Effects of Enriched and Impoverished Housing Environments on the Electrocorticograms (ECoGs) of Middle-Aged Rats

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ABSTRACT. Electrocorticograms (ECoGs) were evaluated in young rats and in middle-aged (19 months) rats housed under three different environments. The standard condition (SC, N=5) indicated the condition where two rats stayed in a standard cage, enriched condition (EC, N=5) meant keeping 6–8 rats in a large cage and impoverished condition (IC, N=5) was referred to as housing a single rat in a small cage. All middle-aged rats were kept under one of these cage conditions for 12 months, starting at 7 months of age. An ECoG was recorded simultaneously from 6 different locations on the scalp and was subjected to comparisons among the SC, EC and IC by means of spectral analysis. The power of the occipital α band (8.1–10.0 Hz) was significantly increased in IC rats. Total occipital power in IC rats was also enhanced as compared to SC and EC rats. These findings demonstrate that ECoG changes are present in the neocortex of middle-aged rats in different environments. In addition, social interaction seems to have a stronger effect than the differences in living capacity. These results indicate that the aging process should be studied from the viewpoint of environmental influences.—**KEY WORDS:** electrocorticogram (ECoG), enriched and impoverished environment, middle aged rat.

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Recently, in the field of clinical veterinary medicine, the care of aged companion animals which have dementia and abnormal behaviors can cause distress to their owners and family in everyday life. It is very important to know how companion animals age, and to understand what accelerates animal aging, from the standpoint of animal welfare. Animals' environments and their experiences may change the structure of the animal brain, as well as neuronal activity [6, 12, 13]. The studies of Rosenzweig and his co-workers strongly suggest that active environments changed the brain anatomy, enzyme activity and behavior of rats [12, 13], and the housing condition of an experimental animal, whether housed in an enriched or an impoverished environment, caused measurable changes in brain anatomy and chemistry. This relationship between the change of environment and that of brain anatomy was shown to be in the occipital cortex of the animals.

The cholinergic system has a strong relationship to learning and the memory function, as well as to synchronization or desynchronization of electrocorticographic (ECoG) activity [9, 15]. Furthermore, behavioral, physiological and anatomical changes are a natural consequence of aging [1, 2, 4, 14], but there are few reports which describe the relation between environmental effects and neuronal activation in rats, and it is not clear whether the ECoG activities of middle-aged animals change under different environments in the course of advancing age.

In the following experiment, we compared the ECoG activities of normal young rats with three groups of middle-aged animals housed under different conditions. The goal of the study was to determine whether the effects of different environments on middle-aged animals caused a change in cortical electrical activity.

MATERIALS AND METHODS

The environmental conditions of the rat cages were determined from a report [12] describing spaces and environments in detail. In these experiments, the standard condition (SC, N=5) was a stainless steel cage (25 cm $\times$ 26 cm $\times$ 18 cm) housing two subject animals (Table 1). The enriched condition (EC, N=5) was a large size stainless steel cage (36 cm $\times$ 59 cm $\times$ 38 cm) housing six rats in which forty types of equipment for rat play were placed and changed everyday. The impoverished condition (IC, N=5) had minimum surrounding stimulation and each rat was housed individually in a stainless steel cage (16 cm $\times$ 31 cm $\times$ 20 cm). On the initial day of the experiment, middle-aged male Wistar rats were purchased from a breeder (SLC Co., Ltd.) at the age 7 months and were kept under these conditions for 12 months in a controlled colony room: temperature $21 \pm 1^\circ\text{C}$, humidity 50–60% and 12 hr (8:00 hr – 20:00 hr) lighting. Pellets (Clea Co., Ltd., CE-2) of food and water were freely available. At the end of the 12 month period, young (8 weeks old) male Wistar rats were also purchased from the same breeder.

