成長に伴うビーグル犬の non-REM 睡眠脳波の変化

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Developmental Electroencephalogram of Non-REM Sleep in Beagle Dogs

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ABSTRACT. Cross-correlation and power spectral analyses along with visual analysis were applied to electroencephalograms recorded during the NREM sleep in a litter of 5 beagles in order to evaluate the changes due to aging, and to establish the age-matched reference data for the clinical application. Based upon results obtained, the developmental course of the NREM sleep during the 1st year of life could be divided into 4 stages; from 0 to 6 weeks, from 6 to 14 weeks, from 14 to 24 weeks and from 24 to 50 weeks of age.—KEY WORDS: beagle dog, cross-correlation, developmental EEG, NREM sleep, power spectrum.

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Electroencephalogram (EEG) in the normal dog was investigated in unanesthetized and physically restrained adults [10, 17], in lightly anesthetized adults [12], and in maturing dog [1, 4–7, 9, 14–16, 18]. They, except Klemm [12], analysed EEGs visually. Recent advances in the medical electronics have succeeded in analyzing the EEG quantitatively and Klemm [12] used an electronic interval-histogram analysis.

It is well known that the stage of non-REM (NREM) sleep is suitable for obtaining artifact-ree records of EEG, and furthermore, electroencephalographic abnormalities are frequently displayed during sleep. On the other hand, regardless of the behavioral state, the stage of maturation of the brain inevitably influences the electroencephalographic pattern.

The aim of the present study was to examine electroencephalographic features of the NREM sleep in the maturing dog by means of the cross-correlation and power spectral analyses and also to establish the age-matched reference data for clinical diagnosis.

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MATERIALS AND METHODS

Animals: A litter of 5 beagles, 3 males and 2 females, was used from 0 week to 50 weeks of age.

EGG recording: Recordings were carried out weekly from 0 to 12 weeks of age, biweely from 12 to 26 weeks of age and at an interval of 4 weeks from 26 to 50 weeks of age, respectively.

Dogs were led into the quiet and dim room maintained at a room temperature of 23 to 29°C after taking postprandial outdoor exercise. After the electrodes were placed at appropriate sites (mention later), the dog was laid in a lateral recumbent position on a table without restraint. When the dog closed eyes, breathed regularly, relaxed without jerking, not responsed to noise and showed apparent slow or slackening (in neonate puppies) waves of the electrical activity, the state was regarded as NREM sleep [7, 11] and the EEG was taken. The respiratory rate was counted and the behavioral state of

the dog during recording was monitored. It was confirmed by this respiratory count that no deflections synchronizing with respiration existed in the EEG wave form.

Anterior and postrior placements were employed on the scalp. The placement of exploring electrodes was as follows. The distance between the zigomatic process of the frontal bone and the external occipital protuberance was divided into three. The site at the anterior one-third on the mid-line of either left or right half of the skull served as the anterior placement: the left and right anterior sites (LA and RA), and likewise the site at the posterior one-third serves as the posterior placement: left and right posterior sites (LP and RP). The reference and ground electrodes were placed on the nose (nasal reference recording [12]) and on the dorsal surface of the neck, respectively.

Electroencephalographic needle electrodes (made by San-ei) were employed for exploring, reference and ground electrodes throughout the experiment. The electrode was a stainless steel needle 15 mm long. They passed through the skin without contact with underlying muscles.

The EEG was amplified at a time constant of 0.3 sec with a high cut-off frequency of 100 Hz by an amplifier (San-ei 331-type medical oscilloscope). As the amplitude of the EEG varied markedly during maturation, the amplification was set at either 100 μV/cm for dogs at 0 to 6 weeks of age and more than 30 weeks of age, or 200 μV/cm for dogs at 7 to 26 weeks of age. The EEG was recorded by a data recorder (TEAC R-61-type) and also by a pen recorder (San-ei RA-101-type) with a pen deflection of 200 to 400 μV/cm at a paper speed of 3 cm/sec.

EEG processing: Output from the data recorder was fed into a signal processor (San-ei 7T07A-type) for the cross-correlation (San-ei program 0–9303–100) and power spectral analyses (San-ei program 0–9302–100) via the amplifier.

