Review

A Physiomics Approach to Reveal Systemic Organ Dynamics in a Rodent

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The central nervous system controls the activity states of the peripheral organs in response to various environmental changes. However, the physiological interactions across multiple organs remain largely unknown. Recently, we have developed an electrophysiological recording system that simultaneously captures neuronal population activity patterns in the brain, heartbeat signals, muscle contraction signals, respiratory signals, and vagus nerve action potentials in freely moving rodents. This paper summarizes several recent insights obtained from this recording system, including the observations that some but not all brain activity patterns are associated with peripheral organ activity in a behavioral test, and that functions across cortical networks can predict stress-induced changes in cardiac function in rats. The evidence suggests that adding information on peripheral physiological signals to behavioral data assists in a more accurate estimation of animals' mental states. The concept of such a research approach opens a new field of large-scale analysis of systemic physiological signals, termed "physiomics," which is expected to unveil further physiological issues involving mind–body associations in health and disease.

Key words local field potential; heart rate; behavior; central–peripheral association

1. INTRODUCTION

To understand the neuronal dynamics underlying psychiatric functions and their pathological changes in mental diseases, behavioral analyses using rodents are among the major experimental approaches. For example, widely utilized behavioral tests include an open field test and an elevated-plus maze (EPM) test to evaluate animals' anxiety-like behavior, and a forced swimming test to evaluate depression-like behavior. The fundamental premises for these behavioral tests are that: 1) a specific behavioral pattern reflects the mental state of animals under experimental conditions; and 2) behavioral patterns representing internal mental states emerge within a short time period (typically several minutes) during these behavioral tests. However, empirical evidence demonstrates that behavioral patterns differ considerably among individual animals and, furthermore, mental states are not always constant but vary across time and conditions even in a single animal. It is thus impossible to estimate animals' mental states accurately from a single behavioral test.

From a neurophysiology viewpoint, brain functions are achieved by neuronal spike patterns at a temporal scale of milliseconds, whereas the majority of behavioral tests are assessed at a range from seconds to minutes. To bridge such differences in the temporal resolution and to account for behavioral patterns caused by the spike dynamics of neurons, experimental approaches that analyze animals' mental states with higher temporal resolutions are required. To address these issues, we have recently developed a physiological recording system to monitor systemic bioelectrical signals from the brain and peripheral organs, which can be combined with

conventional behavioral assessments. This paper presents the methodological concepts of our recording technique and several recent insights obtained using this method, such as anxiety-related peripheral organ activity and stress-related responses to corticocardiac signals.

2. METHOD FOR SIMULTANEOUS RECORDING OF BIOELECTRICAL SIGNALS FROM SYSTEMIC ORGANS

A key experimental technique in neurophysiology research is the recording of extracellular local field potential (LFP) signals that represent spiking and collective oscillatory patterns of the nearby neuronal population.^{1,2)} Recently, this recording method has been greatly improved owing to the use of 3D printers, enabling us to deal with tens of electrodes in the brain of a freely moving animal.^{3–5)} The amplitude of LFP signals fluctuates at a range of approximately 1 mV, and the sampling rate of LFP signals is 10 kHz or greater. In the peripheral system, major electrical biosignals observed at the peripheral organs include heartbeat signals recorded as electrocardiograms (ECGs) and skeletal muscle signals recorded as electromyograms (EMGs). In conventional techniques, these different electrical signals are recorded utilizing independent recording devices; for example, LFP signals are captured and transferred to a multichannel recording device through a communication cable attached to an animal's head, whereas an ECG signal is recorded by a radiotelemetric transmitter implanted in the peritoneal cavity.^{6–8)} After utilizing those experimental techniques, we noted that the peripheral electrical signals have electrophysiological characteristics (e.g., amplitude ranges and temporal resolutions) similar to those of brain LFP signals. Based on this idea, we conceived a recording approach that simultaneously transfers all these bioelectri-

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cal signals to a single recording device (Fig. 1A).

To summarize the methodology briefly, ECG signals are recorded by positioning a pair of electrodes around the heart,^{6–8)} EMG signals are recorded by inserting electrodes into the dorsal neck muscle tissue, breathing (BR) signals are recorded by placing electrodes on the olfactory bulb, and vagus nerve (VN) signals are recorded by wrapping the VN fiber with a cuff-shaped electrode. All these electrical signals are integrated onto an electrical interface board mounted on the animal's head and collected by a multichannel recording device (Fig. 1A). Further details of surgical procedures and recording principles are described elsewhere.^{3,9,10)}

Figure 1B shows representative recorded signals and the corresponding information extracted from those signals. In brain LFP traces, power spectra with frequency bands from 0–100 Hz were constructed by wavelet analysis. In ECG traces, R-wave peaks (time of heartbeats) were detected and R-R (beat-to-beat) intervals were converted to instantaneous heart rates. In EMG traces, the magnitude of the signal relative to the average was computed as the root mean square (RMS). Such EMG signals are utilized to estimate the awake/sleep state^{11,12)} and fear behavior¹³⁾ of animals. In BR signals, power spectra were constructed using the same methodology as for LFP traces, and instantaneous BR rates were estimated based on the maximum power. BR signals enabled us to estimate roughly the relative changes in BR rates such as basal BR rates and transient sniffing behavior with approximately 90% accuracy. In VN signal traces, times of action potentials were extracted. Taken together, the new recording system enables precise analysis of multiple invisible physiological signals as vital signs representing internal mental states in freely moving animals.

