A low-noise flexible integrated system for recording and analysis of multiple electrical signals during sleep–wake states in rats

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Abstract

A low-noise flexible system for the simultaneous recording and analysis of several electrical signals (EEG, ECG, EMG, and diaphragm EMG) from the same rat was constructed for studying changes in physiological functions during the sleep–wake cycle. The hardware in the system includes a multichannel amplifier, a video camera, a timer code generator, and a PC. A miniature buffer headstage with high-input impedance connected to a 6-channel amplifier was developed. All electrical activities devoid of 60 Hz interference could be consistently recorded by our low-cost amplifier with no shielding treatment. The analytical software was developed in the LabVIEW environment and consisted of three major frames: temporal, spectral, and nonlinear analyses. These analytical tools demonstrated several distinct utilities. For example, the sleep–wake states could be successfully distinguished by combining temporal and spectral analyses. An obvious theta rhythm during rapid-eye-movement sleep (REMS) was recorded from parietal to occipital cortical areas but not from the frontal area. In addition, two types of sleep apnea with/without cardiac arrhythmias were observed under REMS condition. Moreover, the evoked potentials of the primary somatosensory cortex elicited by innocuous electrical pulses were modulated by vigilant states, especially under a slow-wave sleep state. These results show that our system delivers high-quality signals and is suitable for sleep investigations. The system can be easily expanded by combining other recording devices, like a plethysmograph. This compact system can also be easily modified and applied to other related physiological or pharmacological studies. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Sleep can be simply classified into two categories based on the characteristics of cortical activity (EEG) and dorsal neck muscle activity (EMG): slow-wave sleep (SWS) with a high-amplitude and low-frequency synchronous EEG as well as decreased nuchal EMG, and rapid-eye-movement sleep (REMS) with low-amplitude and high-frequency desynchronous EEG accompanied by muscle atonia (Steriade and McCarley, 1990; Gotte mann, 1992). Using both EEG/evoked potentials (EPs) in various brain sites and electromyogram (EMG) is satisfactory for investigating sensory information processing under different vigilant states (Arezzo et al., 1981; Cauller and Kulics, 1988; Meeren et al., 2001; Shaw et al., 2001). In studies of sleep disorders, utilizing both electrocardiogram (ECG) and respiratory activity is also necessary for monitoring physiological functional changes (Guilleminault et al., 1983; Sato et al., 1990; Roth and Roehrs, 2000). Most systems, such as, Grass model 79, used to investigate sleep-related issues are expensive, massive, and of low flexibility. In this study, we introduced a low-noise, low-cost, miniature integrated multichannel system to simultaneously record several electrophysiological activities in rats. The utility of this system is illustrated with the successful recording of characteristic waveforms without 60 Hz interference during the slow–wake cycle. Two types of central sleep apneas are also depicted herein. Moreover, it was
observed that the processing of somatic sensory information under various vigilant states markedly differed.

2. Materials and methods

2.1. Fabrication of recording electrodes

Two types of electrodes were used in this study. One was a stainless steel screw (outer diameter, O.D. 1 mm) for recording cortical activity, the other was a teflon-coated 7-strand stainless steel microwire (O.D. 0.23 mm; A-M Systems, #7935) for recording other electrophysiological activities. In an attempt to reduce the restriction on animal activities, one end of a flexible miniature socket was connected to a rat, and the other end soldered with wires and microwires was connected to an amplifier (Fig. 1). The microwires in the socket were used to directly record the activity of the heart, nuchal muscle, and diaphragm muscle via appropriate placement/processing (described below). To increase the flexibility of the microwires placed under the skin of a freely moving rat, straight microwires for recording cardiac and diaphragm muscle activities were alternated with spiral configurations (Fig. 1A). Wires soldered in the socket were used to attach to the screw electrodes on the skull to record brain activity. Finally, the soldered part of the socket was cemented with dental acrylic to avoid deformation before implantation.

