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Changes in Electroencephalograms of Rats during the Development of Stress Ulcer

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Electrocorticograms in restrained rats and in restrained and water(35°)-immersed rats showed a series of cycles fluctuating between low voltage rhythmical waves and high voltage irregular waves including spindles and spikes during a 6-hr stress period, whereas those in restrained and water(25°)-immersed rats showed a succession of low voltage rhythmical waves whose initial increased frequency gradually decreased below the original levels as the stress period was prolonged. Hippocampal electrical activities fluctuated in parallel with the cortical activities. A specific gastric motility, a severe gastric ulceration, and a marked hypothermia were observed only in the restrained and water(25°)-immersed rats. Acute adrenalectomy in the restrained and water(25°)-immersed group more decreased frequencies of the rhythmical low voltage waves and induced abnormal waves such as discharges and wave flattening with high mortality. These changes could be retarded by epinephrine or corticosterone administration but not by ACTH administration.

Keywords——ACTH; adrenalectomy; corticosterone; electrocorticograms; epinephrine; hippocampal electroencephalograms; restraint stress; restraint and water-immersed stress; stress ulcer

Restraint and water(25°) immersion stress on rats and mice provokes gastric ulceration and deteriorates its degree as the stress period is prolonged.[2)] In the process of developing gastric ulcers, a large number of body functions may differ from those in the normal state. We have examined changes in gastric motility as well as in catecholamine contents of the adrenals, glandular stomach, brain, and urine in rats during this stress[3)] and have also investigated changes in serum glucose, serum free fatty acids, and liver glycogen concentrations in mice subjected to the same stress.[4)] The results of these experiments have suggested that the sympatho-adrenal system was activated in the early stage of this stress and that this enhanced activity was decreased along with predominance of the parasympathetic activity as the stress period was prolonged. It is important to study the state of the central nervous system (CNS) in animals subjected to stress, because gastric ulceration with stress is prevented not only by peripherally acting drugs but by centrally acting drugs.[2,5)] Measurement of electroencephalograms (EEGs) and evoked potentials is of much help in studying the state of the CNS continuously. Many electroencephalographic investigations have been conducted to see the state of the CNS under stress. For example, Porter[6)] studied changes in EEGs in cats and monkeys during a period of stress such as epinephrine or insulin administration and hypoxia. Murakami and Minakami investigated changes in EEGs from various regions of the brain in rabbits under restraint and cooling.[7)] Kawakami, *et al.*[8)] reported

1) Location: *Yayoi-cho, Chiba, 280, Japan.*
2) *a*) K. Takagi, Y. Kasuya, and K. Watanabe, *Chem. Pharm. Bull.* (Tokyo), **12**, 465 (1964); *b*) S. Yano and M. Harada, *Jpn. J. Pharmacol.*, **23**, 57 (1973).
3) S. Yano, M. Akahane, and M. Harada, *Jpn. J. Pharmacol.*, **27**, 635 (1977).
4) S. Yano, M. Yamamoto, and M. Harada, *Chem. Pharm. Bull.* (Tokyo), **24**, 1646 (1976).
5) D.A. Brodie, Gastroenterology, **55**, 125 (1968); K. Takagi and S. Okabe, *Jpn. J. Pharmacol.*, **18**, 9 (1968).
6) R.W. Porter, *Am. J. Physiol.*, **169**, 629 (1952).
7) N. Murakami, *Bull. Inst. Const. Med.* (Kumamoto University), **10**, 224 (1959), *ibid.*, **10**, 235 (1959), *ibid.*, **10**, 243 (1959); M. Minakami, *ibid.*, **17**, 256 (1967), *ibid.*, **17**, 270 (1967).
8) M. Kawakami, K. Seto, S. Ishida, M. Yoshida, and M. Yanase, *Saishin Igaku*, **25**, 2118 (1970).

EEGs and evoked potentials in rabbits subjected to restraint stress. Electroencephalographic studies in animals under cold exposure[9] and after forced exercise[10] have also been reported.

Rats are regarded as the first choice of animals especially in the study of stress ulcers. No study, however, has so far been undertaken to observe continuously the state of the CNS in rats subjected to stress capable of developing gastric ulcers in detail. In the present study, continuous measurements of EEGs in rats subjected to restraint and water immersion stress were attempted by means of an apparatus deviced newly for this purpose. Simultaneously, gastric motility was recorded as one of responses of the peripheral organs to stress, because a specific pattern of gastric motility was found to occur under such a severe stress[3,11] and thus, considered to serve as one index which expressed the magnitude of influence of stress on the body. In addition, several experiments were conducted in acutely adrenalectomized rats in order to study influence of the adrenals on EEGs, since the adrenals play an important role in controlling the body functions against stress.

