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Development of a Low-cost, Comprehensive Recording System for Circadian Rhythm Behavior

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Circadian rhythm is defined as a 24-hour biological oscillation, which persists even without any external cues but also can be re-entrained by various environmental cues. One of the widely accepted circadian rhythm behavioral experiment is measuring the wheel-running activity (WRA) of rodents. However, the price for commercially available WRA recording system is not easily affordable for researchers due to high-cost implementation of sensors for wheel rotation. Here, we developed a cost-effective and comprehensive system for circadian rhythm recording by measuring the house-keeping activities (HKA). We have monitored animal's HKA as electrical signal by simply connecting animal housing cage with a standard analog/digital converter: input to the metal lid and ground to the metal grid floor. We show that acquired electrical signals are combined activities of eating, drinking and natural locomotor behaviors which are well-known indicators of circadian rhythm. Post-processing of measured electrical signals enabled us to draw actogram, which verifies HKA to be reliable circadian rhythm indicator. To provide easy access of HKA recording system for researchers, we have developed user-friendly MATLAB-based software, Circa Analysis. This software provides functions for easy extraction of scalable "touch activity" from raw data files by automating seven steps of post-processing and drawing actograms with highly intuitive user-interface and various options. With our cost-effective HKA circadian rhythm recording system, we have estimated the cost of our system to be less than $150 per channel. We anticipate our system will benefit many researchers who would like to study circadian rhythm.

Key words: Circadian rhythm, Mice, Electrical Equipment and Supplies, Software

INTRODUCTION

Circadian rhythm is defined as a 24 hour biological rhythm, which persists even without any external cues but also can be re-

entrained by various environmental cues [1-3]. Accumulating lines of evidence indicate that this biological rhythm is not only important for periodic daily behaviors of organisms, but also critically linked to various forms of pathophysiological processes including metabolism, immunity, neurodegeneration and cognitive function [4-7]. As a result, there has been a growing need of interdisciplinary research on circadian rhythm and other research areas. However, researchers who are newly getting into the fields of circadian rhythm soon face high cost setup fees for the behavioral experiments. For this reason, reducing financial burden for re-

search equipment can be a good way to accelerate interdisciplinary research on circadian rhythm.

In the field of chronobiology, most circadian rhythm behavioral experiments have been based on animal's locomotor activity [8, 9]. For rodents, the most widely accepted method for measuring circadian locomotor activity is wheel-running activity (WRA) recording system. However, cost for commercially available WRA configurations is not easily affordable for many researchers since these instruments come at a cost of tens of thousands of U.S. dollars to hundreds of thousands of U.S. dollars. The factors for high price in commercial WRA configurations could be divided into three categories: 1) custom-developed animal housing environment and cages with running wheel, 2) activity counting/recording devices and monitoring system, 3) high cost commercial analysis software. Since these systems are usually provided as a whole package, there are not many options for consumers to reduce the price.

In addition to high cost, there are also some intrinsic disadvantages of WRA configuration of currently available circadian rhythm recording systems. One of the critical issues on WRA configuration is that the usage of wheel-running behavior itself can alter the animal's endogenous activity patterns, energy balance, food intake, motivation and reward systems, stress, anxiety, depression and even brain structures [10-15]. Moreover, when an animal fails to learn wheel-running behaviors or is unable to utilize wheels because of innate motor defects, WRA configuration cannot measure the animal's circadian rhythm. Therefore, measuring the endogenous circadian rhythms with natural locomotor activities, such as drinking/feeding behaviors and sleep/wake cycles would be much more suitable for monitoring animal's inherent circadian rhythm.

There have been several approaches proposed to monitor natural activities, including vibration detecting system [16-18], home cage system [16, 19, 20], beam break system [16], video monitoring system [16, 21], RFID recording system [16, 22], and piezo-sensor system [23]. While these systems were successful in terms of detecting natural animal behaviors, none of these systems seems to achieve noticeable cost reduction compared to WRA system. In terms of cost, rather simple wheel & wheel-counting sensors are generally cheaper than any of sensors used in the above system. Therefore, currently available systems including WRA system are not capable of recording natural activities at a reasonable cost.

To overcome the issues with currently available systems, we developed a novel circadian rhythm recording system as an alternative option. We provide a novel cost-effective solution for circadian rhythm recording system which monitors natural activities. By simply connecting a metal lid of animal housing cage to an input channel of a standard analog/digital (A/D) converter

by copper wire, we were able to record animal's movement when a mouse touches a metal lid while standing on a metal floor (ground), generating an electrical signal. This idea has been previously described as a low-cost solution to detect drinking behavior in mice with various additional advantages [24]. Since animal's food pellets and water bottle are generally placed above the metal lid of a cage, the recorded electrical signals from animal's "touching behavior" can reflect combined house-keeping activities (HKA) of eating, drinking and some of natural locomotor behavior such as grabbing the metal lid, which are all well-known indicators for circadian rhythm. To convert these electrical signals into scalable activity, we have developed MATLAB-based user-friendly software, Circa Analysis. This software is optimized for HKA recording system and can automatically draw actograms. In combination with a novel hardware configuration and optimized software, our system can achieve simultaneous recordings from 16 mice for duration of indefinite time. By eliminating the need for a running-wheel and commercial software, we were able to reduce the cost down to 1/10th of commercial systems.