To evaluate the degree of the in-phase activity between two EEGs, normalized cross-correlation coefficients (NCC) at $\tau=0$ (zero timeshift) were calculated for pairs from LA, RA, LP and RP on the linear average system. Output from the data recorder was fed into the signal processor at an amplification of 200 μV/cm. The signal delay time was set at 10 msec. The number of arithmetic operation times was set according to an available length of EEGs in relation to the age, that is, at 5 (the total langth of about 30 sec) for 0 to 5 weeks of age, at 9 (about 50 sec) for 6 to 26 weeks of age and at 18 (about 100 sec) for more than 30 weeks of age, respectively.

To evaluate the power of an EEG at various frequency components, the power spectral analysis was employed on the linear average system. Output from the data recorder was fed into the signal processor at an amplification of 200 μV/cm. The signal analyzing frequency resolution was set at 0.195 Hz and the input signal maximum frequency at 50 Hz. The number of arithmetic operation times was the same as in the case of the cross-correlation analysis. Power distributing from 0.1952 to 49.9711 Hz (real frequency) was designated as 100% and the ratio of power at each frequency (Table 1) was expressed as percentage.

In adition, over-all frequencies and amplitudes were measured manually on the original records and the electroencephalographic pattern was analyzed visually.

RESULTS

For cross-correlation analysis means of 5 dogs were used. Interhemispheric cross-correlation was shown in Fig. 1.

At 0 to 5 weeks of age the NCC between the anterior sites was +0.440 at 0 week of age. NCC were low and varied irregularly. Most of them remained below +0.600 dur-

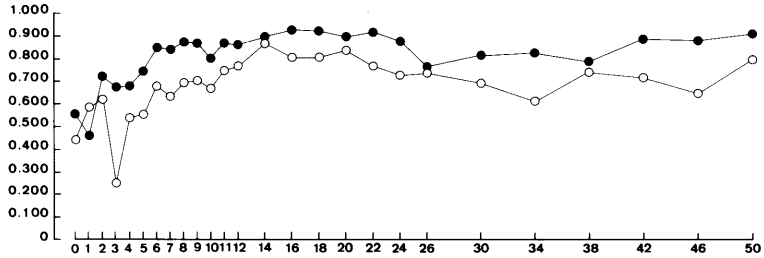

Fig. 1. Developmental course of in-phase activity between the interhemispheric homologous sites. The ordinate indicates the mean cross-correlation coefficient at $\tau=0$ (N=5) and the abscissa the age in weeks. ○—○: Between the left and right anterior sites (LA-RA). ●—●: Between the left and right posterior sites (LP-RP).

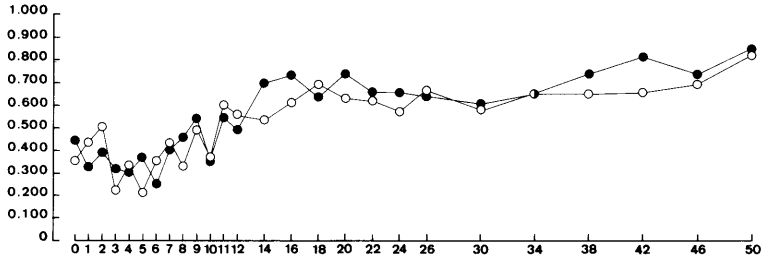

Fig. 2. Developmental course of in-phase activity between the intrahemispheric heterologous sites. ○—○: Between the left anterior and posterior sites (LA-LP). ●—●: Between the right anterior and posterior sites (RA-RP). Details are in Fig. 1.

ing this period. On the contrary, the NCC between the posterior sites increased with increasing age, from +0.557 at 0 week to +0.749 at 5 weeks of age and its increase from 1 to 2 weeks of age was prominent. At 6 to 20 weeks of age the NCC between the anterior sites reached +0.683 at 6 weeks of age and increased gradually until 14 weeks of age to +0.800 or over. High NCC over +0.800 were maintained from 14 to 20 weeks of age. As compared with the anterior site, the NCC bwteen the posterior sites showed a high value of +0.854 at 6 weeks of age and then high NCC of over +0.800 were maintained. Particularly, very high NCC of more than +0.900 persisted from 14 to 20 weeks of age.

At 22 to 50 weeks of age the NCC between the anterior sites was decreased to +0.700 at 22 weeks from +0.842 at 20 weeks of age and further decreased toward a NCC of +0.615 at 34 weeks of age. Thereafter NCC varied around +0.700. The NCC at 50 weeks of age was +0.803. Between the posterior sites, the NCCs were generally higher (>+0.760) but showed a similar decreased from 26 weeks to 38 weeks of age. Subsequently the NCC increased gradually and reached +0.919 at 50 weeks of age.