Experimental

Animals——Male Wistar rats weighing between 280 and 360 g were used. They were deprived of food overnight but were allowed free access to water.

Method——1) Stress Method: Three kinds of stress procedures, namely, restraint, restraint and water(35°) immersion, and restraint and water(25°) immersion, were employed. An ether-anesthetized rat receiving a gastric balloon for recording gastric motility as mentioned in Method 2) was restrained in a stress cage of our making.[11b] This cage was made of stainless steel wire meshes designed in the shape of the back of rats and had enough capacity to allow rats to breath without disturbance. Thereafter, the cage with the animal was placed obliquely in an empty bath and the head of the animal was tightly fixed to a stereotaxic instrument (for rats, Takahashi) which had been attached to the bath, by means of a pair of rubber-covered ear bars. Subsequently, electrodes for measuring EEGs were placed as mentioned in Method 3). At the end of these procedures, the animal was freed from ether exposure and was allowed to stand for 100 min. The termination of this period was considered to be the onset of restraint stress (about noon–1: 00 p.m.). In the case of water immersion, animals were immersed to the level of the xiphoid process by filling the bath with water of 25° or 35° at 100 min after the onset of stress, and the bath was maintained at a given temperature throughout the experimental period.

2) Recording of Gastric Motility: Gastric motility was recorded on a kymographion manometrically by means of the balloon method described by us previously.[11b]

3) Recording of EEGs: After the animal was fixed in the stereotaxic apparatus under ether anesthesia as mentioned in Method 1), a pair of metal screw electrodes measuring 0.78 mm in diameter was placed on the occipital cortex for recording electrocorticograms (ECoGs). Hippocampal EEG activities were taken from the Hippocampus (A: −4.5, L: 4, O: 3—3.5, the stereotaxic atlas by Skinner[12]) *via* bipolar parallel copper electrodes measuring 0.24 mm in each diameter. Infiltration of 1% procaine hydrochloride solution was applied to surgical incisions during the operative period when needed. EEGs were continuously recorded for 460 min after the onset of stress (Sanei, polygraph 141-C). At the termination of each experiment, body temperature was measured in the abdominal cavity.

4) Evaluation of Gastric Ulceration: The severity of gastric ulceration (Erosion index) was evaluated as a summation of the length of ulcers according to our method.[2b]

5) Criteria for Occurrence of the Increased Gastric Motility: Occurrence of the increased gastric motility was expressed as the time when the tone level and amplitude reached the maximal response. In the case where the tone elevation was not so manifest, the time of occurrence of the maximal amplitude was used for the measurement.[3]

6) Adrenalectomy: Bilateral adrenalectomy was made by ventral approach just before balloon insertion.

Drug Treatment——In the experiments of adrenalectomized rats, drugs were administered immediately after excision of the adrenals. *l*-Epinephrine bitartrate (Sigma) was infused through a femoral intravenous polyethylene tube in a rate of 40 μg (base)/kg/hr throughout the experiment and ACTH (Acthar Injection, Armour) was infused likewise in a rate of 1 IU/kg/hr. Corticosterone (Merck) was injected through an

9) H. Negoro, *Yokohama Igaku*, **19**, 299 (1968); S. Yamaoka, *Nippon Seirigaku Zasshi*, **31**, 127 (1969).
10) J. Matsumoto, T. Nishisho, T. Suto, T. Sadahiro, and M. Miyoshi, *Nature*, **218**, 177 (1968).
11) *a*) K. Watanabe, *Chem. Pharm. Bull.* (Tokyo), **14**, 101 (1966); *b*) M. Harada and S. Yano, *Pharmacometrics*, **8**, 1 (1974).
12) J.E. Skinner, "Neuroscience, a Laboratory Manual," W.B. Saunders Company, Philadelphia, 1971.

intraperitoneal polyethylene tube in repeated doses of 3 mg/kg every 1 hr. The control animals received saline administration either at 0.5 ml/kg/hr intravenously or at 0.5 ml/kg intraperitoneally every 1 hr.

