論　文　内　容　要　旨

A novel microcontroller-based system for the wheel-running activity in mice

（マウス輪回し行動測定における新規マイクロコントローラシステムの開発）

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Introduction:

Voluntary wheel-running activity is widely used to assess circadian rhythm and motivation for exercise in the animal studies. Since those neural functions underlie neuropsychiatric disorders such as the sleep disorder, depression and stressor-related disorder, examining the wheel running activity in the animal models of disease would provide deep insight into understanding pathophysiology underlying them. However, long-term recording of the activity within additional animal facility space and associated costs could hamper its use depending on the scale of the study.

Equipment to measure wheel-running activity in mice is available commercially and mainly consists of a running wheel and a data acquisition system, which are placed inside and outside of the cage, respectively. Considering the high-density rack systems with smaller cages that house mice under specific pathogen-free conditions, equipment providing stand-alone operation and remote reporting of the acquired data online would be desirable for wheel-running activity analysis in mice.

To address this, I developed an open-source hardware system named Wheel-Running Activity acQuisition (WRAQ) based on a microcontroller recording mice's voluntary wheel-running activity in their home cage.

Materials and Methods:

WRAQ was built based on a low-profile running wheel. A microcontroller managed data acquisition with a microSD card writer, Adafruit Feather M0 Adalogger, connected to a binary counter. I extended WRAQ to WRAQ-WiFi, which enables online monitoring of ongoing wheel-running activity using a built-in FireBeetle ESP32 IoT microcontroller. ESP32 was set to upload the data to the Ambient IoT data visualization cloud service, enabling users to monitor the ongoing and collected data online. Data analysis can be done by ActogramJ free software which is based on ImageJ. In addition, the raw data of WRAQ-WiFi were converted into the readable file by ActogramJ by custom-made python program.

To apply WRAQ to a mouse model of endotoxemia, a single intraperitoneal injection of lipopolysaccharide (LPS) at a dose of 2.5 mg/kg was administered to the C57BL6 wildtype mice. I also generated male WT mice with injection of AAV8-hSyn-hM3D(Gq)-mCherry to the bilateral suprachiasmatic nucleus (SCN)-subparaventricular zone (SPZ) to activate SCN-SPZ specifically by intraperitoneal injection of clozapine-N-oxide (CNO, 1 mg/kg) during measurement of wheel-running activity. The effect of CNO on neuronal excitability was examined using immunohistochemistry for c-Fos.

Results and Discussion:

Consistent with the nocturnal behavior of mice, wheel-running activity exhibited an abrupt

increase and decreased following the onset and end of the dark period, respectively. Under a 12:12 h light/dark cycle (LD) cycle, the number of wheel revolutions followed a diurnal rhythm with exclusive activity during the dark period. Under altered schedules in light entrainment, particularly constant darkness illumination (DD), WRAQ recorded the free-running in circadian rhythm with a shortening of the period. By contrast, constant light illumination (LL) led to variable changes in circadian rhythm, ranging from free-running with an elongated period to an arrhythmic pattern. In the mouse endotoxemia mode, LPS administration significantly reduced and its gradual recovery of voluntary wheel-running activity supporting the applicability of WRAQ to mouse models of diseases requiring longitudinal observation of long-lasting behaviors in the home cage. I also examined the effect of genetic modulation of the neural pathway using WRAQ-WiFi using mice expressing excitatory chemogenetic probe (hM3D) in the SCN-SPZ. Upon CNO injection, I observed a significant increase of c-Fos-positive cells in the SCN-SPZ. Under the guidance of WRAQ-WiFi, injection of CNO at CT14 induced a significant shift in the onset of the active period lasting at least 7 days. These data reveals that online monitoring with the WRAQ-WiFi system enables studies requiring a temporally-specific genetic or pharmacological intervention.

Conclusion:

Together, these findings indicate that the WRAQ system is a novel and cost-effective solution for the analysis of wheel-running activity in mice. This approach may ultimately contribute to the real-time analysis of rodent behaviors during temporal genetic and pharmacological interventions.