The 19-month-old and 8-week-old rats were anesthetized with pentobarbital sodium (50 mg/kg, *i.p.*) for surgery. The rats were placed in a stereotaxic apparatus (David Kopf Instruments) with bregma and lambda in the same horizontal plane. After checking for deep anesthesia, a local anesthetic (xylocaine, 1.0%) was injected into the rat scalp to eliminate pain. Then six recording electrodes (stainless steel screws, 1 mm in diameter) were symmetrically placed on both sides of the skull at the following coordinates [8]: Right and left frontal (RF and LF), $L = \pm 2.0$ mm from midline, $AP = 3.0$ mm from bregma (Abbreviations: L, lateral; AP, anterior-posterior); Right and left occipital (RO and LO), $L = \pm 4.0$

Table 1. Description of the three rat cage housing conditions used in the present experiment. These conditions are reported by Rosenzweig *et al.* [12]. Forty kinds of equipment for rat play were placed and changed everyday in the enriched condition. A capacity of a cage in each group was calculated. The capacity per rat in each cage was calculated

Group	Cage capacity (cm ³)	Number of animals in a cage	Capacity per rat in per cage (cm ³)	Tool existence
Standard condition	19760	2	9880.0/rat	—
Enriched condition	80712	6	13453.5/rat	40 kinds
Impoverished condition	9920	1	9920.0/rat	—

Fig. 1. The positions of the electrodes in the skull are indicated by dots (A). The monopolar ECoGs (each electrode vs reference electrode) of the enriched condition rats during immobility are shown (B). The electrode positions of the other subjects are the same as in this representation. Abbreviations, LF: left frontal area, RF: right frontal, LT: left temporoparietal, RT: right temporoparietal, LO: left occipital, RO: right occipital, R: reference electrode.

mm, AP=4.5 mm; Right and left temporoparietal (RT and LT), L=±2.5 mm, AP=7.5 mm (Fig. 1). The reference electrode (R, stainless steel screw, 1 mm in diameter) was located in the midline above the cerebellum (Fig. 1). All the screw electrodes were fixed to the cranial bone with dental cement. The experimental measurements were carried out after a recovery period of at least one-week.

The rats were allowed to move freely in a plexiglass cage (31 cm × 25 cm × 18 cm) in a electro-magnetically shielded room (180 cm × 180 cm × 180 cm), and the ECoG data were recorded between 10:00 hr and 13:00 hr for approximately 1 hr. The electrodes were connected to a multichannel amplifier (NEC, Synafit 1000) by a multichannel flexible cable. The bandpass filter was set from 0.5 to 100 Hz. The animal behavior described by the observers' voices and ECoG data were simultaneously recorded on a digital audio tape (DAT) recorder (TEAC, RD-111T) in order to make coincident the off-line data analyses.

The ECoG data on the DATs were fed into a microcomputer with an A/D converter (NEC SAN-EI, 7T18A) which was used to determine the ECoG power spectra. Artifact-free ECoG segments of 8 sec each during immobility were sampled at 128 Hz. Each channel

spectrum, processed by a Hamming window, was averaged 15 times for each subject, and the ECoG spectra were calculated with Fast Fourier Transformation (FFT). Each spectrum was further compressed into four frequency bands: delta waves (δ), 0.0–4.0 Hz; theta waves (θ), 4.1–8.0 Hz; alpha waves (α), 8.1–10.0 Hz; and beta waves (β); 10.1–25.0 Hz [4, 14]. The total power values (N) were calculated and compared among the SC, EC and IC environment groups.

$$N = \sum_a^b (A_n(T))$$

Where N is the total energy of ECoG power, a is the minimum frequency, b is the maximum frequency, T is the averaging number, and $A_n(T)$ is the address of the sampling mean frequency at the n point. The mean frequency (M) of each channel ECoG was also calculated:

$$M = \frac{\sum_a^b (n \cdot A_n(T))}{N} (H_z)$$

$$M = \frac{\sum_a^b (n \cdot A_n)}{\sum_a^b A_n} (H_z)$$

The statistical analysis consisted of estimating the mean, S.D. and range for each parameter measured. The significance of differences ($p < 0.05$) among the SC, EC and IC groups was determined by the Student's t -test.