2.2. Animal preparation and electrode implantation

Adult Long–Evans (hooded) rats were used in the study. Animals were raised in a sound-attenuated room with a 12:12 h light–dark cycle (07:00–19:00 lights on) as well as appropriate temperature (22 ± 2 °C) and humidity control (40–70%). At the time of surgery, body weights of the rats were between 240 and 300 g. The recording electrodes were implanted under pentobarbital anesthesia (50 mg/kg, i.p.). Ketamine was administered as needed to maintain proper anesthetic depth during surgery. After anesthesia, the rat was placed in a standard stereotaxic apparatus. The dorsal surface of the skull was exposed and cleaned. Six stainless steel screws were driven bilaterally into the skull overlying the frontal (A −2.0, L 2.0), parietal (A +2.0, L 2.0), and occipital (A +6.0, L 2.0) regions of the cortex. The parietal lead was placed over the representative area of the rat’s tail (Chapin and Lin, 1990; Shaw et al., 1999a). Each wire of the miniature socket was wrapped around an individual screw. The mean impedance of these skull electrodes was 6 kΩ. A reference electrode was implanted 2 mm caudal to the lambda. Care was taken to prevent electrodes from penetrating the underlying dura. To assure proper functioning of the electrodes, EPs of the parietal lead were elicited by stimulating the rat’s tail using 2-ms duration constant current pulses (Grass S48). Only animals with identifiable positive short-latency EPs were used in the study. In contrast to the monopolar recording of cortical activity, other electrical activities were recorded using a bipolar configuration. Two 7-strand stainless steel microwires were bilaterally inserted into the dorsal neck muscles to record EMG. The ECG was recorded via a pair of microwires placed under the skin of the dorsal part of the body (one was between the cervical and thoracic levels, the other at the lumbar level). To record the EMG of the diaphragm (DEMG), an incision parallel to the last rib on the right side was made and a pair of teflon-coated 7-strand stainless steel microwires was inserted into the crural part of the diaphragm. Appropriate adjustment of the recording

Fig. 1. System setup. (A) Connection module and electrodes. Stainless steel screws were used as skull electrodes. Wires in the socket were individually wrapped to screw electrodes to record the EEG. Microwires in the socket were used to monitor the nuchal EMG, ECG, and diaphragm EMG. A socket-matched miniature headstage with FETs is shown in the right part. (B) Recording apparatus. A 6-channel amplifier was placed over the transparent recording chamber, which allowed us to observe the rat’s behavior. A grounded metal plate was placed under the recording chamber.


microwires achieved a better signal-to-noise ratio, which was visualized by on-line monitoring software. Following suturing to complete the surgery, dental cement was applied to fasten the connection socket to the surface of the skull. During dental cement application, continuous monitoring of the cortical activity was necessary to ascertain a patent contact between the skull electrodes and the wrapped wires. Finally, animals were given antibiotics (Combion-S) and housed individually in cages for recovery.

One week after surgery, animals were placed individually in clear acrylic chambers, so that their behaviors could be easily observed (Fig. 1B). A narrow opening at the top of the chamber permitted unimpeded feeding of the head plug/cable assembly to the skull implant. To allow rats to habituate to the experimental apparatus, each animal was placed in the recording environment at least 2 times (1 h/day) prior to testing. On the day of the recording, a 30 min period was allowed for the rat to become familiar with the chamber. The entire experiment was performed in a sound-attenuated room. The experiment was performed in the 6 h period per day (12:00–18:00). All surgical and experimental procedures were carried out by recommended procedures approved by the Institutional Animal Care and Use Committee of Tzu Chi University.

2.3. Multichannel integrated system

The entire system consists of three major components: a multichannel amplifier for amplification of electrophysiological signals, analytic programs to extract the characteristics of each activity, and a behavior tracking system to record behavioral changes (Fig. 2). A timer code generator added a timer stamp onto the video signal. The timer and data acquisition card are initiated by a synchronized device. Video frames were saved on tape for further analysis. Analysis and video-related devices were kept away from the amplifier to reduce possible external electromagnetic interference. Other specifications of the multichannel amplifier and the hardware and software of the computer are described below.

