Electrocorticogram in Rats Loaded with SART Stress (Repeated Cold Stress)

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Abstract—The electrocorticogram (ECoG) in a SART (specific alternation of rhythm in temperature)-stressed (repeatedly cold-stressed) rat, which is regarded as an experimental model for clinical vagotonic-type dysautonomia, was investigated in the present study by the power spectral technique. 1) Analysis of ECoG in SART-stressed rats during the resting-arousal state indicated a decrease in total power and a decrease in relative power in the δ band, and also an increase in relative power in the θ , α and β bands. 2) In the slow-wave sleeping state, the ECoG of SART-stressed rats indicated a marked increase in total power, an increase in the δ band and decreases in θ , α and β bands. 3) Electric stimulation of the posterior-hypothalamic area evoked alterations of ECoG similar to those caused by SART stress. ECoG response to electric stimulation in SART-stressed rats was less than that in unstressed rats. 4) Lesioning of the posterior-hypothalamic areas prevented SART stress-induced ECoG alterations. SART-stressed rats thus appear to be at a higher consciousness level on awakening but to sleep more soundly. They seem to exhibit greater fluctuation in brain activity than normal rats. There is also the possibility that the posterior-hypothalamic area is responsible to some degree for ECoG alterations in SART-stressed rats.

Numerous reports indicate that experimental animals such as mice, rats and guinea pigs exposed to SART (specific alternation of rhythm in temperature) stress (repeated cold stress) (1, 2), a type of subacute stress, come to show various physiological abnormalities in the periphery (3–9). With respect to the central nervous system (CNS), such rats show certain biochemical changes including decreased content of total acetylcholine and increased activity of related enzymes (10, 11) as well as increased contents of dopamine and norepinephrine in the brain (12, 13). Little, however, is known about the functional states in the CNS.

A SART-stressed animal may be regarded as an experimental model for clinical vagotonic-type dysautonomia, considering the results of the GSR (galvanic skin response), Ashner's oculocardiac and mecholyl tests (1, 14). The hearts of such animals are

sympathicotonic (6). This complicated imbalance in the autonomic nervous system may be involved in the modulating mechanisms in the CNS.

In the present study, we examined the electrocorticogram (ECoG) of SART-stressed rats by the power spectral technique. Moreover, electric stimulation and lesions of the posterior-hypothalamic area were performed so as to gain some understanding of the mechanism of ECoG alterations produced by SART stress.

Materials and Methods

Animals and stress procedure: Male adult Wistar rats weighing between 270 and 310 g were used. For loading SART stress, rats were alternately kept at 24°C and –3°C for 1 hr-periods from 9 a.m. to 4 p.m. and then at –3°C from 4 p.m. to 9 a.m. the following morning. This was continued for 5 con-

secutive days (1, 2), and stopped on the 6th morning. The stressed rats were submitted to experiments at least 1 hr after the cessation of stress.

ECoG recording: Under pentobarbital-Na (40 mg/kg, i.p.) anesthesia, the rats were made stationary by a stereotaxic apparatus (Narishige, SR-5). Stainless steel screw electrodes (1.2 mm in diameter) were implanted on the cortical surface through the skull. Electrode placements were the left frontal (2 mm anterior and 1.5 mm lateral to bregma) and bilateral parietal (3.5 mm posterior and 2 mm lateral to bregma) areas. The reference electrode was placed on the intraparietal bone (1.5 mm posterior to lambda). All electrodes were fixed in place with acrylic dental cement (Nissin Dental Products Inc., Mend-Rex®). Ten days after the surgery, ECoG was monopolarly recorded in both unanesthetized and unrestrained states during resting-arousal and slow-wave sleep. The electrodes were connected to a polygraph system (Nihon Kohden, RM-6000) through a mercury pool swivel which permitted the animal to move freely without entangling the electrode leads. ECoG was drawn on an ink-writing recorder at a paper speed of 25 mm/sec for about 10 min during monitoring for 60 min and simultaneously recorded on magnetic tapes (Sony, SIT-90F) using a cassette data recorder (Nihon Kohden, RMG-5204) for later ECoG analysis. The time constant and high-cut filter were set at 0.3 sec and 100 Hz, respectively.

Electric stimulation and lesions of the posterior-hypothalamic area: The posterior-hypothalamic area was defined according to stereotaxic coordinates derived from the atlas of Paxinos and Watson (3.8 mm posterior and 8.0 mm ventral to bregma and 0.5 mm lateral to the midline) (15). A pair of twisted stainless steel wires with epoxy-resin coating (0.2 mm in diameter and about 0.2 mm in interpolar distance) served as the electric stimulation electrodes. For the electric stimulation experiments, square wave pulses (a duration of 0.1 msec, 3 or 5 V) were sent to the left posterior-hypothalamic area at a rate of 100 pulses per second for 15 sec using an electric stimulator (Nihon Kohden,

SEN-3201) and an isolator (Nihon Kohden, SS-102J). ECoG of the left frontal cortex was registered continuously during the stimulation. For the lesion experiments, radiofrequency destruction of the posterior-hypothalamic areas was bilaterally achieved at 65°C for 3 min using a lesion generator (Radionics, model RFG-4A) and an element of 0.25 mm in diameter (Radionics, type TC). ECoG of the left frontal cortex was recorded.