Table 1. Number of frequency component (k) and its real frequency (Hz)

k	Hz	k	Hz	k	Hz
1	0.1952	11	2.1472	21	4.0992
2	0.3904	12	2.3424	22	4.2944
3	0.5856	13	2.5376	23	4.4896
4	0.7808	14	2.7328	24	4.6848
5	0.9760	15	2.9280	25	4.8800
6	1.1712	16	3.1232	26	5.0752
7	1.3664	17	3.3184	27	5.2704
8	1.5616	18	3.5136	28	5.4656
9	1.7568	19	3.7088	29	5.6608
10	1.9520	20	3.9040	30	5.8560

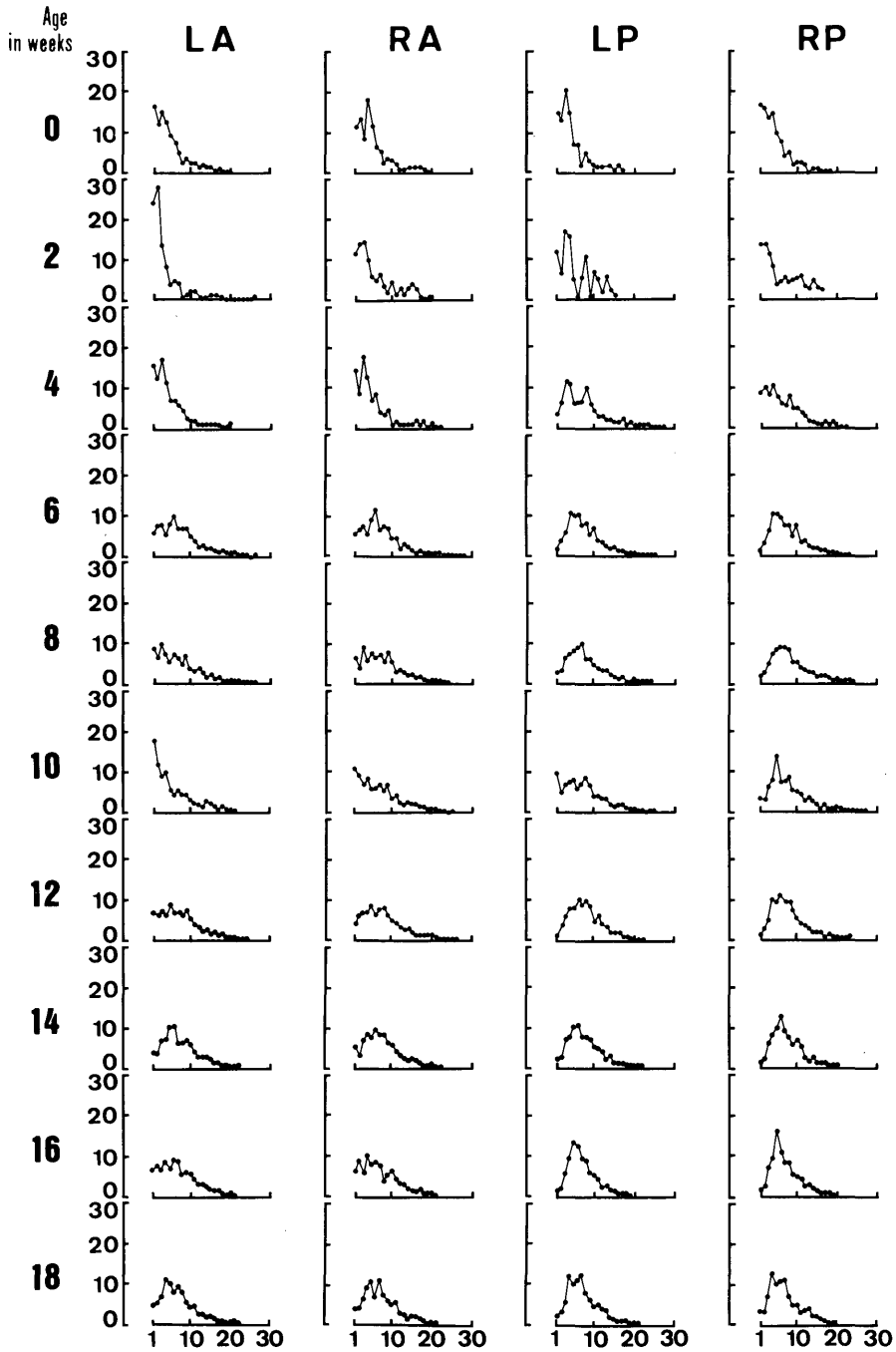