2.3.1. A multichannel amplifier for biopotentials

To minimize the size and cost of the recording apparatus, we developed a multichannel amplification system for the simultaneous recording of 3 EEGs, 1 ECG, and 2 EMGs. An appropriate length (30–50 cm) of recording wire between the animal and the amplifier was needed to reduce the interference generated by animal movement. Two kinds of recording configurations were used: monopolar recording for cortical activity and bipolar recording for the ECG and EMGs. A detailed circuit diagram of the amplification system for biopotential recording combined with the field-effect transistor (FET) headstage is depicted in Fig. 3. In this study, buffered signals from FETs were fed into two operational amplifiers (LF411, National Semiconductor, Santa Clara, CA) for appropriate amplification and bandpass filtering to extract the signal frequency bandwidth of interest. For bipolar recording, an instrumentation amplifier (AD620AN; Analog Device, PMI division) was used for its high CMRR (100 dB) and low-noise voltage density (9 nV/√Hz) to extract a differential signal between two leads. All biopotentials were amplified 10,000-fold, but with different selections of filter bandwidths. The EEG was filtered with 0.3–70 Hz, the ECG with 10–100 Hz, and the nuchal EMG and DEMG with 100–500 Hz. Moreover, the evoked response was filtered with 0.3–1000 Hz. Although these parameters were fixed in our design, these values were deemed satisfactory for the demands of each electric activity after several adjustments. These specifications can be obtained by arranging resistors and capacitors as shown in Fig. 3. Precise resistors (1% error) and multilayer ceramic capacitors were used. Each passive element was carefully fine-tuned using a multimeter to reach the specification of the multichannel amplification system. Finally, the entire circuit was laid out on a single-side printed circuit board then packaged in a $22 \times 4 \times 15 \text{ cm}^3$ metal cage.

Several considerations are helpful for obtaining a low-noise, high-quality recording. To reduce the distortion of biopotentials, a preamplifier with high-input impe-
dance is necessary. In addition, decreasing the impedance of each lead allows the reduction of contamination by external interference (such as 60 Hz). Therefore, nine FETs (MMBF5484, Motorola Semiconductor, USA), which possess high-input impedance ($\approx 10^{12} \Omega$) and light weight, were used here with a common-source configuration. All FETs were fixed on the female connection module (Fig. 1A), which reduces the impedance of each lead, thereby decreasing external interference. In addition, a grounded metal plate was placed under the recording chamber to increase the capacitance between the animal and the ground, which also reduces the interference from external electromagnetic sources (Shaw, 2001). In the implementation of this custom-made amplifier, an accurate layout and proper grounding were also found to be important for noise reduction. Moreover, the power input of the operational amplifier was bypassed with a capacitor to reduce the ripple, especially in the front-end stage. This was also useful for reducing 60 Hz interference.

Removing all AC power sources from the recording chamber was also helpful in reducing the 60 Hz interference. The DC power source of the amplification system was constructed using general regulators LM7808 and LM7908 (National Semiconductor) with large capacitors, and it was located in a separate enclosure. The distance between the multichannel amplifier and all power sources was greater than 150 cm (Fig. 2). Having taken all the above precautions, a shielded environment or shielded cable was not necessary for the experiment (Section 3).

2.3.2. Hardware and software specifications

A personal computer (Pentium III 550) with 256MB of RAM and 40GB of hard disk capacity was equipped with a general-purpose data acquisition card (PCI6023E, National Instruments, Austin, TX) under a Microsoft Win2000 environment. The PCI6023E card has a fast (up to 200 kHz sampling rate) 12-bit analog-to-digital converter with 16-channel single-ended analogue input channels. This set-up met our specifications and was sufficient for multichannel recording and analysis. The system was programmed in an icon-based LabVIEW software development environment. The trigger signal from the stimulator was connected to the TRIG (PFI0) pin of the board to perform a time-lock processing, such as that used for EP studies. The on-line and real-time data acquisition program, which was modified from examples of LabVIEW, can simultaneously display 6-channel electric activity and their derived EEG spectra, RR intervals, and smooth rectified DEMGs. In addition, these data can also be instantaneously recorded on a hard drive, and up to 6 h of data can be saved. The sampling rate for each EEG channel was 200 Hz. The sampling rate of the ECG and EMG was 1 kHz. For EP analysis, the sampling rate was 4 kHz to preserve the temporal waveform. Acquired data were then moved to write-only CDs for further tem-

Fig. 3. Circuit diagram of the multichannel amplifier. Detail circuits of the amplifiers of the EEG (upper) and the ECG or EMG of the nuchal and diaphragm muscles (lower) are shown, respectively. Values of passive components in the EMG or ECG amplifier are indicated at the bottom of the diagram. The length of the recording wire was 30–50 cm.
temporal, frequency, and/or spatial analysis. All analysis programs were also developed with LabVIEW.

