Visual Evoked Potentials in Guinea Pigs with Brain Lesion

Takashi TAKEUCHI, Minoru SUZUKI, Kiitiro SITIZYO, Reiko ISOBE, Toshiyuki SAITO[1)], Takashi UMEMURA[2)], and Akinori SHIMADA[2)]

Departments of Veterinary Physiology, [1)] Veterinary Pharmacology, and [2)] Veterinary Pathology, Faculty of Agriculture, Tottori University, Koyama-cho, Tottori 680, Japan

(Received 10 December 1991/Accepted 6 May 1992)

ABSTRACT. Visual evoked potentials (VEPs) were recorded in 10 adult male guinea pigs with brain lesion. Lesions were produced in 5 animals by superficial suction of the occipital lobe. The other 5 animals were orally administered with hexachlorophene (about 35 mg/kg/day) for 28 days. In the VEP following the ablation of the occipital lobe, the peaks P_{10}, N_{20}, P_{55}, N_{75}, N_{140} and P_{200} disappeared in many cases. The amplitude of the peak N_{40} decreased to approximately one half its control VEP. In the VEP obtained from the animals administered with hexachlorophene, the peak latencies of N_{20}, P_{30}, P_{55}, N_{75} and P_{100} were slightly prolonged after the 7th day following the first administration. On the other hand, there was no change in the latency of N_{40} during the whole period of administration. The peak-to-peak amplitude showed some variability in different peaks. Histologically, diffuse status spongiosis were found in the white matter of the cerebrum, cerebellum, and brain stem. As described above, the ablation of the occipital lobe caused markedly depressed VEPs, however, the responses to the photic stimulation persisted after the injury. On the other hand, the VEPs of animals administered with hexachlorophene showed a high probability of peak appearance, and a decrease in amplitude was not marked.—KEY WORDS: guinea pig, hexachlorophene, occipital lobe ablation, VEP.

J. Vet. Med. Sci. 54(5): 813–820, 1992

Visual evoked potentials (VEPs) are useful indicators of the functional integrity of the nervous system in general, and of the visual system in particular. There have been several reports concerning VEP in guinea pigs [2, 5, 21]. We have studied the VEP in guinea pigs, drawing attention especially to a standard VEP waveform [15], changes in the VEP with stimulus conditions [16], comparison between scalp and dural VEPs [18], development of VEP [17], and changes in the VEP with different photic conditions [19].

To increase the information obtainable from evoked potential studies, it is necessary to understand how changes in VEP peaks reflect the deficit of specific components of the visual system. Some of this knowledge may be gained by characterizing alterations produced by treatments which are selective, specific, or some other way. Dyer *et al.* [6] examined the VEP obtained from focal lesions which were placed at the recording site in the visual cortex, and showed the influence of the lesion. Cohn carried out a study concerning VEP in a brain injured monkey, and found that there was a marked reduction in the amplitude of the electric output: 3 days after operation and that the brain electric output recovered to approximately one-half to two-thirds its preoperative amplitude 17 days after operation [4].

The brain lesion produced by hexachlorophene consisted of cystic spaces that appeared empty. The axons of myelinated nerves are either compressed or destroyed by fluid accumulation in the myelin sheaths [12].

The VEPs from the impaired brain seem to be dependent on the kind of lesion, and the time following the lesion. It, therefore, seemed important to carry out a series of experiments. We studied the VEP in guinea pigs with two kinds of brain impairments, ablation of the occipital lobe, and central nervous system disorders following administration of hexachlorophene. The ablation of the occipital lobe represents an injury to a large volume of nervous tissues. The lesion produced by hexachlorophene represents a destruction of myelinated nerves.

MATERIALS AND METHODS

The experiment was performed on 10 adult male guinea pigs weighing 750–980 g. The animals were anesthetized with sodium pentobarbital (Nembutal, Dainippon Pharmaceutical Co., Ltd., 25 mg/kg body weight, ip). Silver-ball electrodes, 1 mm in diameter, were implanted using aseptic surgical techniques.

Holes of 1 mm in diameter were drilled through

the skull and the exploring electrodes were inserted into the holes in contact with the dura and firmly fixed in the skull with dental cement. The reference electrode was fixed in the skull in the same way. The exploring electrodes (LO, RO) in the occipital areas were placed bilaterally on the middle point of the lambdoidal point and the coronal suture, and 5 mm lateral to the sagittal suture. The exploring electrode at the vertex (V) was placed on the midline, 1 mm posterior to the coronal suture. The reference electrode (N) was placed on the midline, the middle point of the line connecting both eyes. These electrodes were placed so as to correspond to the visual area reported by Zeigler [21], and Campos and Welker [2]. One week after electrode implantation, control VEPs were recorded by use of silver-ball electrodes implanted in the skull.