Results

1) Electroencephalograms under Stress

In the series of these experiments, ECoGs and gastric motility were recorded in order to compare influences of 3 kinds of stress.

a) **Restrained Rats (N=5, Fig. 1)**——Typical ECoG recordings from a single rat are shown in Fig. 1. ECoGs fluctuated throughout the experiment between low voltage rhythmical waves (100—150 μV, 4—5 Hz) and high voltage irregular waves (>150 μV) which included spindles and spikes. When the high voltage irregular waves occurred the animal appeared quiet behaviorally, while the low voltage rhythmical waves were observed when the animal struggled in the cage. Frequency of the rhythmical waves did not decrease up to the end of the experiment.

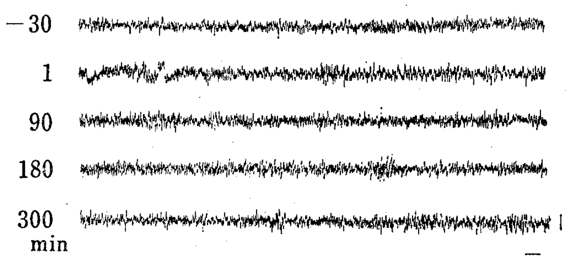

Fig. 1. Electrocorticograms of a Restrained Rat

0 min: Time of water immersion (In this case, water immersion was not carried out). The perpendicular bar and horizontal bar indicate 200 μV and 2 sec, respectively.

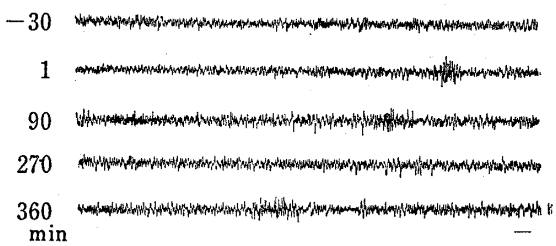

Fig. 2. Electrocorticograms of a Restrained and Water (35°)-immersed Rat

See footnotes in Fig. 1.

b) **Restrained and Water(35°)-immersed Rats (N=6, Fig. 2)**——The changes in ECoGs in this group were similar to those in the restrained group except a transient occurrence of rhythmical waves immediately after water immersion. The animals appeared more quiet behaviorally with a somewhat more frequent occurrence of spindle waves during the whole experiment in comparison with the restrained group.

c) **Restrained and Water(25°)-immersed Rats (N=7, Fig. 3)**——As demonstrated in Fig. 3, ECoGs underwent marked changes as judged from persistence of low voltage rhythmical waves without high voltage irregular waves and spindles after water immersion. Frequency of the rhythmical waves which were the major component of ECoGs after water immersion increased for about 10 min immediately after water immersion and gradually decreased below the levels prior to water immersion. Severe gastric ulcers, specific gastric motility,[3,11b)] and marked hypothermia were observed only in the restrained and water(25°)-immersed group, which showed greater magnitude of this stress in comparison with other two. Erosion index,

Table I. Erosion Index, Occurrence Time of the Increased Gastric Motility, and Final Body Temperature of Three Stressed Groups

Group	No of animals	Erosion index (mm)	Occurrence time (min)	Body temperature (°)
Restraint	5	4.0±1.8	n.o.[a)]	37.9±0.5
Restraint and water immersion (35°)	6	2.6±1.3	n.o.	36.1±0.2
Restraint and water immersion (25°)	7	23.3±4.5	231.7±14.7	28.1±0.6

a) The increased gastric motility did not occur.

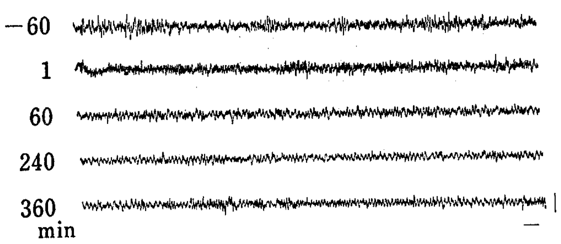

Fig. 3. Electrocorticograms of a Restrained and Water (25°)-immersed Rat

See footnotes in Fig. 1.