Different electrophysiological signals contain various inherent characteristics. Four major types of signals were recorded in this study. The essential analytic functions for EEG activity are as follows.

1. **Overview of the original signal**: The EEG signal was displayed to reveal the contaminated segment. By comparing EEG and nuchal EMG activities with the observed behavior from the videotape, severely contaminated segments could be rejected. This initial evaluation of the EEG was essential for subsequent steps.

2. **Temporal analysis**: Two kinds of analytic tools were established for selected segments. First, statistical moments (mean, variance, skewness, and kurtosis) were used to evaluate the statistical distribution of the magnitude of the selected EEG segments. Moreover, nonlinear characteristics (phase portrait, dimension value, and algorithmic complexity) of the data segments were also established to analyze the dynamics of selected EEG segments under different physiological situations (Shaw et al., 1999c). In addition, the sleep spindle or K-complex was also noted for the differentiation of sleep stages.

3. **Frequency analysis**: Selected 8-s EEG segments were transferred into the frequency domain using Fourier transformation with a Hamming window. Contiguous EEG data segments were overlapped by 50% in a time–frequency display. Discrete frequency bands (delta: 1–4 Hz; theta: 4–8 Hz; alpha: 8–13 Hz; beta: 13–20 Hz; and gamma: 20–50 Hz) were identified from the EEG spectrum, and power density values in these individual frequency bands were summed to obtain absolute powers. The normalized unit of individual frequency bands was calculated from the ratio between the power value of an individual frequency band and the power summation of 1–50 Hz. The analysis of coherence, transfer function, and phase spectrum was also developed to evaluate the relationships between different channels (Shaw et al., 1999b).

The following steps were used for analyzing evoked field potentials.

1. **Recording of EEG-EP epochs**: A segment of EEG activity preceding and following the stimulus was digitized and stored. This operation was repeated about 100–200 times with a random inter-stimulus interval which was larger than 1.5 s.

2. **Selected averaging of EPs**: Stored EP epochs were selected according to specific criteria and then averaged. EP segments contaminated by movement artifacts and large baseline drift, and high-frequency artifacts generated by chewing action, were eliminated under quiescent rest conditions. On the other hand, different patterns of EEG and neck EMG activities appear in different sleep stages (Steriade and McCarley, 1990; Gottesmann, 1992). When a 1-s EEG-EP segment was compared with the neck EMG, a reasonable EP epoch could be selected and then averaged for a characteristic sleep state.

3. **Digital filtering**: Characteristics of the EP waveform were estimated using a finite impulse response filter with different cutoff frequency settings. The frequency components of EPs were computed using a digital filter with zero phase shift (Oppenheim and Schafer, 1989). This allowed us to evaluate variations in the specific peak of EPs in both time and frequency domains. Finally, we could read out the magnitudes and latencies of various characteristic peaks of an averaged EP (Shaw et al., 1999a, 2001).

The essential steps used for the analysis of the ECG signals are described below:

1. **Extracted R-peak**: An adjustable window discriminator permitted the extraction of a 120-ms ECG segment and the rejection of possible artifacts. A peak detection algorithm was then used to select the R-peak from these chosen ECG segments. Finally, the exact R-peak time stamps were verified or corrected by visual inspection of the original ECG trace. The HR was calculated from contiguous R-peak time stamps. In addition, an 80-ms preceding segment with reference to the R-peak was extracted for P-wave identification. Visual examination of the ECG trace was important for obtaining an exact time stamp of the P-wave.