MATERIALS AND METHODS

Animals

Adult male and female mice (>56 days old) from BALB/cByJ were housed in standard distributional type of housing cages in a temperature and humidity-controlled sound proof box on a 12:12h light-dark cycle for 1 week before the beginning of the experiment. The intensity of the LED light that mice experience at the bottom of the housing cage is adjusted to 300 lux. Humidity and temperature for animal maintaining chambers were kept between 40~60% and 25~26°C. All experimental procedures were conducted according to protocols approved by the directives of the Institutional Animal Care and Use Committee of the Institutional Animal Care and Use Committee (Approval number :201603025) of KIST (Seoul, Republic of Korea).

For animal housing floor material, metal grid floor was optimal for long term recording compared to flat metal floor, although both materials provided similar conduction, resistance, and accuracy of electrical signals. This is because mice feces can be easily accumulated in flat metal floor. Moreover, flat metal floor has been shown to hamper adequate footing of animals. Therefore, metal grid floor is suitable for long term recording, considering animal's welfare.

Hardware and software

For animal housing cages, we used metal grid floor type distributional mouse cage with metal lid (JEUNG DO Bio & Plant, Ko-
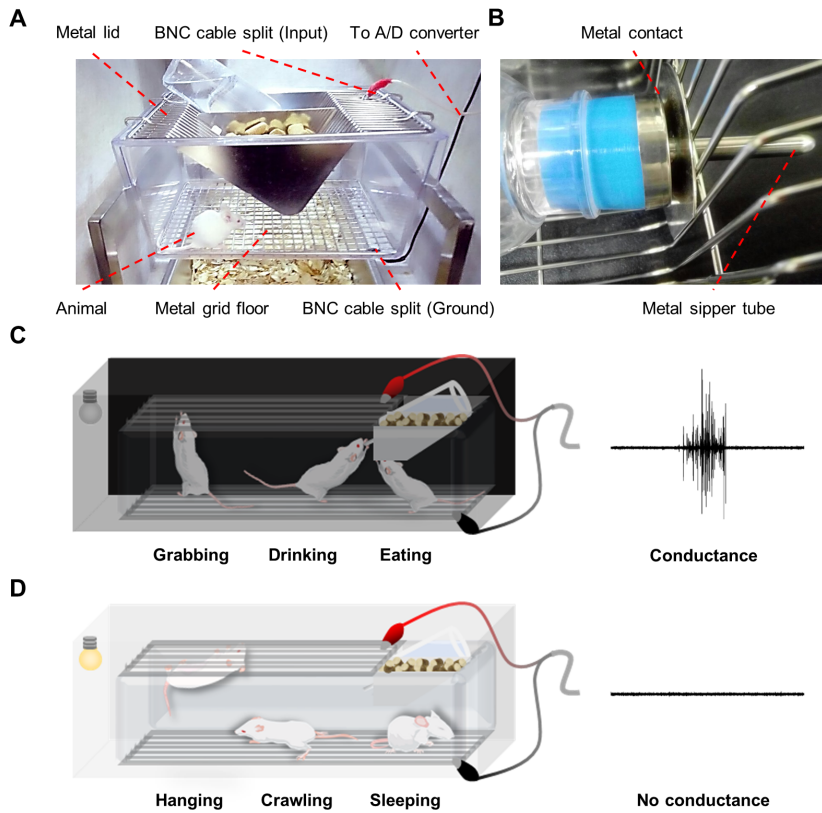
Fig. 1. Experimental design of HKA recording system. (A) Actual setup of house-keeping recording system with animal in distributional housing cage (left) and enlarged view of metal sipper tube in metal contact with metal lid (right). (B) Metal contact acts as a bridge to detect drinking behavior through the metal lid. (C) Type of characteristic behaviors which generate electrical conductance: grabbing, drinking and eating. (D) Type of characteristic behaviors which do not generate a conductance: hanging, crawling and sleeping.

rea) (Fig. 1A). Sound proof LED light-dark chamber was custom ordered from the company (JEUNG DO Bio & Plant, Korea). For standard A/D converter, we have utilized a Digidata 1440a (Molecular Device) for simultaneous 16 channel recording. The recording environment of computer was Intel G2020 2.90 GHz dual core processer with 2 GB RAM and 1.5 TB hard drive running on Windows 7 OS. Our Circa Analysis software was developed on MATLAB version 2017b. For recording software, Axoscope ver. 10.6 (freely available from Molecular Device) was used. With our HKA system, it was possible to record up to 16 channel simultaneously with A/D converter of Digidata 1440a model (Up to 8 channels with 1320a model). Circa analysis software provides up to 16 channel simultaneous analysis.