Fig. 3. Power distribution from 0 to 18 weeks of age. The ordinate indicates the mean power expressed in percentage (N=5) and the abscissa the number of frequency component (See Table 1). LA and RA: Left and right anterior sites, respectively. LP and RP: Left and right posterior sites, respectively.

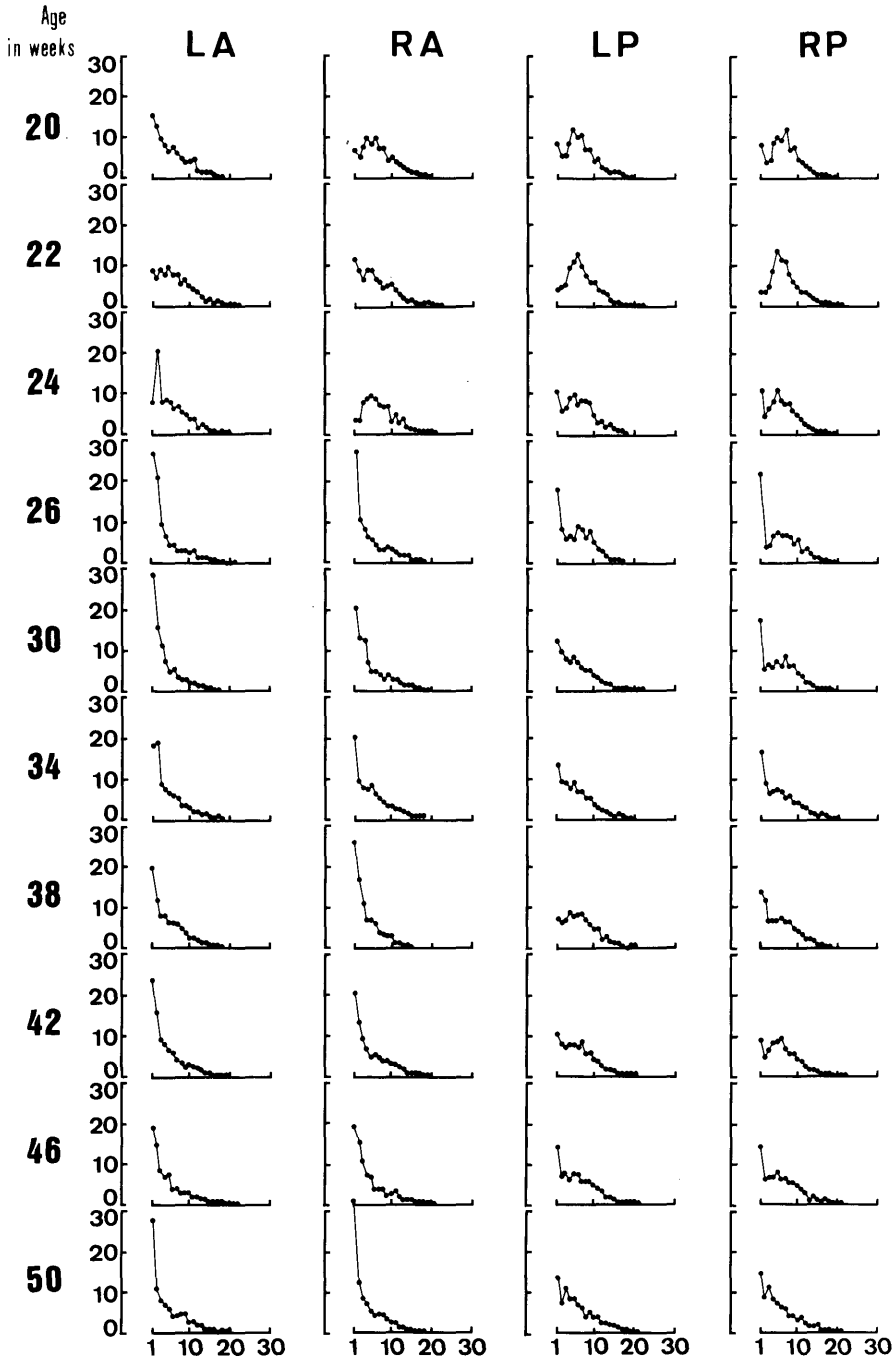

Fig. 4. Power distribution from 20 to 50 weeks of age. Details are in Fig. 3.