2. **Temporal analysis**: Two kinds of analytic tools were established for selected segments. First, the statistical moments (mean, variance, skewness, and kurtosis) were used to evaluate the statistical distribution of the magnitude of the selected RR interval segments. A popular index, SDNN (standard deviation of all normal-to-normal RR intervals), was also obtained from the square root of variance. Moreover, nonlinear characteristics (Poincaré plot, dimension value, and algorithmic complexity) of the data segments were also established to analyze the dynamics of selected HR segments under different physiological situations.

3. **Frequency analysis**: Selected 16-s RR interval segments were interpolated and resampled at the rate of 40 Hz with a sample-and-hold algorithm. The resampled RR interval segment was transferred into the frequency domain using Fourier transformation with a Hamming window. Contiguous RR data segments were overlapped by 50% in a time-frequency display. Several suggestive analytic processes on heart rate variability in the frequency domain have been recommended in a critical review (Task Force of the European Society of Cardiology and the North Society of Pacing and Electrophysiology, 1996). The powers of three meaningful frequency bandwidths were selected: VLF (very low frequency, 0.0025–0.025 Hz), LF (low frequency, 0.3–0.75 Hz), and HF (high frequency, 0.8–4 Hz). In addition, several normalized indices were also
calculated, for example, LF/HF, LF%, and HF%. These indices are useful for assessing functional dynamics of the autonomic nervous system. Analyses of coherence, transfer function, and phase spectrum were also developed to evaluate relationships between the characteristic rhythm of HR and respiration.

The essential steps in the program for analyzing DEMG signals are described below:

1. **Smoothing out of DEMG**: A full-wave rectified process was applied to DEMG signals. Sequentially a 300-point moving average, which is almost equivalent to a 3 Hz lowpass filter in theory, was used to obtain a smooth integrated DEMG (IDEMG).

2. **Temporal analysis**: The IDEMG signal was converted into a binary sequence via a mean-threshold detector. An average respiratory rate was calculated from the binary count divided by the duration of the IDEMG segment.

3. **Frequency analysis**: Selected 16 s IDEMG segments were resampled at the rate of 40 Hz. The resampled IDEMG segment was transferred into the frequency domain using Fourier transformation with a Hamming window. The contiguous IDEMG data segments were overlapped by 50% in a time–frequency display. The analysis of coherence, transfer function, and phase spectrum were also developed to evaluate the relationship about the characteristic rhythm of HR and respiration.

Finally, the tonic activity of the neck muscle activity was quantified by the root-mean-square value of the magnitude of EMG.

### 3. Performance and results

In the 6-h recording period (n = 4), the durations of wakefulness, SWS, and REMS are about 2, 3.3, and 0.7 h, respectively. A representative example of all electrical signals under awake, SWS and REMS situations is illustrated in Fig. 4. Under the awake condition, the EEG revealed a characteristic low-amplitude, high-frequency desynchronous discharge pattern. The neck EMG showed a high tonic discharge. During SWS, the EEG presented a high-amplitude, low-frequency synchronous pattern, and the EMG activity of the nuchal muscle decreased. Under REMS condition, the EEG revealed a low-amplitude and high-frequency desynchronous pattern, which is very similar to that of awake state. By contrast, the neck muscle usually lacked tonic activity, i.e. atonia. Therefore, clear cardiac activity was easily observed in the nuchal EMG trace at this time (the rightmost column of Fig. 4). The neck EMG sometimes also revealed phasic activity, which was correlated with muscle twitch (Figs. 5 and 6). Characteristics of these electrical activities in the sleep–wake cycle have been elucidated in previous studies (Steriade and McCarley, 1990; Gottesmann, 1992). In the present study, we could also record these typical changes (Fig. 4), which indicates that the recording wires were not an obstacle to the rats in our setup, allowing the rats to fall asleep at will. On the other hand, low-noise ECG and DEMG recordings are also clearly shown in Fig. 4. A larger low-frequency fluctuation in the RR interval sequence was illustrated under both awake and REMS conditions. By contrast, a relatively high-frequency oscillation occurred under the SWS condition. The respiratory rhythm was relatively irregular under both awake and REMS situations compared to that of the SWS condition. Sleep apnea is a type of sleep disorder characterized by abnormal respiratory pauses during sleep (Guilleminault et al., 1978). Sleep apneas in rats have been defined as spontaneous cessations of diaphragmatic activity for at least 2 s during sleep (Sato et al., 1990). This definition may be more stringent than a common clinical criterion (Guilleminault et al., 1978), in which apnea is defined as respiratory pauses lasting 10 s or more. Respiratory pauses for 2 s in rats are approximately equivalent to those for 20 s in humans, considering rats’ respiratory rate of 100–150 breaths/min. An example of clear sleep apnea with no cardiac arrhythmia is shown in Fig. 4. Most sleep apneas were shown in the REMS state in rats.