The experiment was performed in a shield room. The animals were awake and were restrained in a restraint box with the head exposed. After dark-adaptation for 15 min, brief flashes from a stroboscope photostimulator (accessory for a polygraph amplifier) placed 20 cm away from the eyes were presented to both eyes, for 5 min with a frequency of 0.5 Hz. The luminous intensity of the flash was approximately 80 luxes at a distance of 20 cm from the photostimulator. The click sounds of the photostimulator generated by the discharge were removed. Details of recording conditions and averaging techniques are published elsewhere [18].

Brain lesions were produced by an ablation of the occipital lobe or an exposure to hexachlorophene. In the first experiment, 5 animals were anesthetized with sodium pentobarbital and surgery was performed under aseptic conditions. The occipital bone was removed, then the occipital lobe was ablated bilaterally from the occipital edge of the hemisphere to 3 mm anterior.

The ablations were carried out by means of suction. The removed bone was replaced by a paraffin wax plate with silver-ball electrodes.

In the second experiment, the brain lesions were produced by the administration of hexachlorophene (Aldrich Chemical Company, Inc.) in 5 animals. The chemicals were administered orally (35 mg/kg/day) for 28 days.

VEPs were recorded on the 3, 5, 7, 14, 21st day after the ablation in the first experiment, and on the 3, 5, 7, 10, 14, 21, 28th day after the first administration of hexachlorophene, in the second experiment. The data were statistically analyzed by using Student's unpaired t test.

After the VEP recordings, the animals were killed, and autopsies were performed. Brains and eyes were fixed in a 10% formalin solution, and the paraffin-embedded sections were stained with hematoxylin and eosin (H-E). Special stain, luxol fast blue (LFB)-HE was used for the tissues in the group of animals administered with hexachlorophene.

RESULTS

Control VEP: The control VEP waveforms are shown in Fig. 1. The VEP had 5 positive peaks (P_{10}, P_{30}, P_{55}, P_{100}, P_{200}) and 4 negative peaks (N_{20}, N_{40}, N_{75}, N_{140}). In accordance with the report by Creel *et al.* [5], it is considered that the peaks up to N_{40} were primary responses, and the other peaks were secondary responses. In some cases, negative peaks appeared following the peak P_{200}. There was considerable variation in amplitude, and it seemed to correspond to the so-called after-discharge. On the other hand, the VEPs obtained from the V-N lead had 6 peaks corresponding to N_{20}, P_{30}, N_{40}, P_{55}, N_{140} and P_{200}. The VEP waveform obtained from the V-N lead was lower in amplitude than those from occipital areas.

Changes in VEP with ablation of occipital lobe: On the 3rd day following the ablation, the VEP waveforms consisted of a negative component having a latency of about 40 msec. This negative component was accompanied by an earlier and a later positive component. These components were considered to correspond to the peaks P_{30}, N_{40} and P_{100} from the viewpoint of peak latencies. The amplitude of N_{40} was markedly depressed. After the 5th day following the ablation, the amplitude of N_{40} gradually recovered (Fig. 1).

The peak latency of P_{30} tended to decrease from the 7th day after the ablation, and it is significantly ($P<0.05$) shorter in the RO-N leading following the 7th day than the control value. In one of the animals, the peak latency of N_{40} tended to prolong up to the 5th day after the ablation. The peak latency of P_{100} showed variability in different leadings. In the LO-N leading, the peak latency of P_{100} from the 3rd day to the 14th day was significantly ($P<0.01$) longer than the control value. On the other hand, there was no significant difference in the peak latency of P_{100} in the RO-N leading (Fig. 2).

Amplitude measurements were made from peak to peak, P_{30}-N_{40} and N_{40}-P_{100}. Following the abla-