3. PHYSIOLOGICAL RESPONSES OF PERIPHERAL ORGANS IN THE ELEVATED-PLUS MAZE (EPM) TEST

In this and the next sections, we give examples of the application of our recording system to the EPM test. In this maze, mice were freely allowed to enter two open and two closed elevated arms (Fig. 2A). On the basis of rodents' habit of avoiding open environments, anxiety-related behavior is quantified by the time spent in the open arms. While the EPM test is a widely utilized behavioral test in neuroscience research,^{14–16)} how physiological activity in the whole body varies during it remains largely unknown. We addressed this

issue using our systemic recording method.¹⁷⁾

On average, mouse heart rates, the magnitudes of muscle contractions, and BR rates in the closed arms were lower than those in the open arms, which is consistent with the general assumption that animals' mental states are more settled in the closed arms. In addition to the averaged characteristics, mice in the closed arms occasionally showed markedly low heart and respiratory rates lasting for several seconds or minutes, whereas in the other time periods, they exhibited higher peripheral activity levels similar to those observed in the open arms. We classified such lowered activity patterns as low-peripheral (LP) activity states of peripheral signals (Figs. 2A, 2B). The emergence of LP states demonstrates that peripheral activity is not always constant even when animals are located in the same behavioral contexts. Notably, LP states more frequently appeared when the animals were located in the distal part of the closed arms. This suggests that EPM tests might be more accurately evaluated when the distance from the center of an EPM, and not only in which arms the animal is located, is taken into consideration. Power spectrum analysis of brain LFP signals demonstrated that these peripheral activity changes were associated with brain activity patterns. For example, during the emergence of LP activity states, the delta and theta powers of cortical LFP signals showed prominent increases and decreases, respectively, demonstrating that cortical activity patterns depend on whether an animal exhibits LP states rather than on where animals are located. Taken together, the results of this representative study suggest that behavioral data might be improved by combining behavioral patterns with physiological signals to evaluate animals' mental states more accurately.

4. VN ACTIVITY IN FREELY MOVING ANIMALS

Peripheral organ activity is mainly controlled by the autonomic nervous system, particularly the VN. Despite the functional importance of the VN, its detailed neurophysiological dynamics remain to be clarified fully. Although VN recordings were performed in early studies,^{18–21)} they were restricted to anesthetized animals, and, to the best of our knowledge, recordings from freely moving animals have not been reported due to technical limitations. We therefore developed a new recording method that can monitor the action potentials of the VN using a cuff-shaped electrode. As shown in Fig. 1, this VN recording configuration could also be integrated into our

Biography

Takuya Sasaki graduated with a Bachelor's degree from Tohoku University under the supervision of Prof. Kohji Fukunaga and completed his Ph.D. in Pharmaceutical Sciences under the supervision of Prof. Norio Matsuki at The University of Tokyo. He then worked in the laboratories of Prof. Juichi Nakai at Saitama University in 2012 and of Prof. Ryuichi Shigemoto at the National Institutes for Physiological Sciences in 2013 as a Japan Society for the Promotion of Science (JSPS) postdoctoral researcher, and in Prof. Jill Leutgeb's laboratory at the University of California, San Diego, as a JSPS postdoctoral fellow for research abroad in 2013. In 2014, he returned to the Laboratory of Chemical Pharmacology, The University of Tokyo Graduate School of Pharmaceutical Sciences, and started working under Prof. Yuji Ikegaya on neuronal circuit dynamics in the cortex and central-peripheral interactions mainly using in vivo electrophysiological techniques. In particular, the development of simultaneous recordings of bioelectric signals from the brain and peripheral organs has enabled Dr. Sasaki to address a number of research issues related to mind–body communication in health and disease.

