Characteristics of the Cortical and Hippocampal EEG Power Spectra of Rabbits during Normal Behavioral States and after Administration of CNS Acting Drugs

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Abstract—The EEG power spectra of the motor cortex (MC) and hippocampus (HPC) in rabbits were characterized, and the effects of CNS acting drugs on the spectra were investigated. The EEGs of rabbits with chronically implanted electrodes were recorded with bipolar leads and simultaneously analyzed for 15 min with a computer to obtain their power spectra. MC spectra had one peak of delta wave, and HPC spectra had two peaks of delta and theta waves, whose peak powers and frequencies were changed in correspondence to the level of consciousness. Pentobarbital (20 mg/kg, i.v.) produced the peaks at 11 and 4 Hz in MC and HPC spectra, respectively. Morphine (5 mg/kg, i.v.) produced the peak at 7 Hz in MC spectra and shifted the theta wave peak of HPC to lower frequencies. Diazepam (4 mg/kg, i.v.) produced the peak at 14 Hz in MC spectra and decreased the two peak powers in HPC spectra. Chlorpromazine (4 mg/kg, i.v.) shifted the theta wave peak of HPC to lower frequencies. Amitriptyline (5 mg/kg, i.v.) increased the peak powers of the delta waves in MC and HPC spectra. These results suggest that each of the five CNS acting drugs produces the characteristic spectra, and they are different from the spectra obtained during normal behavioral states.

Various computer techniques for qualitative and quantitative analysis of EEG signals have been developed and have enabled us to get much information on brain functions. Recently, power spectral analysis is often employed in experimental as well as clinical studies in order to qualitatively and quantitatively clarify the EEG changes caused by CNS acting drugs (1-5) or psychological stress (6) and to diagnose cerebral disorders (7-10). It might be possible to characterize the alterations of the CNS caused by drugs or other agents as alterations of spectra by this analysis, if better techniques were developed.

Sterman and Kovalesky (5) reported that it was important to select standardized baseline conditions in order to produce stable quantitative data. Before employing EEG power spectral analysis, it is also necessary to characterize the EEG power spectra electrophysiologically. Young et al. (11) have reported that the cortical EEG power spectra in rats during the behavioral states of wakefulness, sleep and rapid eye movement sleep, were different both qualitatively and quantitatively. Leung et al. (12) have studied in detail the spectral characteristics of the hippocampal EEG in freely moving rats with regards to different behaviors. However, the characteristics of the EEG power spectra of the cortex or hippocampus in rabbits have not been so clearly delineated.

In the present study, the suitable time for EEG power spectral analysis was investigated, and the EEG power spectra of the motor cortex (MC) and hippocampus (HPC) in rabbits were characterized during normal behavioral states. Furthermore, the effects of five CNS acting drugs, pentobarbital, morphine, diazepam, chlorpromazine and
amitriptyline, on the EEG power spectra were investigated.

Materials and Methods

Animals and surgical procedure: Male Japanese White rabbits, weighing 2.5-3.5 kg, were used. The animals were anesthetized with pentobarbital sodium (30.0 mg/kg, i.v.), and bipolar stainless steel wire electrodes (0.25 mm diameter, insulated except for the last 0.5 mm of the tips; polar distance, 0.5-1.0 mm) were chronically implanted into the HPC (A: -4, L: 4, H: 5) according to the brain atlas of Sawyer et al. (13). Two stainless steel screw electrodes (1.0 mm diameter, silver-plated) were placed subdurally at an interval of 2 mm on the surface of the MC. Each electrode was fixed with dental cement to a perforated hole in the skull and soldered to connector socket. The socket itself was fixed by means of the cement together with screws driven into the skull, and all exposed parts of the electrodes were also covered with the cement. Animals were allowed at least 1 week to recover from the surgery before commencing the experiments.

EEG recording and analysis: The animals were moved in a transparent plastic box (26×42×34 cm) which was placed in a sound-proof, shielded room. The EEGs of the MC and HPC were bipolarly recorded on a polygraph (San-ei Sokki, 142-8), at a time constant of 0.1 sec and low pass filter setting of 25 Hz, concomitantly with observing the behavior under unanesthetized and unrestrained states. The normal behavioral states of wakefulness, rest, slow wave sleep (SWS) and rapid eye movement (REM) sleep were identified by corresponding changes in the EEGs, and the behaviors were observed through a TV camera in a separate room. Simultaneously with the EEG recording, the power spectral analysis of the EEGs was performed with a signal processor (San-ei Sokki, 7T07) followed by Fast Fourier Transformation at frequencies from zero to 25 Hz. The spectra were plotted on an X-Y recorder as histograms at intervals of 0.22 Hz.