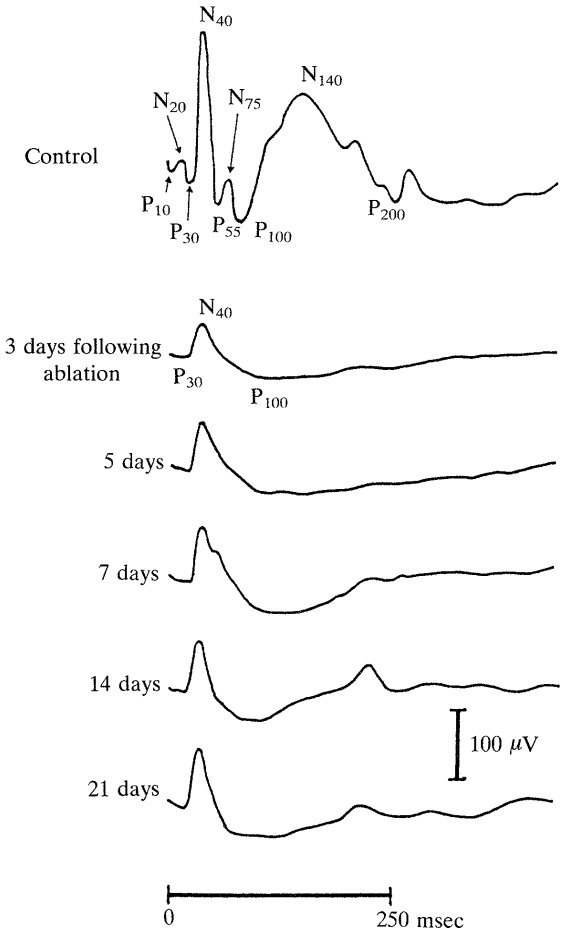

Fig. 1. VEP waveforms in guinea pigs with occipital lobe ablation. Recorded from electrodes placed at LO and N.

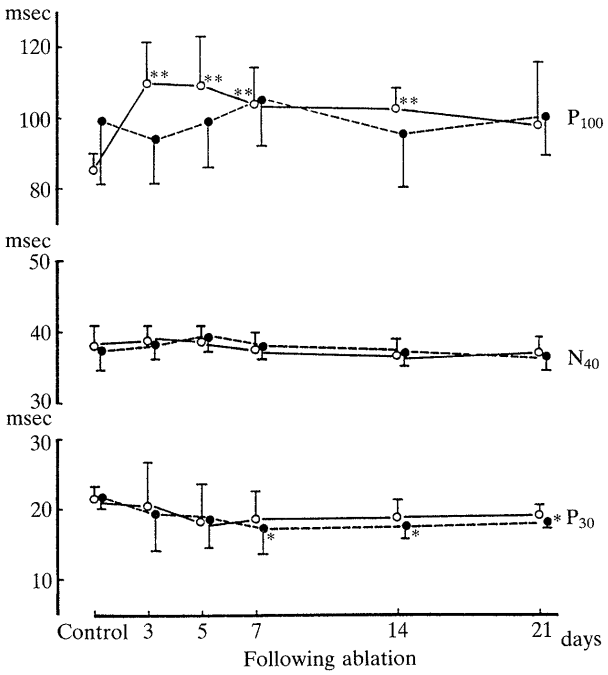

Fig. 2. Changes in peak latencies of VEP with occipital lobe ablation. ○; recorded from electrodes placed at LO and N. ●; recorded from electrodes placed at RO and N. Each value indicates the mean ± S.D. (n=5). *, **; significantly different from control values at $P<0.05$ and $P<0.01$, respectively.

tion, these peak-to-peak amplitudes decreased to about half the control values. The peak-to-peak amplitude of P_{30}-N_{40} was significantly ($P<0.05$) smaller from the 3rd day to the 21st day in the RO-N leading than the control value. That of N_{40}-P_{100} was significantly ($P<0.05$) smaller from the 3rd day to the 7th day in the RO-N leading than the control value. In the LO-N leading, the difference is not statistically significant (Fig. 3).

One of the animals died on the 22nd day after the ablation. The other animals were killed 28 days following the ablation. Autopsy and histopathological examinations were performed on 5 animals.

Macroscopically, the extent of ablation was equal in both hemispheres (Fig. 4-a). In one of the animals, the medullary corpus was incompletely ablated. There was a conspicuous proliferation of connective tissue in the ablated area of the occipital lobe.

Microscopically, a mild purulent meningitis was correspondingly widespread, covering the entire extent of the occipital lobe. The nodular tissues contained hemosiderin-laden macrophages and gliosis, and axonal swelling was found around the ablated area (Fig. 4-b). The frontal lobe, striatum, cerebellum, medulla oblongata, and retina were microscopically normal. These histopathological findings were common among the 5 ablated animals.

Changes in VEP with administration of hexachlorophene: The VEP waveforms in the treated animals were shown in Fig. 5. In 3 animals, 9 peaks appeared in the whole period of hexachlorophene administration. In other animals, new peaks appeared between the peaks N_{40} and P_{55}. In one of the animals, some peaks of the secondary response disappeared on the 20th day following the first administration of hexachlorophene. The after-discharge following the peak P_{200} became clearly observable after the administration of hexachlorophene. In 3 animals, the after-discharge was augmented throughout the course of administration of hexachlorophene. It didn't, however, show any marked increase in amplitude.