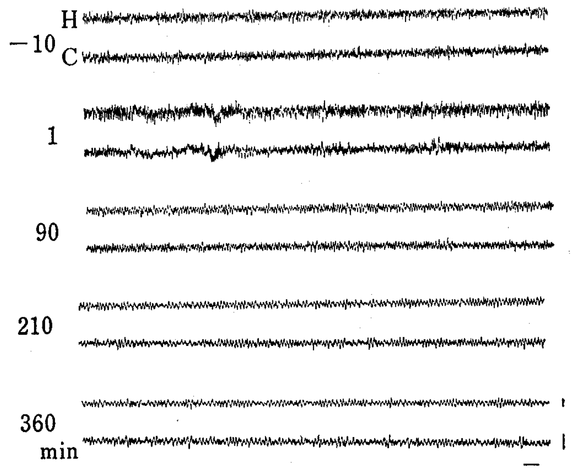

Fig. 4. Electroencephalograms of a Restrained and Water(25°)-immersed Rat

H: hippocampus, C: occipital cortex.
Saline was infused. As to other explanations, see footnotes in Fig. 1.

occurrence time of the increased gastric motility, and final body temperature of the 3 stressed groups are given in Table I.

2) Influence of Adrenalectomy on EEGs under Stress

In the series of these experiments, restraint and water(25°) immersion stress was adopted. In addition to ECoGs, hippocampal EEGs were recorded in order to see the state of the CNS further, gastric motility being also recorded.

a) **Non-adrenalectomized Rats (N=10, Fig. 4)**——Influence of a continuous infusion or repeated injections of saline on EEG activities was not observed. Changes in ECoGs were the same as those obtained in the experiment of Results 1)-c). Hippocampal EEGs fluctuated between rhythmical waves (4—5 Hz) and irregular waves before water immersion and the rhythmical waves were maintained after water immersion with a gradual decrease in their frequencies. Cortical and hippocampal EEG activities changed in parallel.

b) **Adrenalectomized Rats (N=7, Fig. 5)**——Cortical and hippocampal activities in this group during the periods before and after water immersion fluctuated in a similar manner

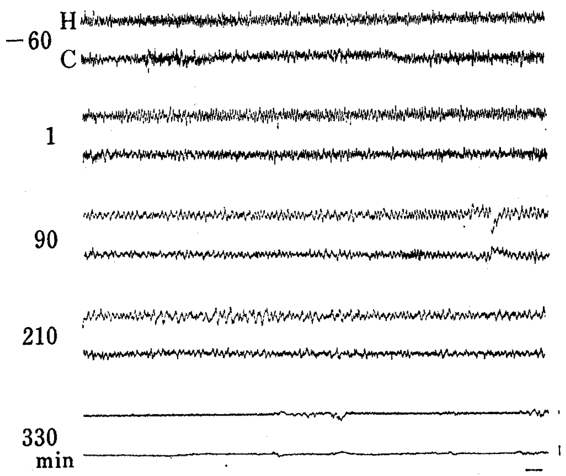

Fig. 5. Electroencephalograms of an Adrenalectomized, Restrained and Water(25°)-immersed Rat

See footnotes in Fig. 4.

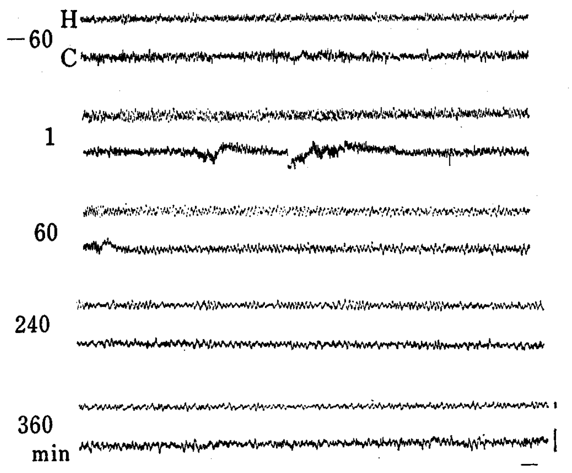

Fig. 6. Electroencephalograms of an Adrenalectomized, Epinephrine-infused, Restrained and Water(25°)-immersed Rat

See footnotes in Fig. 4.

to those in the non-adrenalectomized group. However, after water immersion, frequency of cortical and hippocampal rhythmical waves gradually decreased more compared with that in the non-adrenalectomized group and abnormal waves such as discharges and EEG flattening frequently occurred (in 5 of 7 rats). Among these 5 rats, 4 died before the termination of the experiment.

c) **Adrenalectomized and Epinephrine-administered Rats (N=4, Fig. 6)**——In this group, changes in EEGs were similar to those in the non-adrenalectomized group, although frequency of their rhythmical waves decreased more rapidly, and neither abnormal EEG waves nor death occurred.

d) **Adrenalectomized and Corticosterone-administered Rats (N=4)**——Results obtained in this group were similar to those in the adrenalectomized and epinephrine-administered group.

e) **Adrenalectomized and ACTH-administered Rats (N=4)**——Changes in EEGs in this group were similar to those in the adrenalectomized group. Abnormal EEG waves and EEG flattening occurred in 3 of 4 rats and all of the 3 rats died during the experiment.

