these insects to carry a micro radio transmitter while flying freely outdoors in experiments to be carried out after obtaining the appropriate radio transmission license from the Ministry of Internal Affairs and Communications.

2.2. Anesthesia

As with vertebrates, surgical procedures on moths require anesthesia. Having discovered early that using nerve-block agents such as xylocaine acting directly on the nerves results in total loss of the moth's ability to fly, we were compelled to examine other reversible procedures.
Reducing temperatures to 8–10 °C is known to slow moth metabolism, inducing a state of suspended animation. We placed a moth in a Tupperware® with 10 small holes for ventilation and stored the container in a commercial refrigerator. The moth became completely immobile when left in the refrigerator for approximately 60 minutes, giving us time to perform a 5-minute surgical procedure. After the procedure, the moth was gradually returned to room temperature and able to fly freely in about an hour.

2.3. Surgical procedures

Moths are covered by generous quantities of lamellar and piriform scales. Some procedures involve preparing moths by removing the scales using air sprays to expose the exoskeleton. Based on our extensive experience with surgical techniques, we chose to secure the field of view under a surgical microscope by wetting the surgical field with a diluted antiseptic agent in preparation for the surgical procedure.

The surgical procedure involves embedding multiple electrodes into the body of the moth. For each electrode, a small hole is formed in the dorsal surface of the hard, shell-like exoskeleton using a pin fixed to a celluloid plate. The hole measures approximately 5 mm deep and 0.2 mm in diameter. The procedure does not involve bleeding. After the hole is formed, a 0.18-mm enameled wire prepared beforehand is bent at a 90-degree angle at the tip to allow electrode insertion into the hole to a depth of approximately 4 mm. Solder is applied beforehand to the 2-mm section at the tip of the enameled wire.

The enameled wire must be fixed to the hole to prevent detachment during wing flapping. We applied small amounts of Aron Alpha® instant adhesive (Toagosei Co., Ltd.) for this purpose. (The team led by Prof. Ando used beeswax.) The instant adhesive has the advantage of solidifying together with the piriform scales surrounding the hole, which serve as auxiliary structures for fixation (Figure 1).
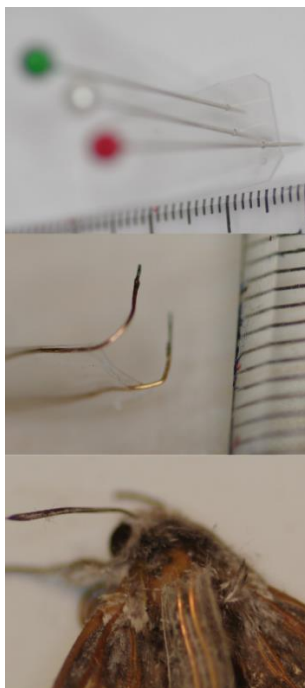

Fig. 1. Inserting and fixing electrodes for EMG.

2.4. Basic anatomy

The muscles involved in wing flapping are the dorsal longitudinal muscle (DLM) for the downstroke and the dorsal ventral muscle (DVM) for the upstroke. These two muscles are called power muscles, but indirect muscles. This is because the curved plate on the thoracic back is deformed and the wings are moved based on the lever principle.

The 3rd axillary muscle is responsible for wing retraction and the subtrochanteric muscle of the wing for abduction and downstroke, these two are called steering muscles, direct muscles those are linking with wings. In this study, we intended to measure two opposing muscles associated with the highest potentials (DLM and DVM) in Figure 2.
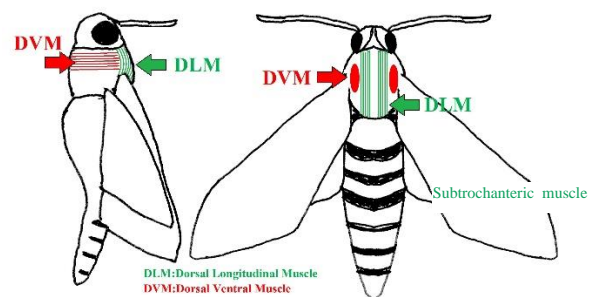

Fig. 2. Anatomical conceptual diagram of muscles involved in wing flapping.