Statistical analysis of the data was carried out for a

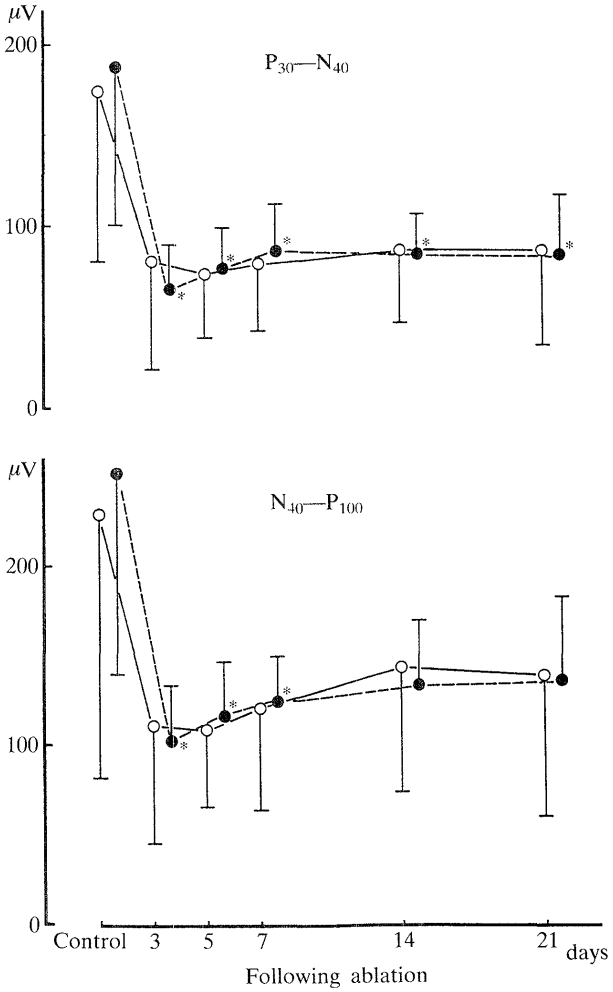

Fig. 3. Changes in peak-to-peak amplitudes of VEP with occipital lobe ablation. ○; recorded from electrodes placed at LO and N. ●; recorded from electrodes placed at RO and N. Each value indicates the mean ± S.D. (n=5). *; significantly different from control values at $P<0.05$.

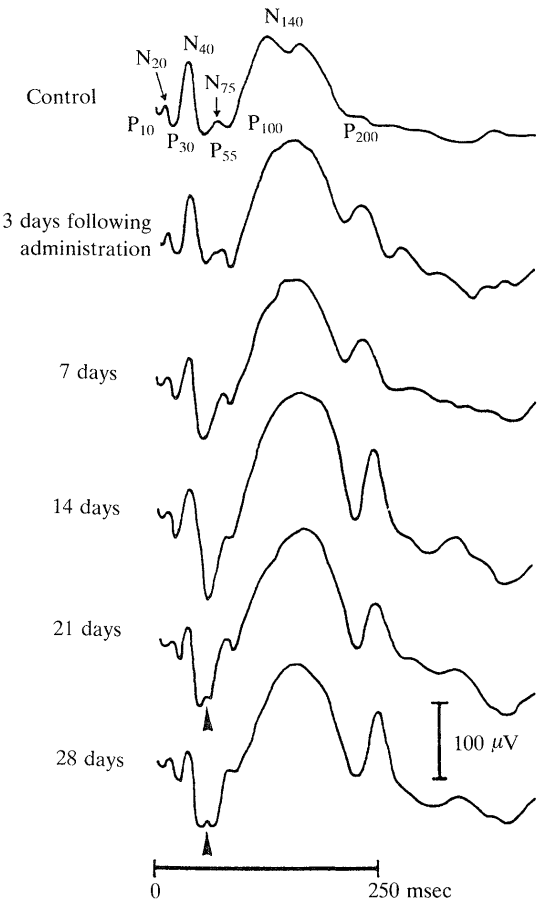

Fig. 5. VEP waveforms in guinea pigs with administration of hexachlorophene. New peaks are indicated by arrowheads. Recorded from electrodes placed at LO and N.