Discussion

All of the present experiments were carried out on rats receiving acutely implanted electrodes and an acutely inserted gastric balloon. EEGs of unrestrained rats with chronically implanted electrodes show a series of sleep-wakefulness cycles.[13] A similar cycle pattern was observed in unrestrained rats with acutely implanted electrodes on the occipital cortex and in neck muscles in our preliminary experiments although rate of occurrence of rapid eye movement sleep pattern was decreased or abolished. Restrained rats in the present study also showed an ECoG cycle fluctuating between low voltage rhythmical waves and high voltage irregular waves including spindles and spikes and frequency of the rhythmical waves decreased a little compared with that of unrestrained rats (5—7 Hz). These ECoG figures were different from those of unrestrained rats in that long trains of high voltage irregular waves including spindle bursts did not appear. Cortical low voltage rhythmical waves, which were obtained from the occipital cortical lead in the present experiments, have been observed in the parietal cortical recording.[14] These waves can be elicited by external stimuli such as pinching the foot and correspond to behavioral arousal. Spikes appearing in ECoGs here have been observed in the cortical sleep pattern of curarized rats.[15] ECoGs in the restrained and water(35°)-immersed rats fluctuated in a manner similar to those in the restrained rats although the former animals appeared more quiet and thus, this finding suggested similarity of the CNS activities between both groups. In the restrained and water(25°)-immersed rats, a succession of cortical low voltage rhythmical waves and of hippocampal rhythmical waves was characteristic together with an increased gastric motility, a severe ulceration, and a marked hypothermia. These EEG waves are considered to originate predominantly from stimuli of severe cooling. Impulses from the exteroceptors as well as interoceptors generated by sustained cold stimuli and impulses from the proprioceptors in shivering muscles contribute to the sustained appearance of the rhythmical waves *via* the ascending reticular activating system and hypothalamic activating system. Gaenshirt, *et al.*[16] reported that mean frequencies of the cat ECoGs fell when hypothermia was induced by cooling and that the changes produced by cooling to 20° were reversible, since re-warming permitted recovery

13) E. Roldán, T. Weiss, and E. Fifková, *Electroenceph. Clin. Neurophysiol.*, **15**, 775 (1963); J. Matsumoto, T. Nishisho, T. Suto, T. Sadahiro, and M. Miyoshi, *Proc. Jpn. Acad.*, **43**, 62 (1967).
14) *a*) C.A. Barraclough and C.H. Sawyer, *Endocrinology*, **61**, 341 (1957); *b*) K. Takagi, Y. Kasuya, and S. Tachikawa, *Yakugaku Zasshi*, **87**, 781 (1967).
15) K. Oshima, T. Miyama, and H. Kawamura, *Jpn. J. Physiol.* **12**, 601 (1962).
16) H. Gaenshirt, W. Krenkel, and W. Zylka, *Electroenceph. Clin. Neurophysiol.*, **6**, 409 (1954).

of a normal EEG pattern. Yoshii, *et al.*[17] also reported that frequency of background ECoG potentials in dogs and cats changed almost linearly with body temperature. Decreases in frequency of the cortical and hippocampal rhythmical waves observed here are considered to relate to decreases in body temperature.

Feldman and Robinson[18] reported that adrenalectomy caused a generalized EEG slowing, an increase in amplitude, and occurrence of bursts of high voltage activities in cortical and subcortical regions in rats with permanently implanted electrodes. In the present study, acute adrenalectomy also affected EEGs under stress as shown by a decrease in frequency, abnormal waves and EEG flattening, and threatened maintenance of life, inducing high mortality. These changes could be retarded by epinephrine or corticosterone administration. Accordingly, both substances contributed to maintenance of EEG levels, of the CNS state, and of life under stress. Moreover, the fact that ACTH was not effective in maintaining them indicated a lack of direct involvements of ACTH in them.

17) N. Yoshi, Y. Koyasu, K. Okazaki, and Y. Hasegawa, *Folia Psychiatrica et Neurologica Japonica*, **9**, 121 (1955).
18) S. Feldman and S. Robinson, *J. Neurol. Sci.*, **6**, 1 (1968).