Implanting of stimulation electrodes and radiofrequency destruction were performed 10 days before stress loading. After the end of the experiments, the brain was isolated and electrode placements and lesions were visually verified.

ECoG analysis: Consecutive 2.56 sec ECoG epochs stored on magnetic tapes were digitized at a rate of 10 msec, and then power spectra were obtained by Fast Fourier Transformations, using a data analyzer system (Nihon Kohden, ATAC-450). The sampling time was 10.24 sec in resting-arousal states and 20.48 sec in slow-wave sleeping states. The analytical time of 10.24 sec in the resting-arousal ECoG was chosen to obtain stable ECoG without meaningless alterations and to avoid movement artifacts, since rats possess relatively fast sleep-awakening cycles during the daytime and move more actively as compared with rabbits and cats (16, 17). In the slow-wave sleeping state, as the stable ECoG recordings can be obtained easily, 20.48 sec ECoG samples were analyzed to improve the precision. Subsequently, to quantify the power spectra obtained, total potency and relative power were calculated according to the method described by Young et al. (18) and Wauquier et al. (19), with minor modification. Total power was obtained by adding the powers from 1.56 Hz to 29.64 Hz, and total potency was expressed as the percentage of the control total power for each animal. The relative power in δ (1.56–3.90 Hz), θ (3.90–7.80 Hz), α (7.80–13.26 Hz) and β (13.26–29.64 Hz) bands was represented as the percentage of total power. After observing the paper ECoG records, several artifact-free epochs were selected and analyzed. The average of these data was taken as the analytical value for each animal.

Statistical analysis: All data obtained were presented as the mean $\pm$ S.E. Significant differences were analyzed by Student's *t*-test.

Results

Resting-arousal ECoG in SART-stressed rats: Direct left-frontal ECoG recordings of a rat during the resting-arousal state before and after SART stress and corresponding power spectra are shown in Fig. 1. As seen in the upper part of this figure, a decrease in δ waves and increase in θ waves were observed after the stress, and the ECoG exhibited low-amplitude fast waves as a whole. The lower part of Fig. 1 shows the histograms of the power spectra. The peak power shifted from 2–4 Hz to 5–7 Hz due to the stress.

In SART-stressed rats, as seen in Fig. 2, total power significantly decreased as compared to normal (before stress) rats, and total potency in the left frontal, left and

right parietal areas was 77, 81 and 84%, respectively. As to the relative power of SART-stressed rats, the δ band significantly decreased, and the θ band significantly increased in all three areas. The relative power of the α and β bands tended to increase, although the magnitude of change differed according to the area.

As described above, there was little difference among these three areas in change caused by stress in total potency and relative power. Then, left-frontal ECoG recordings were shown in the following results.

Slow-wave sleep ECoG in SART-stressed rats: Representative left-frontal ECoG recordings before and after SART stress during the slow-wave sleep are shown in Fig. 3. The ECoG after stress was visually observed and found to have higher amplitude and lower frequency than before stress. The histograms of power spectra for 20.48 sec containing these ECoG samples drawn at

Before

After

Fig. 1. Direct resting-arousal ECoG recordings and their power spectra at the left frontal area of a rat before and after SART stress.

Fig. 2. Power spectral analysis of resting-arousal ECoG in non-stressed and SART-stressed rats. □: Non-stress, ■: SART stress. Values are means±S.E. of 23–27 non-stressed and 7–10 SART-stressed rats. *P<0.05, **P<0.01 and ***P<0.001 (*t*-test).

Fig. 3. Direct slow-wave sleep ECoG recordings and their power spectra at the left frontal area of a rat before and after SART stress.

the upper panel are shown in the lower part of Fig. 3. After stress, most of the ECoG spectral power was evident in the 0–5 Hz

band.
The average total potency and relative power of the left-frontal ECoG in slow-wave

sleep states are shown in Fig. 4. SART-stressed rats indicated a markedly increased total potency of 195%, increased δ band and decreased θ , α and β bands in relative power.

In Fig. 5, to summarize and characterize ECoG alterations in SART-stressed rats, differences between relative powers in left-frontal ECoG before and after stress are expressed as Δ relative power. The ECoG of SART-stressed rats was characterized by faster waves in resting-arousal states and slower waves in slow-wave sleeping states, compared to unstressed, normal rats.

Fig. 4. Power spectral analysis of slow-wave sleep ECoG at the left frontal area in non-stressed and SART-stressed rats. $\square$: Non-stress, $\blacksquare$: SART stress. No. of rats: 12/group. ** $P<0.01$ and *** $P<0.001$ (t -test).