Drugs: The drugs used in the experiment were amitriptyline (Lantron, Yamanouchi Pharm.), chlorpromazine hydrochloride (Wintermin, Shionogi Pharm.), diazepam (Cercine, Takeda Pharm.), morphine hydrochloride (Tanabe Pharm.) and pentobarbital sodium (Somnopentyl, Pitman-Moore). All the drugs were administered intravenously into the ear vein.

Results

Time for EEG analysis

The EEGs of the MC and HPC were sampled during 0.25, 1, 5 and 15 min periods, and they were analyzed. The histograms at intervals of 0.22 Hz in the spectra from the 0.25 or 1 min samples were not consistent in view of density; therefore, it was difficult to characterize their peaks and features. However, the histograms in the spectra from the 5 or 15 min samples were consistent in densities, so it was possible to characterize the peaks and features. In the MC and HPC spectra, the peaks and features could be defined more clearly as the time for analysis was increased. Therefore, the analysis time of 15 min was employed in the present experiment.

Characteristics of EEG power spectra

The EEG power spectra of the MC and HPC analyzed for 15 min periods during the normal behavioral states of wakefulness, rest and SWS are shown in Figs. 1 and 2.

Fig. 1. Power spectra of motor cortical EEGs for 15 min during the normal behavioral states of wakefulness, rest and slow wave sleep (SWS) in rabbits.
The MC spectra consisted of powers ranging from zero to frequencies higher than 20 Hz, and there was one peak at 1.94±0.12 Hz (mean±S.D. n=5) in the spectra. The peak power of the delta wave and total power were most increased in the SWS state and most decreased in the wakefulness state. The power in the rest state changed in correspondence to the level of consciousness; however, the values fell between the powers in the SWS and wakefulness states. There was no significant spectral difference between the REM sleep and wakefulness states.

The HPC spectra consisted of powers ranging from zero to 15 Hz, and the spectra had two peaks at 1.63±0.29 and 5.95±0.46 Hz (mean±S.D., n=5), whose peak powers were changed competitively with each other in correspondence to the level of consciousness. In the wakefulness state, the peak power of the theta wave was increased, and the peak power of the delta wave was decreased. In the SWS state, the peak power of the delta wave was increased, and the peak power of the theta wave was decreased. In the rest state, the peak powers of delta and theta waves changed in correspondence to the level of consciousness, but the values fell between the powers in the SWS and wakefulness states. Though the features of the spectra in the REM sleep state were similar to those in the wakefulness state, the frequencies of the theta wave peaks in the REM sleep state were 1.54±0.33 Hz (mean±S.D., n=5) higher than that in the wakefulness state.

**Effects of CNS acting drugs**

The changes of the MC and HPC spectra of the rabbit before and after treatment with saline are shown in Fig. 3. The animal showed the wakefulness state for some time.
since it was placed in the box, and then it showed the rest or SWS states as it adapted to the environment. The spectra were changed in correspondence to the level of the consciousness as described above. After treatment with saline, the spectra also only changed in correspondence to the level of consciousness, and no characteristic changes were observed in the spectra. Similarly, the effect of each dose of the five CNS acting drugs on the spectra were investigated in two or three rabbits.

1) Pentobarbital: Pentobarbital, 5.0 mg/kg, i.v., produced no significant spectral change. At the dose of 10.0 mg/kg, i.v., it tended to increase the peak power of the delta wave and produced a new peak in the MC spectra and a new high peak in the HPC spectra. At the dose of 20.0 mg/kg, i.v., these effects of pentobarbital were clear, marked and long-lasting (Figs. 4 and 5), and similar spectral changes were observed in three rabbits. The peak powers of the delta wave in MC spectra showed a 73.66±37.00% increase compared with those during the SWS states, and the frequencies of the new peaks in the MC and HPC spectra were 11.44±0.42 Hz and 4.15±0.28 Hz, respectively (mean±S.D., n=3).

2) Morphine: Morphine, 1.0 mg/kg, i.v., produced no significant spectral change; and at the dose of 2.0 mg/kg, i.v., it tended to increase the peak power of the delta wave of the MC and shift the peak of the theta wave of the HPC to lower frequencies (which was changed to a sharp peak). At a dose of 5.0 mg/kg, i.v., these effects of morphine were clear and marked, and it produced a new peak in the MC spectra (Figs. 4 and 5). Similar spectral changes were observed in three rabbits, but the production of the new peak was observed in two rabbits and it was not clear in one rabbit. The peaks of the theta waves were shifted 1.01±0.32 Hz (mean±S.D., n=3) to lower frequencies as compared with the peak before treatment, and the frequencies of the new peaks were...
7.33±0.58 Hz (mean±S.D., n=2).