Intrahemispheric cross-correlation was presented in Fig. 2. At 0 to 12 weeks of age low and fluctuating NCC were obtained during this period. Most of them remained less than +0.500 but they increased with age. NCC did not different between the left and right sides.

At 14 to 30 weeks of age the NCC exceeded +0.500 after 14 weeks of age on either the left or right sides. During this period, the NCC remained stable ranging approximately from +0.600 to +0.700. At 34 to 50 weeks of age an increase in the NCC started again at 34 weeks of age and the NCC increased gradually from +0.659 at 34 weeks of age to +0.822 at 50 weeks of age on the left side and from +0.656 to +0.845 on the right side, respectively.

For power spectral analysis means of 5 dogs were used. Percent values above 0.5% could not observed at any frequency component higher than 5.856 Hz (k=30) (Table 1 and Figs. 3, 4), therefore, the abscissa of the power spectral figures were illustrated within k=30 (Figs. 3, 4).

At 0 to 4 weeks of age, much different power distributions were observed even between the homologous sites (Fig. 3). Power at frequencies less than 1 Hz occurred frequently at 10 to 20% levels and amounted to more than 50% of the entire power with the exception of the posterior sites at 4 weeks of age (Fig. 5).

At 6 to 18 weeks of age, difference in the power distribution between the homologous sites almost disappeared at 6 weeks of age and the similarity between the homologous sites increased with age (Fig. 3). Furthermore, the power distribution was shifted from a biased pattern to a dome-like one due to a decrease in power at frequencies less than 1 Hz and an increase in power at frequencies of 1 to 2 Hz (Fig. 5). At 14 weeks of age, the power distribution showed a very similar patern between the 4 sites, at LA, RA, LP and RP (Fig. 3).

At 20 to 24 weeks of age the dome-like pattern began to get collapse from 20 weeks of age at the anterior sites and from 24 weeks of age at the posterior sites (Fig. 4). This resulted from an increase in power at frequencies of less than 1 Hz rather than a decrease in power at frequencies of 1 to 2 Hz (Fig. 5).

At 26 to 50 weeks of age, a marked similarity of the power distribution between the homologous sites was noted (Fig. 4). The power distribution was more biased at the anterior site than at the posterior site (Fig. 4). After 26 weeks of age the power at frequencies less than 1 Hz clearly increased at the anterior sites and slightly at the posterior sites resulting in more than 50% of the entire power at the anterior sites and more than 40% at the posterior sites. On the other hand, power at frequencies of 1 to 2 Hz decreased (Fig. 5).

Visually EEGs were of low amplitude (10 to 50 μV) and occasionally equipotential at 0 week of age. Waves with the frequency of 2 to 6 Hz and the amplitude of 100 to 180 μV were rarely observed. Fluctuations with the frequency less than 1 Hz and the amplitude less than 50 μV was also observed. The spindle-like activity at frequencies ranging 8 to 14 Hz and of a low amplitude occurred irregularly. At 2 weeks of age equipotential periods and fluctuations of less than 1 Hz decreased or disappeared. Slow waves of 1.5 to 4 Hz were first seen during this week. Slow waves appeared at all the site but they were still of low amplitude of 20 to 50 μV. Spindle-like activity at 12 to 13 Hz and of 20 to 40 μV was superimposed on the slow activity.

At 4 weeks of age slow wave activity at 2 to 4 Hz and of 100 to 200 μV was clearly noted at all the sites, particularly at the posterior site. Faster activity of 5 to 10 Hz and of 40 to 60 μV was also observed.