Formula used in data processing

$$Filtered\ Data = \text{HPF}[\ Raw\ Data\ ] \qquad \text{Formula 1}$$

$$Absolute\ Data_n = |\ Filtered\ Data_n\ | \qquad \text{Formula 2}$$

$$Reduced\ Data_n = \frac{1}{S_f}\sum_{k=S_f(n-1)+1}^{S_f n} Absolute\ Data_k \qquad \text{Formula 3}$$

$$\text{When,}\ h'(T_h) = \frac{h(X_{peak})-h(X_{end})}{X_{peak}-X_{end}} \qquad \text{Formula 4}$$

$$Bianary\ Data_n = \begin{cases} 1, & Reduced\ Data_n > T_h \\ 0, & Reduced\ Data_n \leq T_h \end{cases} \qquad \text{Formula 5}$$

$$Activity\ Counts_n = \frac{1}{B_i}\sum_{k=B_i(n-1)+1}^{B_i n} Bianary\ Data_k \qquad \text{Formula 6}$$

*HPF: High Pass Filter, Butterworth 5th order, 1 Hz cut-off

*Rosin's: Rosin's thresholding algorithm

*S_f: Sampling frequency

*T_h: Threshold

*B_i: Bin Interval

*$h(x)$: Histogram of samples, x: Amplitude

Digitizer speculation and recorded data size calculation

The A/D converter used in our HKA system has 16-bit resolution, 16 input channels, 1 to 250 kHz sampling rate range, -10.00 to +10.00 V of input dynamic range, 1MOhm of input resistance. Since the sampling frequency of our recording condition was 20-Hz. We recorded 16 channel simultaneously, therefore the precise calculation of the single data size is 52.7 MB.

16 (bit)×20 (Hz)×86,400 (second)×16 (channel)=442,368,000 (bit)

442,368,000 (bit)×0.125=55,296,000 (byte)

55,296,000 (byte)÷10^6=52.7 (MB)

RESULTS

The principles and experimental design of HKA recording system

A previous study has shown that simply connecting standard A/D converter with metal sipper tube of water bottle and metal grid floor can precisely detect animal's licking behavior [24]. In detail, a junction potential is generated whenever animal licks the tube and closes the electrical circuit for the duration of the tongue–sipper tube contact [24]. Junction potentials occur wherever dissimilar conductors are in contact. Based on this idea, we hypothesized that connecting the metal grid floor and the metal lid of the animal housing cage would generate electrical signals composed of any

lid-touching behaviors. To test this hypothesis, we have plugged one side of BNC cable with input of the standard A/D converter and another side with animal's housing cage, by connecting BNC core part at the metal lid and BNC shell part at the metal grid floor (Fig. 1A). Next, we placed laboratory mouse inside the cage and monitored animal's behavior and signal generation. The signals are generated when animals grab, eat and drink (Fig. 1C). Whenever animals are just crawling the floor, hanging on the cage ceiling, or sleeping, signals were not generated (Fig. 1D). For drinking behavior to generate electrical signals, the metal lid and the metal sipper tube must form metal contact (Fig. 1B). Note that the signals were generated only by direct physical contact between animal and metal materials, but not by the induction. This was confirmed by putting the thin plastic barrier between the cage between metal lid and cage body (data not shown). Taken together, the recorded
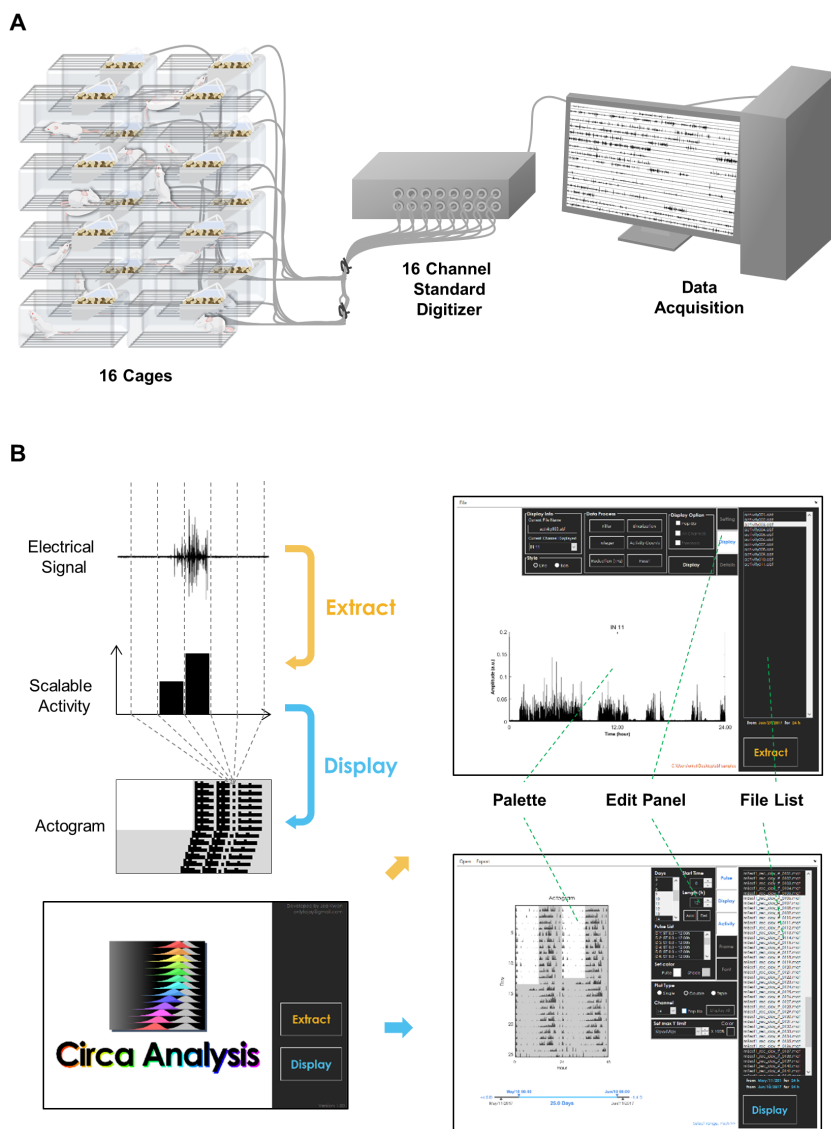