3) Diazepam: Diazepam, 1.0 and 2.0 mg/kg, i.v., tended to increase the peak power of the delta wave and produce a new peak in the MC spectra; and it decreased the peak powers of the delta and theta waves in the HPC spectra. At the dose of 4.0 mg/kg, i.v., these effects of diazepam were clear and marked (Figs. 4 and 5), and similar spectral changes were observed in three rabbits. The frequencies of the new peaks were 14.31±1.04 Hz (mean±S.D., n=3).

4) Chlorpromazine: Chlorpromazine, 1.0 and 2.0 mg/kg, i.v., tended to increase the peak powers of the delta waves of the MC and HPC; it shifted theta wave peak of the HPC to lower frequencies and changed it to be high and sharp. At the dose of 4.0 mg/kg, i.v., these effects of chlorpromazine were clear and marked (Figs. 4 and 5), and similar spectral changes were observed in three rabbits. The peaks of the theta waves were shifted 1.01±0.16 Hz (mean±S.D., n=3) to lower frequencies as compared with the peak before treatment.

5) Amitriptyline: Amitriptyline, 1.0 mg/kg, i.v., produced no significant spectral change; and at the dose of 2.0 mg/kg, i.v., it tended to increase the peak powers of the delta waves of the MC and HPC. At the dose of 5.0 mg/kg, i.v., these effects of amitriptyline were marked and long-lasting (Figs. 4 and 5), and similar spectral changes were observed in three rabbits. The peak powers of the delta wave of the MC during 1 hr after administration were increased 18–20% over that during the SWS state.

Discussion

In the present study, the cortical and hippocampal EEG power spectra of rabbits were characterized electrophysiologically, and the effects of CNS acting drugs on the spectra were investigated.

First, the suitable time for EEG power spectral analysis was investigated in periods from 0.25 to 15 min, because the power spectra consist of power in the ordinate and frequency in the abscissa, but there is no factor of time in the spectra. It was interesting that the peaks and features of the spectra could be defined more clearly as the analysis time was increased. This result demonstrates that each frequency of the EEG signals is controlled at a regular power level to constitute the spectra. If the analysis time was very long, i.e., 1 or 2 hr, the spectra would change grossly in correspondence to the level of consciousness. Therefore, it was decided to set 15 min periods for the analysis in the present study. In other studies, various times, 2 sec to 10 min, were set for the analysis (2, 3, 5, 6, 9–12). The analysis time should be set according to the purpose of the experiments; however, some summation of EEG signals seems to be necessary.

The MC and HPC spectra were compared, and it was found that they each had characteristic peaks, and the peak power or frequency increased or decreased in correspondence to the level of consciousness. The alteration of the peaks of the MC and HPC spectra during normal behavioral states are shown in Fig. 6. The MC spectra had only one peak at about 2 Hz and the peak power changed. The HPC spectra had two peaks of delta and theta waves, and the two peak powers and the peak frequency of the theta wave changed. These results suggest that the alterations of the MC and HPC EEGs corresponding to the level of consciousness can be transformed into the alterations of spectra by power spectral analysis, probably because the amplitude of each frequency of the EEG signals is regarded as more important for power spectral analysis than the appearance time of each frequency of the EEG signals. Young et al. (11) reported that the cortical EEG power spectra of rats during the states of wakefulness, sleep and REM sleep were qualitatively and quantitatively different, and the spectra during the wakefulness and sleep states are similar to that of rabbits in the present study. However, in the REM sleep state, the spectra of rats and rabbits are different, because the REM sleep of rats was associated with a predominant peak of spectral power in the 6 to 9 Hz band (11). Leung et al. (12) studied in detail the hippocampal EEG power spectra during different behaviors in rats, and they found that the three types of EEG pattern, irregular slow activity (0–25 Hz), rhythmical slow activity (4–10 Hz) and fast wave (20–
100 Hz), coexist in different behavioral states. The characterizations of the hippocampal EEG in their study are different from those in the present study.

The five CNS acting drugs used in the present study produced characteristic alterations of the peaks in the MC and HPC spectra, and the alterations caused by the drugs are shown in Fig. 7 and Table 1. These alterations can be classified into three patterns: 1) production of a new peak in the MC spectra, 2) shift of the theta wave peak in the HPC spectra and 3) change of the peak power.