2.5. Hardware

We recorded EMG and angular velocity (force) of wing flapping together with high-speed video of the moth suspended in air and constrained by string. Figure 3 presents a conceptual diagram of the experiment. The angular velocity sensor is grounded via the pin, which also serves as the fourth electrode (neutral point) of the EMG amplification circuit. The wiring to the angular velocity sensor consists of three 0.18-mm enameled wires, connected perpendicular to the pitch angle so as not to interfere with pitch angle measurements.
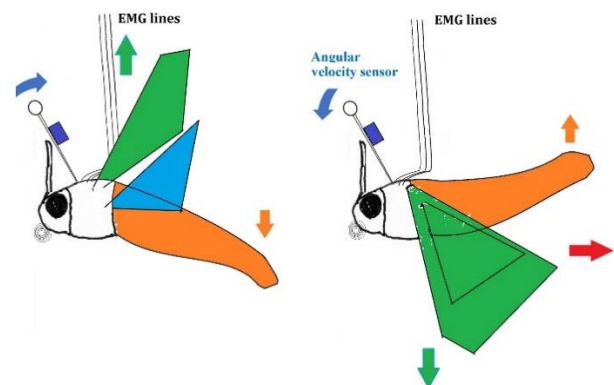

Fig. 3. Conceptual diagram of experiment.

The moth is suspended in air and constrained with an enameled wire. However, it can still flap its wings and the counteraction of the flapping motion will manifest as vertical vibrations of the moth's abdomen to retain balance. This movement can be measured on the thorax and can be recorded as pitch angle using the angular velocity sensor.
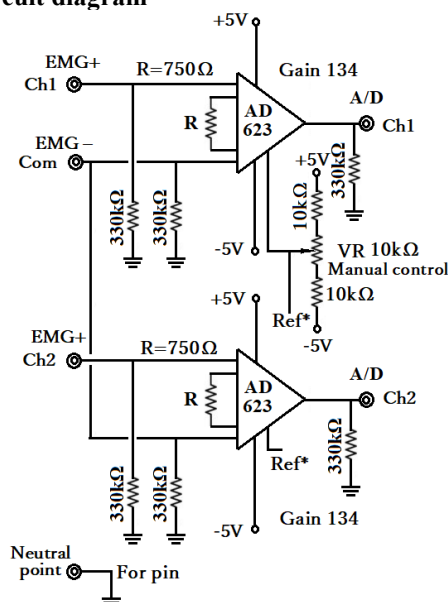
2.6. Circuit diagram

Fig. 4. Circuit diagram of EMG with two instrumentation operational amplifiers (OP AMP AD623) [16].

Since the neutral point is grounded, the zero potential of the operational amplifier is adjusted manually after inserting the electrode. Once this is adjusted, the output should remain within the range of −5 to +5 when the amplification factor (gain) is 134 and fall within the range of the A/D conversion element. Based on the frequency characteristics provided in the specifications for AD623, when the GB product is 800 kHz and gain is 134, the upper limit of the band frequency will be 70 kHz. Since 70 kHz is far higher than the sampling rate of 20 kHz of the A/D converter, using the AD623 should pose no issues for this amplification circuit with respect to circuit design.
An angular velocity sensor, which relies on the principle that a vibrating body with angular velocity will exhibit the Coriolis force, was used to measure pitch angle. This compact piezoelectric vibrating gyro sensor features a simple cap-base structure measuring approximately 0.1 cc in size and contains a ceramic bimorph vibrator. This gyro sensor was welded perpendicularly on the pin fixed to the moth. The pin doubled as a neutral point for the EMG amplification circuit. The LF411 operational amplifier was used with an amplification degree of 13 and zero bias being controlled using a variable resistor (Figure 5).
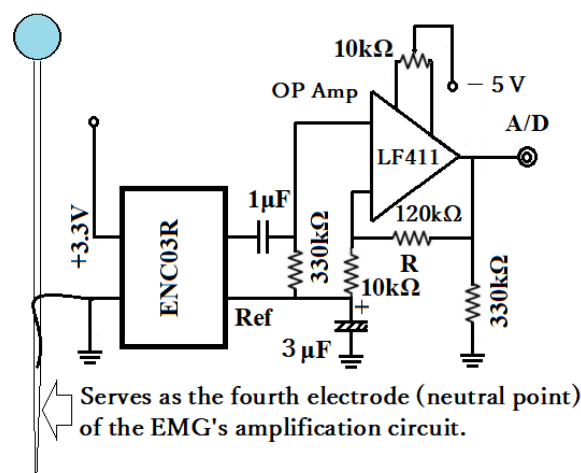

Fig. 5. Circuit diagram for angular velocity measurement using the Murata ENC03R [17].

In Figure 5, the pin inserted into the dorsal thorax is welded to the enameled wire and grounded and also serves as the fourth electrode of the EMG amplification circuit.