RESULTS

The rats with enriched experiences had considerably greater behavior activities in the conditioned cage and in the experimental box. The rats with impoverished experiences, however, moved not only in the conditioned cage but also in the experimental box. the behavioral activity of the SC rats was the same as that of the IC rats.

The mean frequencies of the ECoG in the three conditioned rat groups were slower than in the young rats, except for the LF of the IC rats (Table 2). In the conditioned rats, the mean frequencies of the ECoG in the bilateral-occipital areas tended to be slower than in the other areas. In the young rats, however, the mean frequencies of the ECoG did not differ significantly.

The alpha band power values of the bilateral-occipital areas of the IC rats were greater than those of the SC and EC rats (Fig. 2). The bilateral power distributions of the EC rats were not identical in the LF and RF, EC rats, in particular, the beta band value of the LF in the EC rats was higher than that of the SC rats ($p < 0.05$) and the theta band value of the LF in the EC rats was lower than that of the SC rats ($p < 0.05$). The power distributions of the bilateral-occipital areas were greater than those of the bilateral-temporoparietal and frontal areas in all environment conditions.

The total power values of bilateral-occipital and temporoparietal areas in the IC rats tended to be greater than those of the SC and EC rats (Fig. 3) and the temporopar-

Table 2. Mean frequency of ECoG activity recorded during immobility in the bilateral-frontal, -temporoparietal and -occipital areas of SC, EC, and IC middle-aged and young rats. Calculation method is described in the text. There are no significant differences among SC, EC and IC rats

Group	Mean frequency (Hz)					
	Frontal		Temporoparietal		Occipital	
	Left	Right	Left	Right	Left	Right
SC	6.1±0.1 ^{a)**}	6.3±0.6	6.5±1.0	6.3±0.2 ^{**}	5.6±0.4 ^{**}	6.0±0.9
EC	5.8±0.4 ^{**}	6.4±0.9	6.1±2.2	6.3±0.5 ^{**}	5.9±0.4 ^{**}	5.7±0.5 ^{**}
IC	6.8±0.8	5.7±0.9	6.3±2.6	6.5±0.7	6.0±0.3 ^{**}	6.0±0.6
YG	6.7±0.3	6.5±0.3	6.9±1.8	7.1±0.2	6.9±0.4	6.9±0.5

a) Mean±S.D.

^{**} $p < 0.01$ vs young rat.

Fig. 2. Spatial distribution of relative power of ECoG in SC, EC and IC middle-aged rat. Locations of recording electrodes are shown in Fig. 1. *, vs SC group and $p < 0.05$; **, vs SC group and $p < 0.01$; +, vs EC group and $p < 0.05$. Abbreviations, LF: left frontal area, RF: right frontal, LT: left temporoparietal, RT: right temporoparietal, LO: left occipital, RO: right occipital, δ : delta waves (0–4 Hz), θ : theta waves (4.1–8.0 Hz), α : alpha waves (8.1–10.0 Hz), β : beta waves (10.1–25.0 Hz), SC: Standard condition, EC: Enriched condition, IC: Impoverished condition.

Fig. 3. Spatial distribution of total power values of ECoG in middle-aged and young rats in the three experimental conditions. Values from corresponding electrodes of the left and right hemispheres were averaged. Recordings were made from six locations in Fig. 1. *, vs SC group and $p < 0.05$; +, vs EC group and $p < 0.05$; #, vs Young group and $p < 0.05$; ##, vs Young group and $p < 0.01$. SC: Standard condition, EC: Enriched condition, IC: Impoverished condition; YG: Young Group.