Morphine, pentobarbital, and diazepam produced the new peak at about 7, 11, and 14 Hz in the MC spectra, respectively. It was easy to identify the new peaks produced by pentobarbital and diazepam. However, it was not so easy to identify the new peak produced by morphine, because the new peak was near the peak of the delta wave, and the productive effect was different between individuals. Kareti et al. (1) reported that morphine caused a few predominant peaks in the 2 to 8 Hz band in the cortical EEG spectra of rats, but the peak EEG frequencies were not clear. Yamamoto et al. (4) reported that the power of the cortical EEG spectra of cats increased and the peak EEG frequency was found at about 13 Hz in the awake stage after administration of diazepam. Ishikawa et al. (14) reported that pentobarbital increased the power in the 8 to 16 Hz band in the cortical EEG spectra of rabbits. These results are similar to those in
the present study. Sterman and Kovalesky (5) reported that diazepam decreased the power in the 4 to 7 Hz band in the cortical EEG spectra of rhesus monkeys, and pentobarbital increased the power in the 16 to 24 Hz in the spectra. The drug effects on the EEG spectra may be different between rhesus monkeys and rabbits.

Both morphine and chlorpromazine shifted the theta wave peak of HPC to lower frequencies; however, chlorpromazine heightened the peak and morphine did not. On the other hand, the theta wave peak of the HPC was shifted to higher frequencies during the REM sleep. Moreover, psychological stress increased the power of the theta wave of the HPC to higher frequencies (6). The frequency as well as the power of the theta wave peak may be related to the level of consciousness. Pentobarbital shifted the peaks of the delta and theta waves to at about 4 Hz in the HPC spectra and produced a new high peak, and these spectra was not similar to that during the normal SWS state. Ishikawa et al. (14, 15) reported that chlorpromazine showed slight decrease of the total power of the hippocampal EEG spectra of rabbits, and pentobarbital increased the power in the 0 to 4 Hz band and decreased the power in the 4 to 16 Hz band in the spectra. However, they did not describe the alterations of the frequency and power of the theta wave peak, whose alterations can not be shown by the alterations of the total power or the power in the 4 Hz band width.

Diazepam decreased the peak powers of both delta and theta waves in HPC spectra, for which spectral changes were not observed after administration of other drugs. Yamamoto et al. (4) reported that diazepam decreased the power in the 2 to 6 Hz band in the hippocampal EEG spectra of cats, and the peak EEG frequency was lowered. In the present study, however, the lowering of the peak EEG frequency was not so clear as in rabbits.

Amitriptyline only increased the peak powers of delta waves in the MC and HPC spectra, respectively. The spectra produced by amitriptyline was similar to that during the normal SWS state. Sakai and Matsui (16) reported that the cortical and hippocampal EEG spectra of rats at arousal and slow wave stages after administration of antidepressants, mianserin or imipramine, were not different from that after treatment with the vehicle. However, the increase of the peak power of the delta wave in the MC spectra produced by amitriptyline tended to be more marked than that during the normal SWS state. Pentobarbital also produced a similar increase

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**Table 1. Characteristic effects of CNS acting drugs on power spectra of motor cortical and hippocampal EEGs in rabbits**

| Drug          | Motor cortex | Hippocampus |
|---------------|--------------|-------------|
|               | peak power   | peak        | peak power   | peak frequency |
|               | delta wave   | 5-25 Hz     | delta wave   | theta wave     |
|               |              |             | delta wave   | theta wave     |

Normal behavioral states

| State          | Motor cortex | Hippocampus |
|----------------|--------------|-------------|
| Wakefulness    | ↓             | ↑           |
| Rest           | ↑             | ↓           |
| Slow wave sleep| ↑             | ↑           |
| REM sleep      | ↓             | ↓           |

Pentobarbital  ↑ 11 Hz  ↑  ↑  ↓  →  →
Morphine       ↑  7 Hz    ↑  ↑  ↓  →  →
Diazepam       ↑  14 Hz  ↓  ↓  ↓  →  →
Chlorpromazine ↑  ↓     ↑  ↑  ↓  →  →
Amitriptyline  ↑  ↓     ↑  ↓  ↓  →  →

The peak power and frequency were compared with that of the spectra during the rest state (→). →: No production of peak or no change of frequency, ↑: Increase, ↑: Marked increase, ↓: Decrease, →: Shift in higher frequencies, →: Shift in lower frequencies.
for a short time.

The present findings suggest that the cortical and hippocampal EEGs of rabbits during normal behavioral states and after administration of CNS-acting drugs can be qualitatively characterized by power spectral analysis.

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