2.7. Analog-to-digital conversion

We recorded data to a PC using a 3-channel analog-to-digital converter with a USB connection at a sampling rate of 20,000/sec and 16-bit resolution potential variation (y axis). We used Microsoft Excel to analyze the linear data, using programs for calculating EMG amplitude and PP intervals created with VBA.

2.8. Results of experiment

The data files for wing flapping for 100 moths consisted of recordings lasting 49 seconds at a sampling rate of 20 kHz and 24 seconds at a sampling rate of 40 kHz. Since we processed the data in Excel, recording duration was restricted by the maximum number of rows permitted in an Excel sheet. The data obtained is explained below.
Figure 6 shows a characteristic 1-second section taken from 3-channel data recorded for 49 seconds.
The data presented in Figure 6 corresponds to a 1-second section illustrating the transition from a phase in which the moth slowly begins to flap its wings to the active flapping phase. As the amplitude of the pitch angle increases, a transition from a double-peaked waveform to a single-peak waveform occurs. The baselines (DC components) for both the EMG1 (for the dorsal longitudinal muscle) and the EMG2 (for the dorso-ventral muscle) switches to a decreasing trend at the transition. We believe this mode change in the DC component will be useful in assessing the relationship between the movement of the muscle as a whole and the depolarization of the inserted electrodes. The DC component cannot be obse

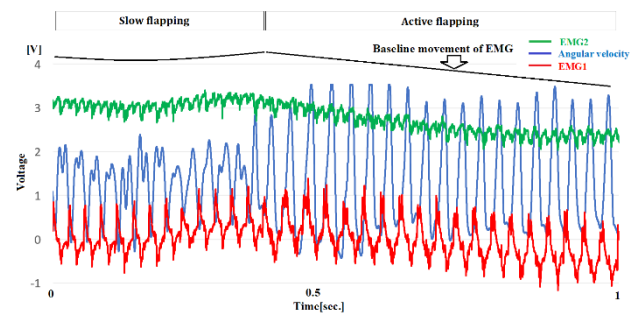rved with the AC amplification circuit due to capacitive co upling.

Fig. 6. Digital conversion performed at sampling rate of 20,000/sec and 16-bit potential variation (y axis) for 1 second.
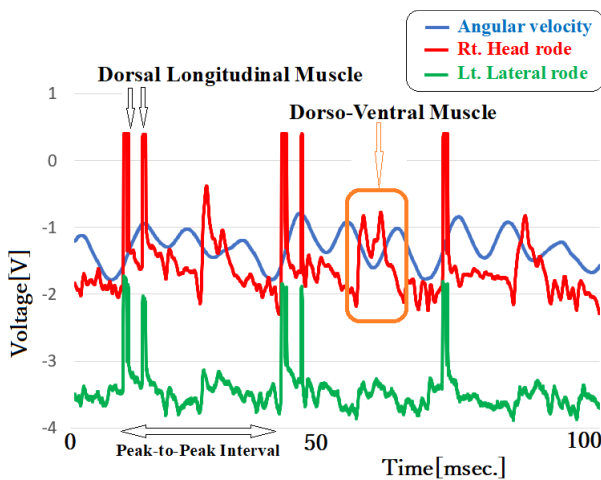

Fig. 7. Signals associated with DLM and DVM and PP intervals of EMG.

Figure 7 represents an enlarged 100-msec section of the plot above. We confirmed two peaks in the downstroke muscles, attributed to the difference in timing of the wingbeats for the left and right wings. The potential variation is most likely generated by the dorsal longitudinal muscle.

Between these peaks, there is a signal for the upstroke, which we believe is associated with the dorso-ventral muscle.

Figure 8 shows the data for 100 EMG amplitudes and the corresponding amplitudes of angular velocity. The correlation coefficient of the regression curve is R = 0.792, indicating generally high correlation (does not intentionally exclude outliers). This demonstrates that greater muscular strength correlates with greater angular velocity, suggesting that continuous EMG measurements can be used for physiological functional evaluations of moth flight.