A second type of sleep apnea with cardiac arrhythmia was observed under REMS condition although its frequency of occurrence was not high (Fig. 5; Guilleminault et al., 1983). In this example, the atrioventricular block was accompanied by irregular respiratory cessations. Remarkably, the high-frequency powers in all EEG leads were relatively high, especially in the gamma frequency bandwidth (~45 Hz in Fig. 5). Noticeably, a clear theta rhythm (7.5 Hz) only occurred at both parietal and occipital EEGs (arrowheads in Fig. 5), but was not obvious in the frontal EEG. This suggests that the cortical field potentials in different recording sites exhibit specific characteristics under the REMS condition. It should be pointed out that no apparent 60 Hz interference appeared in the EEG spectrum or temporal sequence under the REMS state (Fig. 5). Moreover, the background noise level of the amplifier was smaller than 3 μV, which could be measured from the EMG trace in REMS (muscle atonia). Our system consistently produced a high-quality performance throughout the entire recording period. Thus, low-interference activity could be robustly recorded by our system without a shielding device.

To depict the dynamic changes of these electrical activities under sleep–wake states, a 4 min data segment was analyzed. The original traces and analyzed results are illustrated in Fig. 6. The occipital EEG was selected to represent the cortical activity, and the correlated EEG spectrum was shown underneath. A clear theta peak is shown in a number of EEG spectra during
REM5. In the analysis of the power distribution of various typical frequency bands of EEG, a higher ratio of power in the delta band was found under SWS. An increased power ratio in the gamma band occurred in REM5. A transient increase of power in the alpha band was noticed at the transition from SWS to REM5. This phenomenon is due to the presence of spindle activity in this intermediate state. On the other hand, a stable RR interval and regular respiratory rhythm were observed under the SWS condition. At this time, the spectral activity of the rhythm of the RR interval and the respiratory rhythm were highly correlated (Fig. 6). By contrast, a larger fluctuation in the RR interval and irregular respiratory pattern was found in both REM5 and awake conditions. Interestingly, changes of the EEG in the intermediate state preceded alterations of cardiac and respiratory fluctuation in most cases.

In an attempt to understand the somatic sensory processing of the cortex during sleep–wake stages, a 2 ms constant-current electrical pulse was applied to the middle part of the rat’s tail. Averaged EPs of the parietal lead under awake, SWS, and REMS conditions are shown in Fig. 7. The characteristic P1–N1 morphology of the somatic EP was presented under awake condition (Fig. 7), which is consistent with observations of previous studies (Caulier and Kulics, 1988; Shaw et al., 1999a, 2001). In all cases, the P1 peak was preserved but with a small time delay during both SWS and REMS states. More interestingly, the architecture of the EP was obviously changed under the SWS situation. A long-latency (hundreds of milliseconds) response was present. In contrast, a similar waveform of EP under the REMS condition analogous to that of an awake EP was observed. After processing a 30 Hz highpass zero-phase filter to EP, a clear difference was found between the awake and REMS conditions and the SWS condition. On the other hand, gamma activity was significantly attenuated during the SWS state (Fig. 7B).

