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 “physiolomics,” 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-
eral 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, 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.

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 and fear behavior 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 of avoiding open environments, anxiety-related behavior is quantified by the time spent in the open arms. 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 and fear behavior 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.

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, 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 system.
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-
fered 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 solely on changes in behavioral patterns such as decreased social interactions and increased anxiety-like behavior. 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 “physiomics” 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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