At 6 weeks of age slow waves with the frequency of 2 to 4 Hz and the amplitude of

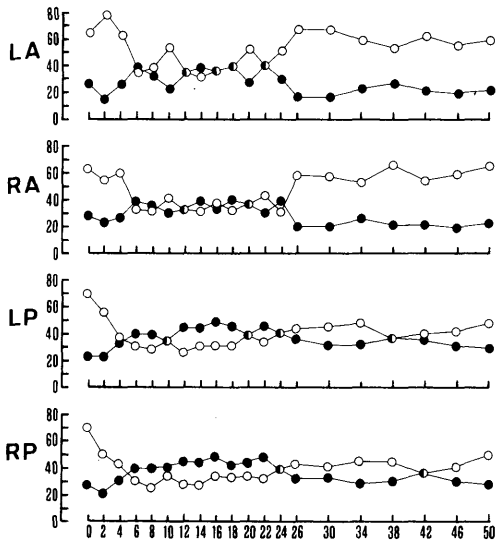

Fig. 5. Developmental course of mean power at frequencies of less than 1 Hz and 1 to 2 Hz. The ordinates indicate the mean power expressed in percentage (N=5) and the abscissa the age in weeks. ○—○: Sum of mean power at frequencies of less than 1 Hz (0.1952–0.9760 Hz, See Table 1). ●—●: Sum of mean power at frequencies of 1 to 2 Hz (1.1712–1.9520 Hz, See Table 1). LA and RA: Left and right anterior sites, respectively. LP and RP: Left and right posterior sites, respectively.

100 to 200 μV at the anterior site and slow waves with the frequency of 1 to 3 Hz and the amplitude up to 300 μV at the posterior site were observed. Spindle activities were interspersed and slight activities at the frequency of 5 to 10 Hz were also superimposed. An increase in the amplitude and a decrease in the frequency at the posterior site were characteristic.

At 8 weeks of age the amplitude of slow waves increased further, reaching about 300 μV at the anterior site and approximately 500 μV at the posterior site. Dominant frequency was 1 to 4 Hz and in particular, activities at 1 to 2 Hz with a high amplitude were marked.

At 10 to 18 weeks of age continuity of the slow wave activity increased. Difference in the amplitude of slow waves between the anterior and posterior sites disappeared after 14 weeks of age due to an increase in the amplitude at the anterior site, and the over-all amplitude of slow waves was 400 to 500 μV.

At 20 to 26 weeks of age continuity of slow wave activity decreased. Furthermore, the amplitude of slow waves decreased with age, especially at the anterior site. At 20 weeks of age, the amplitude was reduced to 200 to 300 μV at the anterior site and to 250 to 400 μV at the posterior site. At 26 weeks of age, it was approximately 100 μV at the anterior site and 250 to 400 μV at the posterior site. Dominant frequency was 2 to 4 Hz at the anterior site and 1 to 3 Hz at the posterior site. Incidence of waves less than 1 Hz was low.

After 30 weeks of age, slow waves appeared sparsely and were of low amplitude, usually 50 to 100 μV either at the anterior or posterior sites. No distinguishable changes could be observed visually throughout this period.

Abnormal electroencephalographic waves were not observed throughout the experimental period.

DISCUSSION

In this study, two kinds of electronic analyses were employed for finding the electroencephalographic features in growing dogs, the NCC at $\tau=0$ which indicated the degree of the in-phase activity between 2 sites and the power spectrum which showed the distribution pattern of power in relation to the frequency. Furthermore, visual analysis was employed for detecting the features of the original wave forms. The results obtained indicate that developmental course of EEG in the NREM sleep during the first year of life in beagle dogs can be divided into 4 stages of 0 to 6 weeks, 6 to 14 weeks, 14 to 24 weeks and 24 to 50 weeks of age as follows.

The NCC between the anterior sites increased from +0.440 at 0 week to +0.683 at 6 weeks of age and, similarly, the NCC between the posterior sites increased from +0.557 to +0.854. These indicated that the interhemispheric in-phase activity between the homologous sites became marked at 6 weeks of age. On the other hand, the discrepancy in the power distribution between the homologous sites, which was particularly remarkable at 0 and 2 weeks of age, almost disappeared at 6 weeks of age. It was also revealed visually that slow waves of low amplitude began to occur at every site from 2 weeks of age and slow waves of high amplitude were observed particularly at the posterior site at 6 weeks of age.