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Fig. 1. Recordings of Systemic Bioelectrical Signals in a Freely Moving Rat

(A) Recording scheme (left) and picture (right). All bioelectrical signals (local field potential [LFP], electrocardiograms [ECGs], electromyograms [EMGs], breathing [BR], and vagus nerve [VN] signals) are collected in the device mounted on the animal's head. (B) Representative recording data simultaneously recorded from a freely moving rat. Top to bottom: Cortical LFP trace and corresponding color-coded power spectrum (black, lower power; white, higher power). ECG trace and corresponding instantaneous heart rate changes. EMG trace and corresponding root mean square (RMS) values to define arousal states. Transient movement evokes large deflection of the signal. BR trace and corresponding respiratory rate. VN signal trace and corresponding instantaneous VN spike rates.

systemic recording system.²²⁾

An example of a VN recording from a EPM test is shown in Fig. 2A, revealing that: 1) VN spikes were not perfectly associated with the location of animals in the maze; and 2) the frequency of VN spikes was higher immediately after animals were placed in the EPM (initial phase) (Fig. 2C). When the animals became familiar with the maze conditions (final phase), VN spikes decreased significantly, especially in the closed arms. While the time spent in the open and closed arms in an EPM is assumed to represent the animals' anxiety levels, our results suggest that VN spikes may not be simply explained by the level of anxiety. Rather, they are associated with novel conditions in which an animal's locomotor activity

is generally higher than that under familiar conditions. We are now addressing the detailed mechanisms underlying this relationship between VN spikes and behavior.

5. STRESS-INDUCED RESPONSES IN THE BRAIN AND PERIPHERAL ORGANS

This section summarizes our recent study of acute stress-induced systemic physiological responses related to cortical LFP signals (Fig. 3A). LFP signals from six cortical regions, ECG signals, and EMG signals were each recorded from a number of rats subjected to acute social defeat stress from an aggressor rat. Notably, stress-induced heart rate changes dif-

Fig. 2. Peripheral Organ Activity in an Elevated-Plus Maze (EPM) Test

(A) Left, schematic illustration of the EPM. Right, changes in the instantaneous heart rate and VN spike rate. Upper bars indicate the maze arms in which an animal remained. Low-peripheral (LP) states of peripheral signals are represented by gray areas. Typical recording periods in the initial and final phases are shown. (B) Probability of observing LP states in an EPM. (C) Distribution of VN spike rates in the initial (0–150 s) and final (450–600 s) phases of the EPM test, showing that VN spikes in closed arms are markedly decreased in the final phase (gray) compared with the initial phase (black).

Fig. 3. Stress-Induced Corticocardiac Interactions

(A) Schematic illustration. Correlational activity patterns across multiple brain regions before the application of social defeat stress predicted whether animals would become stress susceptible or resilient. (B) Typical changes in heart rate variability in response to social defeat stress loads in stress-susceptible and -resilient animals. (C) Left, locations of cortical areas in which LFP signals were recorded. PL, prelimbic cortex; S1, primary somatosensory cortex; PPC, posterior parietal cortex; HPC, hippocampus; RSC, retrosplenial cortex; V1, primary visual cortex. Right, average LFP delta power correlations computed between individual pairs of cortical areas. Thick lines represent higher correlations.

ferred considerably among individual animals; some defeated animals exhibited unstable cardiac cycles, including arrhythmia and increased heart rate variability, after a stress load (Fig. 3B). Based on the irregularity of stress-induced heartbeat signals, individual rats were divided into stress-susceptible and -resilient groups. We then analyzed whether multidimensional cortical LFP signals could explain the differences in stress susceptibility.

Our analyses using principal component analysis and the support vector machine revealed that animals with higher correlational changes in LFP delta power across multiple cortical regions were more likely to be stress susceptible after experiencing defeat stress (Fig. 3C). Conversely, rats with higher theta power correlations showed stress resilience. In previous studies, stress susceptibility was evaluated based solely on changes in behavioral patterns such as decreased social interactions and increased anxiety-like behavior.^{23–27} In addition to those previous studies, our finding demonstrates

that animals subjected to social defeat stress exhibit heterogeneous responses in cardiac physiological signals, and that individual differences in cortical activity may be a mechanism that causes abnormal activity of peripheral organs in response to mental stress episodes.

6. PERSPECTIVES FOR FUTURE PHARMACOLOGY

In traditional pharmacology textbooks, drug effects are grouped into subdivisions describing the effects on a single organ or function, such as the brain and cardiac, respiratory, and digestive systems. However, all organs are intimately connected with each other, suggesting the need to study how disease states and therapeutic drugs affect interactions across multiple organs from the systemic viewpoint. To address this issue, our research approach is applicable to a number of biological issues related to mind–body communication.

Specifically, our results suggested that monitoring periph-

eral physiological signals such as heartbeat signals and VN spikes is crucial to an accurate understanding of the mental state of animals in addition to their simple behavioral patterns. Moreover, they highlight the importance of an “omics” approach, where large-scale physiological data from both the central and peripheral organs are combined. In addition to a growing body of recent omics studies such as genomics, proteomics, and metabolomics, our approach of “physiolomics” will help deepen our understanding of brain-body associations on a macroscopic time scale, including development, aging, and progression of disease.

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Conflict of Interest The author declares no conflict of interest.

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