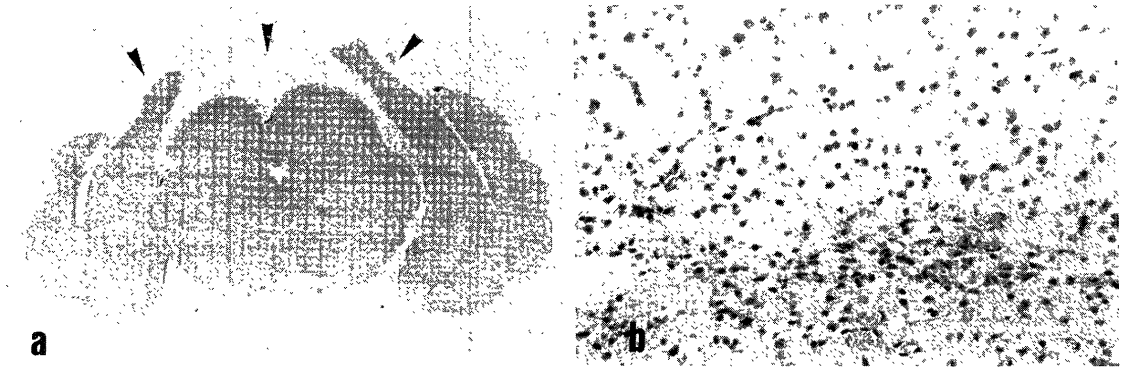

Fig. 4. The lesion in the cerebrum by occipital lobe ablation. a: Macroscopically, the ablated regions are indicated by arrowheads. b: Gliosis is marked around the ablated part in the cortex. HE stain. × 100.

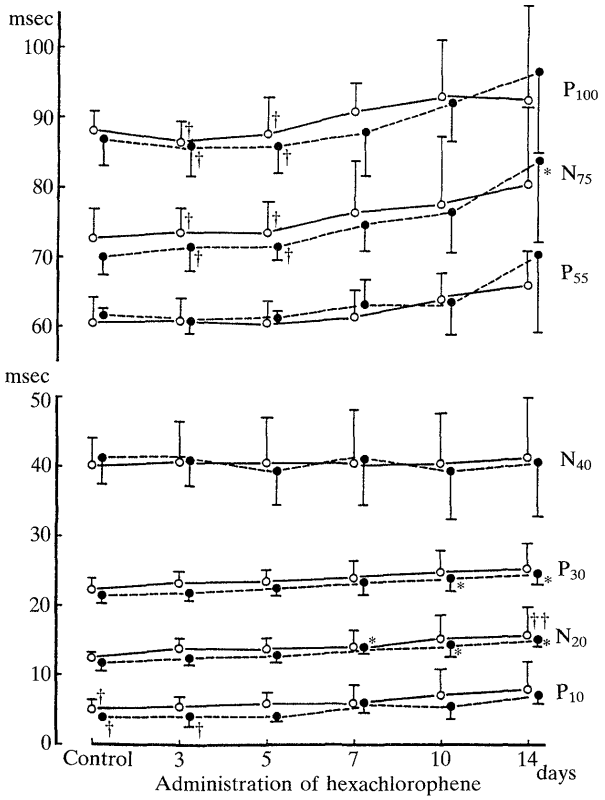

Fig. 6. Changes in peak latencies with administration of hexachlorophene. ○; recorded from electrodes placed at LO and N. ●; recorded from electrodes placed at RO and N. Each value indicates the mean ±S.D. (n=5, †; n=4, ††; n=3). *; significantly different from control values at $P<0.05$.

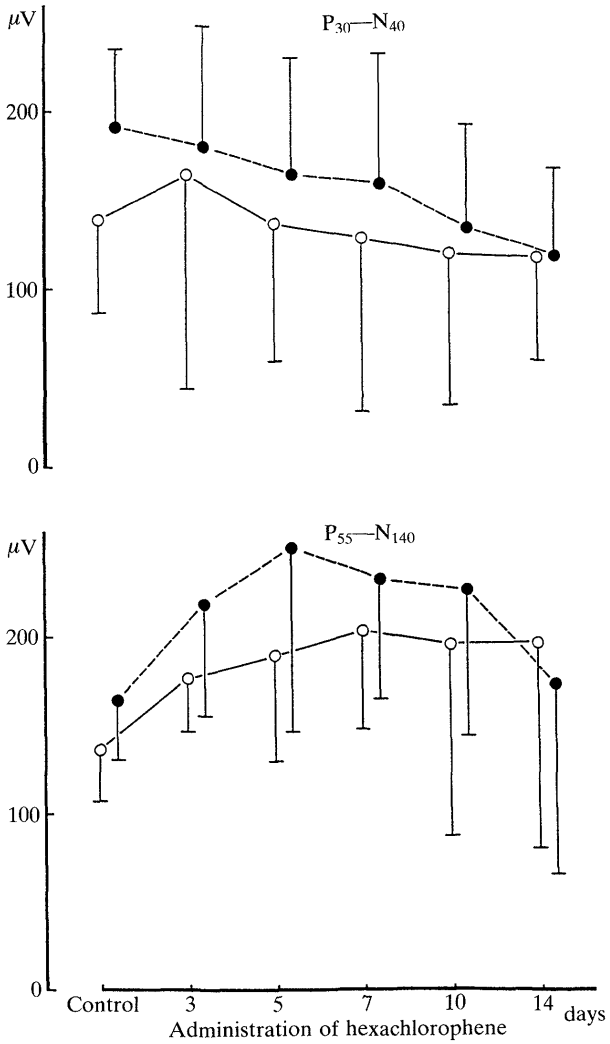

Fig. 7. Changes in peak-to-peak amplitudes with administration of hexachlorophene. ○; recorded from electrodes placed at LO and N. ●; recorded from electrodes placed at RO and N. Each value represents the mean ±S.D. (n=5).