Next, we analyzed frequency fluctuations. Figure 9 plots the data for 100 PP intervals and the amplitude of angular velocity for the corresponding intervals. The PP interval here is similar to the RR interval in ECG and represents the

correlation between angular velocity and the interval between wingbeats.
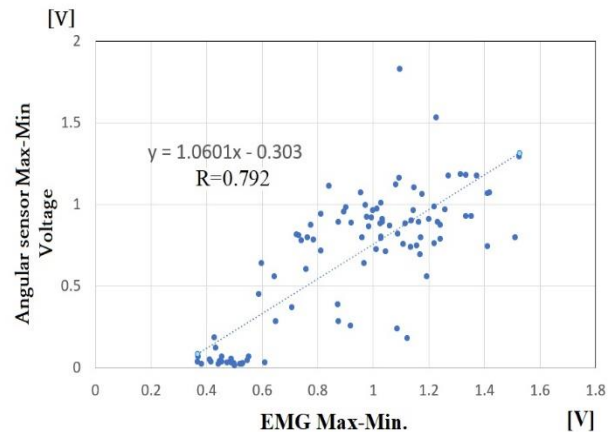

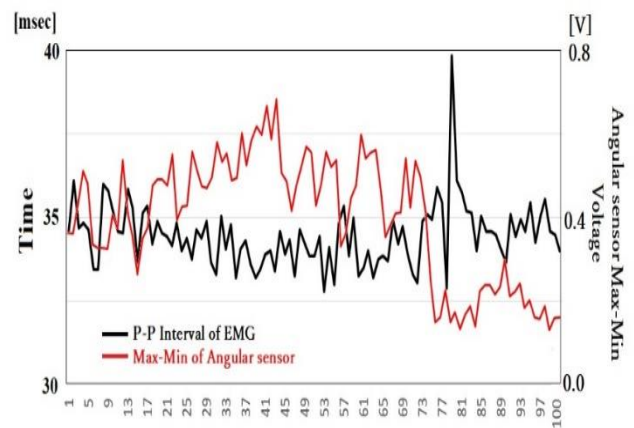Fig. 8. Correlation between EMG and angular velocity.

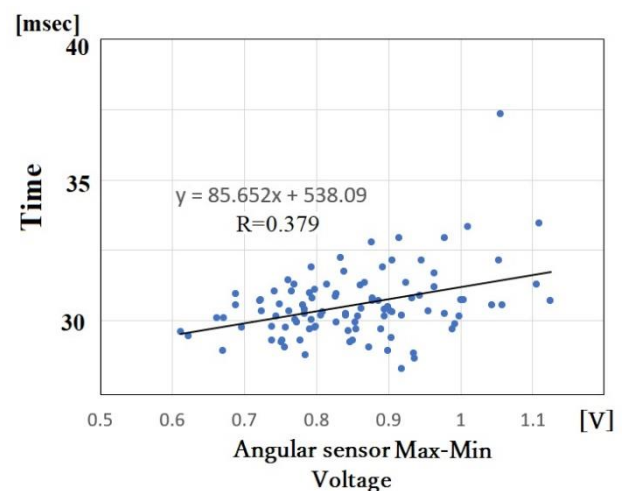Fig. 9. 100 EMG amplitudes and corresponding angular velocity amplitudes.

Fig. 10. Correlation between 100 PP intervals and angular velocity amplitudes.

The correlation coefficient for the correlation between the fluctuation for the 100 PP intervals and the amplitude of angular velocity is R = 0.379 shown in the Figure 10. The

accuracy of the regression curve is relatively poor (does not intentionally exclude outliers).

Although angular velocity is a factor that contributes to the fluctuation of the lift provided by wing flapping, our investigation showed a poor correlation between PP interval fluctuations of flapping motion and the fluctuation of the lift, suggesting that the former does not directly contribute to lift. This experiment failed to identify the physiological mechanism responsible for the PP interval fluctuations of flapping motion.

III. DISCUSSIONS

3.1. Physiological functional evaluations of insects

The experiment proved a correlation between angular velocity in the pitch direction of insects and EMG signals. Previous studies have analyzed insect flight using cameras and wind tunnels or EMG itself. However, these studies have been unable to establish a correlation between pitch angle and motor physiological data (corresponding to the EMG signal amplitude in this study). Our experiment corroborates this correlation and demonstrates the potential usefulness of EMG in functional evaluations of moths. Needless to say, physics supports the existence of a linear correlation between pitch angle and lift provided by wings. For agrochemicals, the exposure limits for chronic low-level exposure have been defined as 1/100 the LD50 value, the dose at which half of the exposed population dies in the case of acute exposure. No current standard for insects is based on physiological functional evaluations.

Even at doses far below 1/100 of the LD50 value, a decrease observed in EMG potential may indicate an onset of functional disorder. We believe this points to the potential for novel screening methods capable of evaluating the effects of repeated exposure to multiple agrochemicals. The application of such a method would not be limited to agrochemicals. It could also be used to assess exposure dose in radiation exposure incidents, such as the Fukushima-Daiichi nuclear power plant accident, based on functional evaluations of insects [18]-[24]. After the Fukushima-Daiichi nuclear power plant accident, professional journals reported numerous observations of anomalous white spots (depigmentation) on the wings of butterflies of the Lycaenidae family.