Fig. 2. Comprehensive circadian rhythm recording system with hardware and software. (A) 16 channel simultaneous HKA recording system using standard A/D converter. (B) MATLAB-based user-friendly software, Circa Analysis, optimized for circadian rhythm analysis of HKA. General process of Circa Analysis software (left top). The main window of Circa Analysis program (left bottom). Both Extract window and Display window has simple interface composed of palette, edit and file list (right).

signals reflect HKA of an animal which can serve as a marker for circadian rhythm.

Comprehensive circadian rhythm recording system

The currently available WRA-based circadian rhythm systems have cost issues and intrinsic limitations due to the dependence of a running-wheel. The cost issues arise from the need of purchasing additional sensors and recording equipment. Intrinsic limitation of WRA system is that wheel-running behavior itself alters the animal's activity patterns in some animals. To overcome these issues caused by wheel dependency, we have designed a HKA-based comprehensive circadian rhythm recording system with hardware and software (Fig. 2).

Circadian rhythm behavior experiments require relatively long period of recording time ranging from one month to a year. Thus, the stability of recording environment, temporal precision and convenience of management are important. In our HKA system, since the only required recording device is an A/D converter and itself serves as a sensor, signal quality and data acquisition stability were determined by A/D converter. For our HKA configuration, we utilized a standard A/D converter which was originally designed for electrophysiology (see details in Materials and Methods). This A/D converter, Digidata 1440a, can digitize 16 independent channels simultaneously, at sampling rates up to 250 kHz, and supported by AxoScope 10 software. AxoScope is a freely available, easy-to-use, full-featured data acquisition program for Windows. With these resources, we developed a HKA-based hardware configuration (Fig. 2A). We recorded signals in gap-free mode at the sampling frequency at 20 Hz, because this particular sampling frequency reliably detected "touching activities" while maintaining the reasonable size of the recorded files. Files were saved at 24 hour interval as ".abf (axon binary format)" file which is the default file format of Axoscope. For 16 channel recordings with duration of 24 hours, the size of files for 16 channels was 52.7 MB, indicating that at least 1.6 GB storage space is required for 30-day experiment.

To extract animal's touch activities from the raw electrical signals and visualize animal's circadian rhythmic activity patterns, we developed MATLAB-based user-friendly software, Circa Analysis (Fig. 2B). We also made a demonstration video to show step-by-step procedure of how to use the program (Supplementary Video 1). With "Extract" function, Circa Analysis converts raw signals to scalable "touch activities". For example, when an animal touches the metal lid three times (for 2 s, 25 s and 15 s) during 0.1 hour (=360 s) of time bin, the total duration of touch activities is 42 s per bin. With "Display" function, Circa Analysis displays converted scalable "touch activities" to draw actogram by aligning the daily activities

in a series (Fig. 2B). Taken together, with a unique combination of hardware and software based on HKA, we have developed a comprehensive circadian rhythm system.