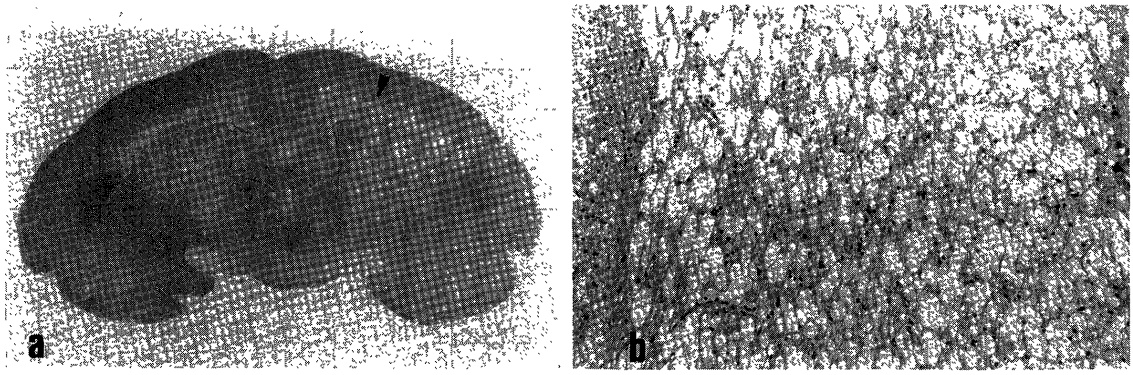

Fig. 8. The lesion in the cerebrum caused by administration of hexachlorophene. a: Macroscopically, many lesions in the white matter are recognizable, and are shown by the arrowhead. b: Severe spongiosis degeneration in the white matter is indicated. HE stain. × 100.

period of 14 days following the first administration. The peak latencies of N_{20}, P_{30} and N_{75} were significantly ($P<0.05$) longer than the control values. The peak latencies of P_{55} and P_{100} also tended to prolong after the 7th day following the first administration, but the differences are not statistically significant. On the other hand, there was no changes in the peak latency of N_{40} during the whole period of administration (Fig. 6).

Changes in peak-to-peak amplitude of VEP with administration of hexachlorophene are shown in Fig. 7. The peak-to-peak amplitude of P_{30}-N_{40} was slightly reduced in the time course of administration. That of P_{55}-N_{140} tended to increase up to the 5th day. In general, the peak-to-peak amplitudes showed striking variability in different individuals. So the differences are not statistically significant.

One animal died on the 14th day and another on the 20th day, and the remainders were killed on the 28th day following the first administration. An autopsy and a histopathological examinations were performed on 5 animals administered with hexachlorophene.

Macroscopically, cerebral swelling was found in all animals. Many lesions in the white matter were recognizable (Fig. 8-a). Microscopically, diffuse status spongiosis in the cerebrum, cerebellum, and brain stem were correspondingly widespread, in the extent of white matter (Fig. 8-b). Disruption of myelin was also seen in the white matter. The gray matter was almost normal. Severe spongiosis was seen mainly in the occipital lobe. The degree of the lesion was severer with the time elapsed after the administration of hexachlorophene.

DISCUSSION

VEP reflects the incoming afferent volleys and the resultant synaptic events that occur in the underlying cortical tissue. In mammals, most of the retinal ganglion cells project to the dorsal lateral geniculate nucleus (LGN_d) of the thalamus. The LGN_d sends a strong projection to the ipsilateral occipital lobe via an optic radiation. This primary visual area is known as the striate area. In the present study, the early negative component (N_{40}) appeared in all animals with ablated occipital lobe. The primary response is made up of the presynaptic potential produced by the activity of thalamocortical fibers and the postsynaptic potential produced with a postsynaptic discharge of the cortical neurons [3].

In cats [7], some of extrastriate areas receive input from the LGN_d and its associated nuclei. Globus and Scheibel [9] found that the apparent termination of primary visual afferents was shown directly upon cortical pyramids in rabbits. Cohn demonstrated that the recovery of summated electric activity following the ablation of occipital lobe might have been the result of a volume conduction phenomenon from adjacent "normal" tissue through the *in situ* destroyed tissue [4].