Government-specified exposure dose limits are calculated from the estimated total lifetime dose over 70 years and do not take into account functional evaluations of insects. As with LD50 values, calculations of these dose limits are not based on physiological evidence.

3.2. Δf fluctuations

We confirmed a correlation between EMG amplitude and amplitude of angular velocity. What factor accounts for the fluctuations observed in PP intervals, a frequency component? We believe the answer will prove crucial in producing a parameter for screening functional disorders caused by chronic low-level exposure.

In human ECGs, the RR interval reflects the status of autonomic nervous system regulation. Similarly, we believe that fluctuations in the PP interval for wing flapping of the moth may reflect the state of regulation. We believe chronic low-level exposure to agrochemicals may manifest as anomalies in Δf fluctuations.

In the present experiment, we constrained the moths to keep them from ascending or flying forward, a factor that may have inhibited their free flapping motions. Additionally, our focus was on the two large muscles (DLM and DVM). We may have failed to make proper assessments of the movement of the two other relatively minor muscles, which are known to be associated with hovering and rotation in flight. Thus, the cause of Δf fluctuations for these muscles may only be identified through monitoring of EMG during free flight.

In the next study, we expect to investigate the fluctuations of the two steering muscles (the 3rd axillary muscle and the subtrochanteric muscle) and fluctuations of PP-Interval, which are directly linked to the wings.

For subsequent experiments, we devised a transmitter using microchips and batteries for wrist watches (1.5 V) and carried out free-flight experiments after obtaining appropriate licenses for radio transmission from the Ministry of Internal Affairs and Communications. The experiment carried out by Prof. Ando's team, which preceded ours, achieved 2-channel transmission using an 80 MHz band FM radio transmitter [13]. We plan to pursue research on Δf fluctuations to analyze the role of the remaining two muscles as part of efforts to establish a screening method for chronic low-level exposure.

IV. CONCLUSIONS

Based on this study, we reached two conclusions (1 and 2 below). We also present two themes for future study, 3 and 4.

1. We observed a correlation coefficient (R = 0.792) between the lift provided by wing flapping (estimated from angular velocity) and EMG signal amplitude.

2. The correlation coefficient for the correlation between PP intervals and lift (estimated from angular velocity) provided by wing flapping was R = 0.379. Thus, based on

the results of the present processing method, we conclude no correlation between the two can be observed.

3. The flapping mechanism appears to incorporate a dynamic gear change between different modes of muscle employment. This suggests we should consider nonlinear analytical techniques, such as the analysis of whip motion using the Lagrangian equation of motion.

4. If the Δf fluctuation can be measured in addition to EMG amplitude, it should be possible to evaluate the status of moth flight using the two parameters of potential and frequency axes. Physiological evaluations based on amplitude and frequency fluctuations may lead to an indicator that potentially replaces the LD50 value used to assess agrochemical toxicity.

Acknowledgements

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imaging data formats. Her team has established the technology to streamline colorization within Pathology 3D, i.e., H&E, immuno-florescent, immuno-histochemistry, and florescence in situ hybridization imaging. Once the colorization is mapped, it can be correlated with another modality such as radiology, for a holistic analysis that will improve patient care and outcomes. The team also created 3D histology images of a single organ using thousands of slides.

Her works further enriches our knowledge of disease by integrating computational pathology data with other specimen-related data (genomics, proteomics, radiographic imaging, etc.). This brings an unprecedented breadth and depth of information to each individual case and yields a comprehensive, multidimensional analysis that would otherwise be impossible.

Authors

Isao Nakajima is a specially appointed professor of Seisa University and a visiting professor of Nakajima Labo. at the Dept. of Emergency Medicine and Critical Care, Tokai University School of Medicine. He got the Doctor of Applied Informatics (Ph.D.), Graduate School of Applied Informatics University of Hyogo 2009, and the Doctor of Medicine (Ph.D.), Post Graduate School of Medical Science Tokai University 1988, and the Medical Doctor (M.D.) from Tokai University School of Medicine 1980. He has been aiming to send huge multimedia data from moving ambulance via communications satellite to assist patient's critical condition. A board member of the Pacific Science Congress, a Rapporteur for eHealth of ITU-D SG2.

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Since joining MSK, she has led pioneering work using MicroCT, Whole Slide Imaging (WSI) and Confocal imaging to connect multi-dimensional and multi-modality images (e.g., single-cell to whole-body analysis). She participated in creating image viewers for several imaging modalities and established new