4. Discussion

In this study, we describe an integrated system to record and analyze several electrophysiological activities and track animal behaviors. All materials used here, including electrodes and electronic elements, are commercially available (Appendix A). For amplification, we only used two operational amplifiers and several passive elements in each channel to amplify specific biopotentials (Fig. 3). The entire design could be packaged in a 22 × 4 × 15 cm3 metal cage. Compared with a commercial 4-channel amplifier (Grass model 79, 160 × 60 × 50 cm3), our 6-channel amplification system is definitely smaller. Our amplifier could be easily placed with the animal nearby; it would lead to a high-quality recording without external electromagnetic interference. Nevertheless, the background noise level of our amplifier was smaller than 3 μV, which measured from the EMG trace.
in REMS (muscle atonia). With this design, a miniaturized, low-cost device can be easily fabricated, which delivers a high-quality signal with low 60 Hz interference in the absence of any shielding (Fig. 5). In addition, our analytic programs were established in the LabVIEW environment, which is a popular graphic-based platform worldwide. So this software is flexible and can easily interact with that of other labs. Several examples provided in this study demonstrate that our system is able to successfully record low-noise activity and extract several inherent characteristics of each signal under different vigilant conditions (Figs. 4–7). Material is reduced and quality is improved at a minimal cost in terms of time and money.

Our experiments show that the somatic EP was markedly influenced by various vigilant states (Fig. 7). The P1 component of the EP originates from a thalamocortical projection to the cortical layer IV (Arezzo et al., 1981; Cauller and Kulics, 1988). The P1 peak was preserved under all situations, but with a small delay under SWS and REMS compared to that under the awake condition. This phenomenon has been described in previous studies, which used electrical stimulation in the somatic-related central nucleus (Arnaud et al., 1979; Gottesmann, 1992; Steriade et al., 1997). On the other hand, the long-latency component has not been well studied. A definite morphological change in long-latency EP was observed under SWS in this study (Fig. 7). An enhancement of the low-frequency, long-lasting positive component was found in the somatic EP under SWS, instead of the N1 component. The morphology of EP under SWS was close to that recorded under light anesthesia (2 h of Fig. 2A in Shaw et al., 2001), which implies that the somatic information might be distorted in the cortex. In addition, the gamma oscillation was blocked under the SWS situation, but was preserved under REMS. During REMS, gamma oscillation waveforms have also been recorded in other studies (Llinas and Ribary, 1993; Buzsáki, 1998), indicating that information processing during REMS was unimpeded similar to that of the awake condition.

Our study also demonstrates that the theta rhythm occurred in the parietal and occipital but not the frontal

Fig. 5. Sleep apnea with cardiac arrhythmia during REMS. Several arrests of ventricular contraction (lacking R-peaks) in sleep apnea periods are marked (**). Two HR values were calculated from RR interval (solid line) to PP interval (dashed line), respectively. Note a clear theta peak in both parietal and occipital spectra, but it is not obvious in the frontal spectrum. No apparent 60 Hz interference was found in our recording.
lobes during the REMS condition (Fig. 5). The hippocampus usually reveals a theta rhythm under REMS and voluntary movement (Steriade and McCarley, 1990; Gottesmann, 1992). In rats, the hippocampus is located just under the parietal and occipital parts of the neocortex. There is a greater chance for theta rhythmic activity to propagate from the hippocampus to the parietal and occipital cortical areas (Gerbrandt et al., 1978). This may account for the different presentations of theta rhythmic activity in various cortical areas. An alternative possibility is the differential functional processing of characteristic rhythms in various cortical areas, including theta rhythms (Basar and Schürmann, 1994).

Two types of sleep apneas with/without cardiac arrhythmia during REMS were observed in rats (Figs. 4 and 5). They have also been reported in previous studies from rats (Sato et al., 1990) and humans (Guillemainault et al., 1978, 1983). Sleep apneas can be classified into three classes: obstructive, central, and mixed types. Most data concern the obstructive sleep apnea syndrome. In our preparation, we addressed the central type of sleep apnea to delineate the role of the central nervous system. Further, our system is flexible and compatible with other devices, e.g. a plethysmograph, for measuring the movement of the chest wall. Thus, the present system is useful for investigating the mechanisms of all types of sleep apneas.

In summary, we herein introduce a low-cost, low-noise, miniature multichannel system for sleep study. Several examples are illustrated to substantiate the compatibility and superiority of our system for rats. The entire system may also be easily combined with other devices, such as a plethysmograph, and applied to other investigations as well, e.g. epileptic seizures.

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Appendix A


References