In the present study, histological findings revealed that the ablated region ranged from one-third to two-fifths of the whole occipital lobe, and the temporal lobe was not damaged. It is, therefore, considered that the primary response following the ablation of occipital lobe appeared to have resulted from a volume conduction from normal tissues.

It is stated that the amplitudes of VEP correlated with the amount of excited neurons, integration of excited neurons, and the activity of reverberating circuits interconnecting distant structures [10]. In the present study, the ablation of the occipital lobe caused a great decrease in the peak-to-peak amplitudes of P_{30}-N_{40} and N_{40}-P_{100}. These depressed VEPs seem to have resulted from a decrease in the amount of excited neurons.

Dyer *et al.* [6] found that in rats, one week after producing a focal lesion, P1, P2 and N2 amplitudes were significantly depressed when recorded from the electrode placed on the lesion, whereas N1 and P3 were significantly increased. The peak N1 which was reported by Dyer *et al.* seems to correspond to the peak N_{40} obtained in this study, in the view point of the peak latency. However, the amplitude of peak N_{40} had no significant increase in the present study.

Cohn's [4] study was concerned with the VEP in monkeys with ablation of three-fifths of the occipital lobe. In 3 days postoperation, there was a marked reduction of amplitude of the output over the left occipital region with 5 and 10 per second light stimulation. In 17 days postoperation, the brain electric output on the left side recovered to approximately one-half to two-thirds its preoperative amplitude.

In the present study, 21 days after surgery, the amplitude of the VEP recovered insufficiently. Nevertheless, 3 days after surgery, responses to the photic stimulation appeared. These responses in the few days after surgery, suggest that the disappearance of light sensation by the ablation of the striate area may be still partially complete.

Hexachlorophene had been used extensively as a germicidal agent and recommended for use in agriculture as a broad spectrum fungicide and bactericide. In the course of toxicity studies [11] conducted in rats on a diet containing high concentrations of hexachlorophene they developed flaccid paralysis of their hindquarters as a result of the effect on the brain. For the purpose of experimentally producing lesions in the brain, we administered hexachlorophene to guinea pigs.

The lesions produced by hexachlorophene in this experiment represented that spongiosis were widespread, covering the extent of the white matter, which resembles diffuse spongy degeneration of the white matter of the brain described in the previous studies [1, 8, 11–13, 20]. The severity of the lesions increased with the period of the administration of hexachlorophene. The peak-to-peak amplitude of primary response (P_{30}-N_{40}) slightly decreased in the time course of administration, and this decreased amplitude was in accord with that reported by Santolucito [14]. This decreased amplitude seems to be caused by the brain lesion. In the group of the animals administered with hexachlorophene, the VEP waveform had 9 peaks in many cases. This high probability of peak appearance is quite different from that in the group of animals with ablated occipital lobes. This difference in peak appearances between the two groups suggests that the disappearance of peaks in the case of occipital lobe ablation is caused by the decrease in the amount of excited neurons.

Histological study revealed a spongiosis degeneration and disruption of myelin particularly in the white matter. This disruption of myelin seems to cause the peak latency to prolong in the later components in the range of 4–14 msec, while there were almost no changes in the peak latencies of early components (P_{10}, N_{20}, P_{30} and N_{40}). So the peak latencies of early components were hardly affected by the lesion produced by hexachlorophene.

It is stated that the separation of peaks was caused by the variation of conduction velocity in the visual pathway [10]. In the present study, new peaks (Fig. 5) appeared between the peaks N_{40} and P_{55} following the administration of hexachlorophene. This appearance of new peaks suggests a change in the conduction velocity in the visual pathway. However, it is not clear whether the new peak was produced by the delay of the N_{40} component or by the shortening of the P_{55} component.

In some cases, the augmentation of after-discharge was seen throughout the course of administration of hexachlorophene. Iwase [10] gave an account of these augmentations in the after-discharge. He stated that there is a correspondence between the frequency of the after-discharge and spontaneous electrical activity in the cortex, and that the after-discharge appeared to have resulted from an augmentation of spontaneous electrical activity. This augmentation of spontaneous electrical activity was shown also in some animals administered with hexachlorophene in the present study. So the augmentation of after-discharge observed in the present study may be correlated with the cortical activity.

As described above, the ablation of the occipital lobe caused markedly depressed VEPs and seems to have resulted from a decrease in the amount of excited neurons. However, the responses to the photic stimulation persisted after the injury. On the other hand, the VEPs recorded from animals which were administered with hexachlorophene showed a high probability of peak appearance, and a decrease in amplitude of VEP was not marked. These results were in accord with the histopathological findings that the disruption of myelin was limited in the white matter, and the gray matter was almost normal.