The influence of electric stimulation and lesions of the posterior-hypothalamic area on resting-arousal ECoG: Figure 6 shows the results from the power spectra analysis of resting-arousal ECoG in the left frontal area induced by electric stimulation of left posterior-hypothalamic area in non-stressed and SART-stressed rats. In non-stressed rats, the total potency decreased in a voltage-dependent manner by stimulation. The relative power of the δ and θ bands showed voltage-related decrease and increase, respectively. Also, significant increase in α and β bands was noted by a stimulation of 3 V and/or 5 V. In the SART-stressed rats, a slight decrease in total potency occurred by electric stimulation, but was not significant. The relative power of the δ and θ bands showed voltage-related decrease and increase, respectively, but the magnitude of change in SART-stressed rats was less than that in non-stressed rats. Moreover, the ECoG alterations elicited by 3 V-electric stimulation in the non-stressed rats were similar to those caused by SART stress.

The results obtained from power spectral analysis of resting-arousal ECoG in the left frontal area of posterior hypothalamus-lesioned rats before and after stress loading are presented in Fig. 7. The bilateral posterior-hypothalamic lesions caused a decrease in the δ band and a slight increase in the θ band in non-stressed rats. When the rats were loaded with SART stress after posterior-hypothalamic lesions, no change in total potency and relative power caused by SART

Fig. 5. Changes of rat ECoG at the left frontal cortex caused by SART stress. A: The value after SART stress, B: The value before SART stress. Values are means $\pm$ S.E. of 9 or 12 rats/group. ** $P<0.01$ and *** $P<0.001$ (t -test).

Fig. 6. Influence of electric stimulation at the left posterior-hypothalamic area on resting-arousal ECoG at the left frontal area of rats. N: Non-stress, S: SART stress. No. of rats: 3 or 4/group. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, compared to non-stressed control, # $P < 0.05$ and ## $P < 0.01$, compared to SART-stressed control, and ## $P < 0.01$, compared to respective non-stressed group (t -test).

stress could be detected, and the data obtained were essentially the same values as before the stress.

Discussion

In the present study, the ECoG of SART-stressed rats was investigated by the power spectral technique. Resting-arousal ECoG alterations in SART-stressed rats were expressed as decreased total potency, and decreased δ band and increased θ , α and β bands in relative power. These results show SART-stressed rats to have low-amplitude fast waves. It has been reported that power spectra are influenced by functional changes in the brain (20, 21), various CNS acting drugs (19, 22–24) and stress (25). Yamamoto

Fig. 7. Influence of bilateral posterior-hypothalamic lesions on resting-arousal ECoG at the left frontal area of rats. N: Non-stress, S: SART stress. Values are means $\pm$ S.E. of 4–6 rats/group. * $P < 0.05$ and ** $P < 0.01$, compared to non-stressed control (t -test).

(24) reported that in rabbits, a decrease in total power accompanied by that in peak power occurred with enhancement of the consciousness level. It is evident that SART-stressed rats were at an enhanced consciousness level, since a decrease in total power accompanied by a shift to higher frequency of peak power was noted in SART-stressed rats. In the human EEG, the suppression of α rhythm and dominant fast waves have been observed when the brain activity level is augmented by mental excitement and attention. From the GSR test results (1), SART-stressed rats fall into hypersensitivity by external stimuli. Considering low-amplitude fast waves of resting-arousal ECoG in SART-stressed rats, the SART-stressed rats may possibly be in a state of mental tension.

Various types of stress have been reported to evoke changes in the ECoG of experimental animals (25–28). Harada et al. (27) reported that the ECoG in rats exposed to restraint stress fluctuated between low-voltage

rhythmical waves and high-voltage irregular waves including spindles and spikes, and that rats restrained in 25°C water showed only low-voltage rhythmical waves, the frequency of which increased in the early stages of the stress, gradually decreasing below the levels prior to the stress. According to Iwata and Mikuni (25), the relative activity in the range 7.5–13.0 Hz markedly increased in rats exposed to fear-induced stress. When rats were kept at –10 to –15°C for 12 hr, no change occurred in ECoG (26). ECoG alterations in SART-stressed rats, therefore, markedly differ from those evoked by restraint, fear-inducement and acute cold stress.

Recently, many patients with psychosomatic diseases have been reported to show irregular α waves and fast dominant waves in their EEG (29). Therefore, it is of much interest that low-amplitude fast waves were seen in SART-stressed rat ECoG in this study.

Slow-wave sleep ECoG in SART-stressed rats showed higher-amplitude slower waves as compared with those in normal rats. SART-stressed rats may sleep more soundly than normal rats. REM-sleep ECoG and the sleep-awakening cycle in SART-stressed rats remain to be investigated.

It is well-known that the arousal response of EEG is induced by electric stimulation of the posterior hypothalamus. In this study, ECoG alterations evoked by 3 V-stimulation in normal rats were similar to those caused by SART stress. Response to electric stimulation in SART-stressed rats was less than that in normal rats. In contrast, lesioning of the posterior-hypothalamic areas inhibited ECoG alterations caused by SART stress. From these results, the posterior-hypothalamic area appears to be involved at least in ECoG alterations in SART-stressed rats, although it will be necessary to examine brainstem reticular formation and other parts of the brain.

In conclusion, SART-stressed rats may possibly be at a higher consciousness level on awakening and sleep more soundly. There is greater fluctuation in brain activity than in normal rats.

We are currently investigating effects of some drugs on these alterations of ECoG

and behavioral characterization in SART-stressed animals.

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