Fig. 4. High voltage spindle recorded from one of the 19-month-old rats during immobility. Amplitude of HVS in frontal areas was approximately 400 μ V and more than four times that of the irregular activity during immobility. The occurrence of HVS from the bilateral-frontal and -temporoparietal areas in the middle aged rat was more marked than in the young rat. Locations of recording electrodes are shown in Fig. 1. Abbreviations, LF: left frontal area, RF: right frontal, LT: left temporoparietal, RT: right temporoparietal, LO: left occipital, RO: right occipital, R: reference electrode.

ietal area power values of the IC rats were also higher than those of the other conditions, but there were no significant differences among them. In the power value of the bilateral-frontal area, there were side-differences in the EC and IC rats and the total power value in the IC rats tended to be more markedly different when compared with those of the other conditions.

In this study, all the rats used displayed a clear high voltage spindle (HVS) (Fig. 4) and HVSs occurred almost exclusively during immobility. The characteristics of HVS were as follows: 1) HVSs were observed during immobility; 2) the electrode positions associated with definite HVS appearance were those in the frontal and temporoparietal areas; 3) HVSs were inhibited by sound stimuli such as hand clapping.

DISCUSSION

The present study was designed to investigate the effects of environment on the ECoGs of middle-aged rats. The general aspects of ECoGs included a tendency for slow waves to appear in middle-aged rats more than in young rats (not shown in figures). The major findings of this study are that the environments affected middle-aged rat ECoG components, and the environment condition particularly affected the occipital-cortical area in middle-aged rats.

These data reveal that rats living in impoverished conditions for 12 months from 7 months of age, have different ECoG changes from rats living in other environmental conditions, and that the most marked difference of environmental condition among the rats in the EC, IC or

SC seemed to be social interaction with other subjects in the experimental cage (Table 1). The cage capacities per rat between the SC and IC were quite similar, but the IC rats were individually housed for 12 months. In the SC and EC, the capacity per rat was quite different, but the occipital area ECoGs and the other ECoG characteristics did not differ greatly (Table 1, Figs. 2 and 3). An identical element of the SC and EC was social interaction among the subjects, and there was a tendency for the ECoG characteristics of rats in these conditions to be similar.

Buzsáki *et al.* suggested that the effects of aging in the rat cortex appeared in the temporoparietal and occipital regions, as shown by electrophysiological methods [4]. Morphological studies have also shown that cell death and the degeneration of cholinergic neurons in the occipital cortex occurred earlier than in the frontal and temporoparietal cortex [12, 13]. Furthermore, a characteristic of the aged-rat ECoG is that the alpha band power value of the occipital cortex is greater than that of the young rat [4, 14] which may suggest that the changing of the ECoG alpha band power value is one of the aging markers of the rat.

The present results of the power distributions in the occipital area are consistent with those of the morphological investigations and other reports of ECoG of the aged rat [4, 12, 14]. The occipital region was more affected by the difference of environments than the other regions, which indicated a greater difference between the enriched and impoverished experiences. Buzsáki and the other researchers found that the HVS of the rat was one of the major parameters of aging [3, 4, 10, 11, 14], and our results also demonstrated that HVSs from the frontal and temporoparietal regions, and the occurrence of HVSs in middle-aged rats was more marked than in young rats. Whilst other groups have shown that cortical spindles such as HVSs are related to the epilepsy model in inbred rat strains [5, 7], there is no clear correlation of the HVSs, including cortical spike-wave discharges, to enriched or impoverished experiences and our hypothesis is therefore that the ECoG components, the spindle waves excepted, are strongly related to aging elements and environmental conditions in the rat.

The problem of companion animals' aging is an inevitable phenomenon. The present study suggests that the progressive elements of degenerative brain disorders, such as memory deficits and/or dementia in aged animals are influenced more by social interaction than by the living space. In terms of animal welfare therefore, it is necessary for us to investigate the aging process of the animal brain focusing on social interactions.

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