The NCC between the anterior sites increased gradually from +0.683 at 6 weeks to more than +0.800 at 14 weeks of age and also the NCC between the posterior sites increased more gradually but exceeded +0.900 at 14 weeks of age. The NCC between the intrahemispheric heterologous sites exceeded +0.500 after 14weeks of age. Considerablly high interhemispheric and intrahemispheric in-phase activities indicate that EEG from the entire cerebral cortex takes a similar phase relation at 14 weeks of age. At this age, the power distributions at the 4 sites showed a very similar pattern and furthermore, the power distribution was shifted from a biased pattern to a dome-like one. Visually, the amplitude of slow waves increased with increasing age from 4 to 14 weeks of age and continuity of slow waves increased. At 14 weeks of age, the over-all amplitude of slow waves was similar between the anterior and the posterior sites. A gradual increase in the amplitude of slow waves with age was previously reported to occur up to 8 weeks [1] or 10 weeks [4–7, 15, 18] of age.

The NCC between the anterior sites was more than +0.800 from 14 to 20 weeks of age and it decreased gradually toward +0.615 at 34 weeks of age and thereafter the NCC fluctuated around +0.700. The NCC between the posterior sites remained at approximately +0.900 from 14 to 24 weeks of age, showed a small decrease from 26 to 38 weeks of age and gradually increaased again. Thus, the phase relation between the interhemisphric homologous sites were not simple. The NCC between the intrahemispheric heterologous sites remained rather stable from 14 to 30 weeks of age and thereafter showed a gradual increase from +0.659 to +0.822 on the left side or from +0.656 to +0.845 on the right side during a period of 34 to 50 weeks of age. This indicated that the intrahemispheric in-phase activity was further augmented with increasing age. The power distribution was again shifted from a dome-like pattern to a biased one at both the anterior and posterior sites after 24 weeks of age. According to the visual analysis, slow waves decreased in their continuity and amplitude after 20 weeks of age. Considering these electroencephalographic features, the period between 14 and 50 weeks of age may be divided into two at 24 weeks of age. However, physiological basis inducing these spectral changes is still unclear.

After all, the electroencephalographic maturing process of the NREM sleep during the first year of life in beagle dogs was divided into 4 stages, that is, the first stage from 0 to 6 weeks of age, the second stage from 6 to 14 weeks of age, the third stage from 14 to 24 weeks of age and the fourth stage from 24 to 50 weeks of age.

The EEG pattern is influenced by various factors inducing morphological development of the brain. In the period of 2 to 4 weeks of age, the neuronal elements of the cerebral cortex showed dramatic changes such as development of dendritic components, reduction of the neuronal density, a rapidly occurrence of myelination and a marked increase of the brain weight [2, 3,

8]. The above-mentioned striking electroencephalographic changes during the first 6 weeks of life seem to correspond to the reported dramatic morphological changes. The intrahemispheric in-phase activity was low and the power distribution was different between the heterologous sites (for example, between LA and LP) even at 6 weeks of age. After the rapid morphological brain development occurring in the first 2 to 4 weeks of life, the development still continues at a slower rate till the structures of the central nervous system are almost completed at 10 weeks of age [8]. The EEG after the first 6 weeks of life seems to reflect this later morphological maturation.

Senba *et al.* [18] observed that the amplitude of the slow waves of 0.5 to 3 Hz continued to decrease during a period from 13 to 50 weeks, and assumed that increasing thickness of the skull and the temporal muscles would diminish the amplitude. In this study, a similar decrease was observed after 20 weeks of age. Since age-related changes in the cross-correlation and the power distribution were observable till 50 weeks of age, the diminution of the slow waves would not solely be attributable to such anatomical changes.

Final maturation of the NREM sleep in the dog was reported to occur at 3 months [9] or 50 weeks of age [18]. The definitive time of maturation was not find out in this study due to a lack of data on dogs beyond 1 year. Further studies are necessary to clarify it.

The results obtained here may serve as an age-matched reference in evaluating diseases diagnosed by slow waves.

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要　約

成長に伴うビーグル犬の non-REM 睡眠脳波の変化：高橋明男・稲田七郎（鹿児島大学農学部家畜生理学教室）――同腹5頭のビーグル犬の non-REM 睡眠の記録を目視的に生後1年間観察するとともに，相互相関分析およびパワースペクトル分析によって定量的に解析した。non-REM 睡眠の成長に伴う変化は，脳波上，4期に分けられ，第1期は0～6週齢，第2期は6～14週齢，第3期は14～24週齢，第4期は24～50週齢であった。