Algorithms for converting electrical signals into scalable touch activity

To convert raw electrical signals into scalable touch activity, we applied various signal processing algorithms to the raw data. We recorded HKA with 20 Hz sampling frequency, and our raw data was masked by low frequency baseline noise (Fig. 3A, H, I) and this noise was completely removed when we applied Butterworth 1 Hz cut-off High Pass Filter (Fig. 3B and Formula 1). Although junction potential due to touch activity should be a positive value, our filtered data showed both positive and negative values. This is probably caused by HPF artifacts. To get rid of negative values, we have absolutized the signals (Fig. 3C and Formula 2). Next, we reduced the data size by averaging the number of samples at lower sampling frequency (Fig. 3D and Formula 3). For example, since our original sampling frequency was 20 Hz, averaging values of 20 consecutive samples made one data point per one second. This process simplified the handling of data. Next, to automatically discriminate the real touching signal from the baseline, we have applied a threshold based on Rosin's method [25] (Fig. 3G and Formula 4). Rosin's method is known as an effective auto-thresholding algorithm for unimodal histograms. It turned out that the amplitude frequency histogram of the reduced data showed a distribution of unimodal histogram, and therefore, optimal for applying Rosin's method. To explain briefly, the algorithm first draws a straight line from the peak to the end of the unimodal histogram. Second, the algorithm draws an orthogonal line from the peak-to-end line to each bin value of the histogram, until it finds the bin with a maximal distance from the peak-to-end line. When the distance is maximal, then Formula 4 becomes true. The threshold is determined as the amplitude value at this bin with maximal distance. Using this algorithm, we were able to search the optimal threshold automatically for each channel data. Using the threshold, we converted the reduced data into binary data, assigning the value of one of each data point if amplitude is greater than threshold, otherwise the value was set as zero (Fig. 3E and Formula 5). To quantify the duration of touch activities, we summated touch durations per bin interval (Fig. 3F and Formula6). In our analysis, we set the bin interval to 0.1 hour (=360 s). Thus the value of y axis represents total duration of touch activities per bin interval of 360 s. Taken together, with these six signal processing steps, we were able to automate the process of extracting numerical values for touch activities.
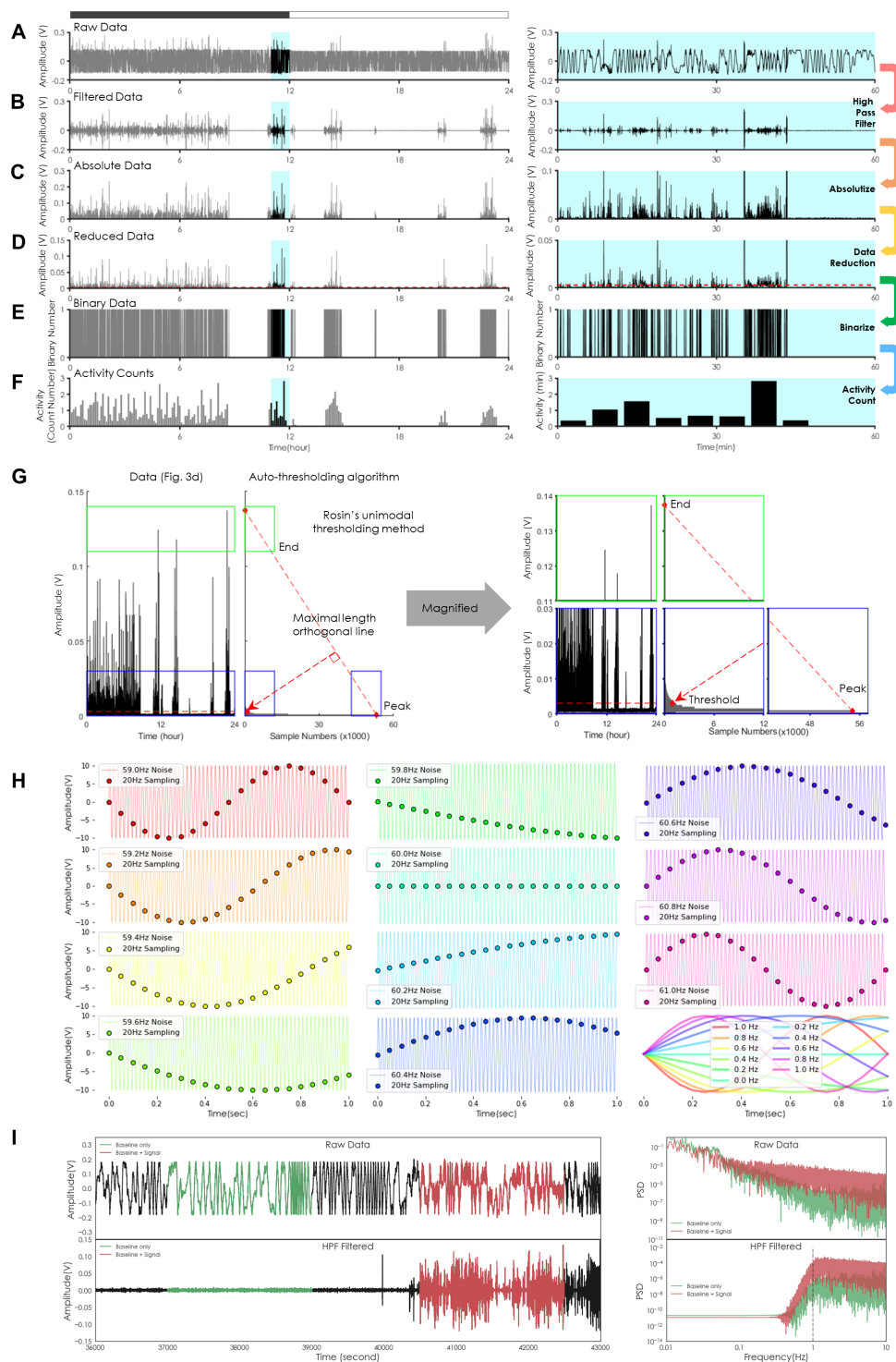
Fig. 3. Algorithm applied for converting electrical signals into scalable touch activity. (A~F) continuous HKA for 24 h (left) and magnified 1 h activity highlighted with sky blue background (right). (A) Raw data file containing electrical noise. (B) Filtered data file showing clear touching signal after 1 Hz cutoff high pass filtering. (C) Absolutized data for further analysis. (D) Data reduction. Red dotted line shows threshold selected by Rosin's unimodal thresholding method. (E) Binary data (contact as 1, no contact as 0 determined by threshold) of continuous HKA for 24 h (left) and magnified 1 h activity highlighted with sky blue background (right). (F) Activity counts of continuous HKA for 24 h (left) and magnified 1 h activity highlighted with sky blue background (right). (G) Rosin's unimodal thresholding method used for auto-threshold detecting algorithm. (H) 20 Hz sampling rate simulation during 60 Hz Powerline Noise drift (59~61 Hz). Low frequency signals ranging from 0 to 1 Hz generated by drift of 60 Hz powerline noise simulation (bottom right). (I) Raw data trace (left top) and 1 Hz cutoff HPF filtered trace (left bottom). Power spectrum density plots of raw data (right top) and HPF filtered data (right top). Green colored traces are baseline without HKA signals and Red colored traces are baseline with HKA signals.