REFERENCES

1. Booth, N. H. 1977. Drug and chemical residues in the edible tissues of animals. pp. 1149–1205. *In*: Veterinary Pharmacology and Therapeutics (Booth, N. H. and McDonald, L. E. eds.), Iowa State University Press, Ames.
2. Campos, G. B. and Welker, W. I. 1976. Comparisons between brains of a large and a small hystricomorph rodent: capybara, *hydrochoerus* and guinea pig, *cavia*; neocortical projection regions and measurements of brain subdivisions. *Brain Behav. Evol.* 13: 243–266.
3. Chang, H. T. 1963. The evoked potentials. pp. 299–313. *In*: Handbook of Physiology, Section 1: Neurophysiology (Field, J., Magoun, H. W., and Hall, V. E. eds.), American Physiological Society, Washington, D. C.
4. Cohn, R. 1969. Visual evoked responses in the brain injured monkey. *Arch. Neurol.* 21: 321–329.
5. Creel, D. J., Dustman, R. E., and Beck, E. C. 1973. Visual evoked responses in the rat, guinea pig, cat, monkey, and man. *Exp. Neurol.* 40: 351–366.
6. Dyer, R. S., Jensen, K. F., and Boyes, W. K. 1987. Focal lesions of visual cortex-effects on visual evoked potentials in rats. *Exp. Neurol.* 95: 100–115.
7. Fuchs, A. F. 1989. The visual system: Neuralprocessing beyond the retina. pp. 442–474. *In*: Textbook of Physiology, 21st ed. (Patton, H. D., Fuchs, A. F., Hille, B., Scher, A. M., and Steiner, R. eds.), W. B. Saunders, Philadelphia.

8. Gaines, T. B. and Kimbrough, R. D. 1971. The oral and dermal toxicity of hexachlorophene in rats. *Toxicol. Appl. Pharmacol.* 19: 375.
9. Globus, A. and Scheibel, A. B. 1967. Synaptic loci on visual cortical neurons of the rabbit: The specific afferent radiation. *Exp. Neurol.* 18: 116–131.
10. Iwase, Y. 1967. Evoked potential. pp. 25–54. *In*: Japanese Handbook of Physiology, V (Tokizane, T. ed.), Igaku-Shoin Ltd., Tokyo (in Japanese).
11. Kennedy, G. L. Jr., Dressler, I. A., Richter, W. C., Keplinger, M. L., and Calandra, J. C. 1972. Reversibility of effects caused by hexachlorophene in the rat. *Toxicol. Appl. Pharmacol.* 22: 276.
12. Kimbrough, R. D. and Gaines, T. B. 1971. Hexachlorophene effects on the rat brain. *Arch. Environ. Health* 23: 114–118.
13. Kimbrough, R. D. 1973. Review of recent evidence of toxic effects of Hexachlorophene. *Pediatrics* 51: 391–394.
14. Santolucito, J. A. 1972. The electroencephalogram and visual evoked potential of the squirrel monkey fed hexachlorophene. *Toxicol. Appl. Pharmacol.* 22: 276.
15. Suzuki, M., Sitizyo, K., and Takeuchi, T. 1990. Visual evoked potential of guinea pigs. *J. Physiol. Soc. Jpn.* 52: 47–53 (in Japanese).
16. Suzuki, M., Sitizyo, K., Takeuchi, T., Hashimura, T., Tsuchida, T., Mitsuyama, T., Nakao, T., and Saito, T. 1990. A basic study of VEP in guinea pigs by binocular stimulation. *Bull. Fac. Agric. Tottori Univ.* 43: 209–215 (in Japanese).
17. Suzuki, M., Sitizyo, K., Takeuchi, T., and Saito, T. 1990. Development of visual evoked potentials in guinea pigs. *J. Physiol. Soc. Jpn.* 52: 206–211 (in Japanese).
18. Suzuki, M., Sitizyo, K., Takeuchi, T., and Saito, T. 1991. Visual evoked potential from scalp in guinea pigs. *J. Vet. Med. Sci.* 53: 301–305.
19. Suzuki, M., Sitizyo, K., Takeuchi, T., and Saito, T. 1991. Changes in the visual evoked potentials with different photic conditions in guinea pigs. *J. Vet. Med. Sci.* 53: 911–915.
20. Udall, V. 1972. Drug-induced blindness in some experimental animals and its relevance to toxicology. *Proc. R. Soc. Med.* 65: 197–200.
21. Zeigler, H. P. 1964. Cortical sensory and motor areas of the guinea pig "Cavia Porcellus". *Arch. Ital. Biol.* 102: 587–598.