The nature of HKA signals and recording condition

According to Nyquist-Shannon theorem, one's sampling rate should be twice greater than the target signal's bandwidth frequency ($f_S \geq 2f_B$). Therefore, to set the optimal range of recording sampling rate, it is crucial to understand the property of signals that we are handling. In our HKA system, we are recording the

animal's touching activity. Since HKA signals are generated by animal's touching or licking of metal materials, the expected HKA signal bandwidth would be determined by maximal frequency of those behavior. Previous studies have reported that animal's licking frequency range is 4~7.5 Hz [24, 26] and touching frequency range is 0.1~1 Hz [27]. Therefore, we expected the bandwidth of HKA
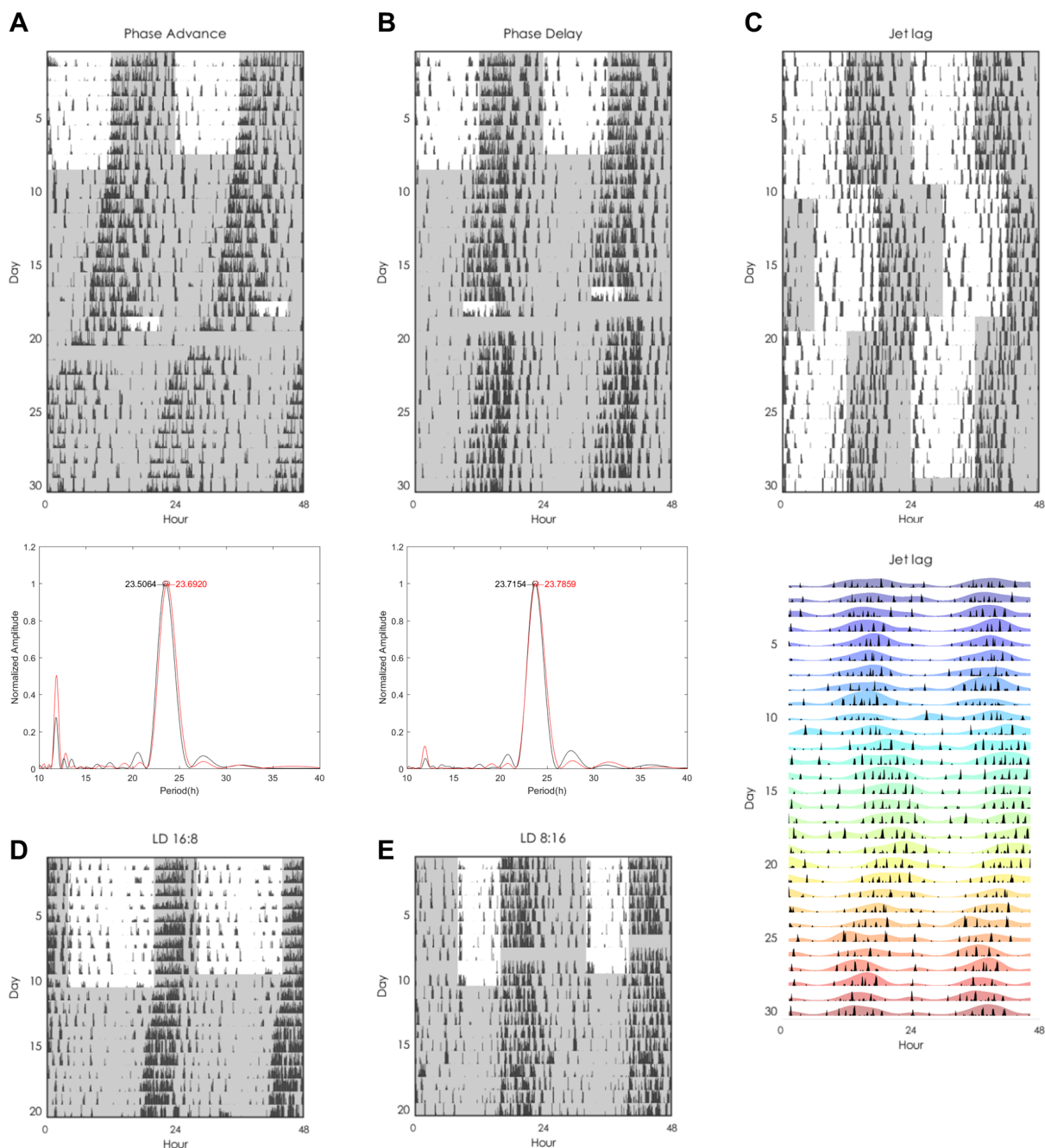

Fig. 4. Representative actograms applied with various circadian rhythm experiment protocols. (A) Actogram (top) and lomb-scargle periodogram (bottom) of phase advance protocol with 6 hour light pulse applied at ZT 15 to ZT 21. (B) Actogram (top) and lomb-scargle periodogram (bottom) of phase delay protocol with 6 hour light pulse applied at ZT 9 to ZT 15. (C) Actogram (top) and activity center plot (bottom) of jet-lag protocol by 6 hour delay/advance. (D) and (E) Actogram of seasonal variation protocol by LD 16:8 and LD 8:16.

signals to be less than 10 Hz and 20 Hz of sampling rate would be sufficient for detecting the HKA signals.

With 20 Hz sampling rate, the HKA signals were accurately detected along with animal's metal contact. However, as shown in the Fig. 3A and 3I, our signals of interest were contaminated with unidentified low frequency noise (<1 Hz). We found that the source of this noise was 60 Hz powerline interference signal which was aliased down to low frequency signal. The reason why 60 Hz signals were aliased down to under-1 Hz-signals was because our 20 Hz sampling rate is subnyquist sampling rate for 60 Hz signals. Since theoretically calculated aliasing frequency of 60 Hz signal sampled with 20 Hz is 0 Hz, we should not observe any baseline noise in recordings. However, 60 Hz powerline in real life has been shown to vary as much as ±1 Hz [28], and this drifts in 60 Hz frequency seem to be the major cause for our HKA system's baseline noise generation.

To confirm this idea, we simulated recording of drifting 60±1 Hz powerline interference with 20 Hz sampling rate (Fig. 3H). When 60 Hz signal was sampled with 20 Hz signal, the aliasing frequency was 0 Hz. Along with increase or decrease in 60 Hz powerline frequency, aliasing frequency increased exactly same with the step size. As long as drifts of 60 Hz signals are restricted to a range of ±1 Hz, our baseline noise frequency is less than 1 Hz. Based on simulation result, it is possible to remove low frequency baseline noise by 1 Hz HPF.

Before applying 1 Hz cutoff HPF to remove baseline noise, we had to confirm that whether our HKA signals would retain after filtering. By investigating the frequency domain and comparing "*Baseline only*" window and "*Baseline with Signal*" window of raw data traces, we noticed that both of the window shares similar and high amplitude in the frequency range of 0.01~0.1 Hz, whereas the range of 0.1~10 Hz showed marked difference in power level (Fig. 3I). This was consistent with the findings of simulation result that low frequency domain less than 1 Hz seem to be baseline noise. On the basis of our observations, we assumed that the frequency domains of 0.01~0.1 Hz as baseline noise window, 1~10 Hz as HKA signal window, and 0.1~1 Hz as mixed window. Therefore, the cutoff frequency for removing baseline noise, should be selected in the range of 0.1~1 Hz. In our recording condition, we selected 1 Hz as a cutoff frequency of HPF, and filtering with this condition showed clear HKA signals (Fig. 3I).

Although our post-processing method provide much simpler way to setting up HKA system, note that adding a low-pass filter with cutoff frequency less than 60 Hz before ADC could have removed powerline interference at the beginning.

Circadian rhythm behavior recording with various protocols

To verify that our system is working properly, we applied various protocols for circadian rhythm behaviors. First, we performed a phase advance experiment by applying 6 hour light pulse stimulation at Zeitgeber time (ZT) 15 of 11th day of constant dark period (Fig. 4A). Our HKA recording system successfully recorded mouse's phase advance (CT 3.1 h) of circadian rhythm without changing taus (before:23.5, after:23.69). Similarly, phase delay

Table 1. Comparison of required components between WRA and HKA configurations

Categories	Object	WRA configuration	HKA configuration
1) Animal housing	Housing cage	Running-wheel mountable tailored type	Distributional Type with grid metal floor 75.5$ / animal[A]
2) Physical parameter counting	Locomotion detector	Running-wheel	Not required
3) Transducer sensor	Locomotion detector	Wheel-counter	Not required
	Drinking detector (Optional)	Lick-sensor	Not required[B]
	Feeding detector (Optional)	Feeding-monitor	Not required[B]
4) Data converting	A/D converter	Tailored type of A/D converter compatible with wheel-counter	Any distributional[C] 1025$ / 16 animal[A]
5) Data processing	Software	Company provided	No cost (Shareware)

Comparison of essential components between WRA system and HKA system shows that WRA system requires running-wheel as a "2) Physical parameter counting" and wheel-counter as a "3) Transducer sensor", whereas HKA system do not require any of those. In terms of "1) Animal housing" and "4) Data converting", HKA configuration utilizes distributional types which are readily available, whereas WRA system requires tailored types which are optimized only for WRA system. For HKA system, we provide Circa Analyzer program for "5) Data processing" as shareware, whereas WRA system software is generally provided by company with a certain price.
[A]The real cost in our HKA system. Total cost for 16 channel recording was 2225.0$ (139$/animal); [B]It is also possible to measure feeding and licking exclusively by separating metal sipper, food container and metal cage ceil; [C]Note that it is possible to measure electrical signals for natural activities, but our current software is only compatible for specific models of A/D converters provided by specific company (Molecular Devices). This standard A/D converter (Digidata 1440A) we used here is secondhand product, and this model is not available anymore at official distributors since new versions are released.

experiment by light pulse stimulation at 10th day of constant dark period showed mouse's phase delay (CT 3.5 h) of circadian rhythm without changing taus (before:23.7, after:23.8) (Fig. 4B). Next, we tested a jet-lag protocol (Fig. 4C) and showed actogram and Gaussian-filtered activity center plot. During jet-lag delay and advance period, animal spent 5 days and 4 days each to adapt. Lastly, seasonal variation protocols with light hour:dark hour ratio of 16:8 (Fig. 4D) and light hour:dark hour ratio of 8:16 (Fig. 4E). All of these experiments were stably and reliably recorded with HKA system. In addition, we confirmed that at least it is possible to record simultaneously from 16 mice over 150 consecutive days (data not shown). This indicates that HKA system can provide highly stable recording system for circadian rhythm behaviors.

Cost-effectiveness and simplicity of HKA system

To assess the cost-effectiveness of HKA system, we compared required components for WRA system and HKA system. As shown in Table 1, one can save the cost required for physical parameter counter (running-wheel) and transducer sensor (wheel-counter) with HKA configuration. Moreover, since HKA configuration utilizes distributional types for housing cage and A/D converter which are readily available in markets, HKA system can also benefit from not using company-tailored types which are optimized only for specific WRA system. Lastly, since we have developed a software which is optimized for this system, we were able to save additional cost for analysis software which is generally provided by company with a certain price. Overall, due to its simplicity of hardware composition, HKA system can be a highly cost effective system for recording circadian rhythm behavior.

DISCUSSION

For several decades, WRA configuration has been the most widely used system for circadian rhythm behavior research. In terms of the equipment price, however, it has been a financial barrier for some researchers. In this study, we present a new circadian rhythm behavior recording system that is easier to operate and more cost-effective than the existing WRA configurations (Fig. 1, Table 1). We have shown the principles of HKA recording system and developed a user-friendly software, Circa Analysis (Fig. 2). Our software provides automatic conversion of electrical signals into scalable touch activities in 6 signal processing steps (Fig. 3). In combination with various light-dark protocols, we have confirmed that the HKA recording system is capable of drawing actograms (Fig. 4) which are similar to those of currently available WRA configurations.

One of the great feature of HKA system is that there is no re-quirement of specific sensor. Generally, acquisition of data in most systems is mediated by following steps: 1) physical parameter detection (ex. wheel-running), 2) transducer sensor (ex. wheel counter), 3) A/D converter, 4) computer. However, in HKA system, simple A/D converter directly detects animal's natural behavior without any additional devices. The simplicity of data acquisition process is where HKA system has huge benefits over other recording systems that uses specific sensors [16-23].

As a result, this simple hardware composition in HKA configuration brings forth appreciable cost benefits. Since A/D converter not only serves as analogue to digital converter, but also as transducer sensor in HKA system, the complete HKA system can be established at a very low cost which is A/D converter. In this system, the estimated cost for recording circadian rhythm was less than 150$ per animal. If an A/D converter is already available, HKA configuration can be implemented at virtually no cost. Although our current HKA system utilizes a specific model of A/D converter and a recording software provided by a specific company (Molecular device), this can be overcome by replacing the A/D converter with one that can write ".abf" format files. This will allow the A/D converter to be compatible with our software, Circa Analysis. Therefore, future direction of our HKA system is to find a cost-effective A/D converter and develop complementary recording software for software compatibility with Circa Analysis.

HKA recording system is versatile and flexible. One of the great benefits for experimenters is that the maintenance of HKA system is much more convenient compared to WRA configurations because there are no accessories to maintain. Moreover, animals do not require additional habituation period for running-wheel housing cage. Since our system is recording natural house-keeping behaviors such as eating, drinking, and grabbing, there is virtually no possibility of changing animal's biological features caused by strenuous activities such as wheel-running [10-15]. Furthermore, HKA configuration is functionally expandable. One can record each animal's grabbing, drinking, and eating behavior separately by isolating the metal lid, metal food container, and metal sipper of water bottle and connecting to separate A/D converter channel to each metal part. Considering the fact that multifunctional metabolic cages cost tens of thousands of U.S. dollars per cage, HKA configuration system could be a great replacement for cost-effective multi-functional HKA recording system.

One of the disadvantages of our HKA configuration is that it is difficult to use any kind of wire-connected head mounting devices, such as fiber optic cables for optogenetics, wires for single unit-recordings and EEG recordings, and plastic tubings for microdialysis. However, this disadvantage applies equally to the WRA configurations. Using wireless devices could be a simple solution

for this problem.

In conclusion, we propose our novel HKA-based circadian rhythm recording system as a low cost solution for circadian rhythm behavior experiment. Our HKA system reliably records circadian rhythm activities for a long duration without affecting animal's intrinsic properties.

ACKNOWLEDGEMENTS

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