INFORMATION TO USERS

This reproduction was made from a copy of a document sent to us for microfilming. While the most advanced technology has been used to photograph and reproduce this document, the quality of the reproduction is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help clarify markings or notations which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure complete continuity.

2. When an image on the film is obliterated with a round black mark, it is an indication of either blurred copy because of movement during exposure, duplicate copy, or copyrighted materials that should not have been filmed. For blurred pages, a good image of the page can be found in the adjacent frame. If copyrighted materials were deleted, a target note will appear listing the pages in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed, a definite method of "sectioning" the material has been followed. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For illustrations that cannot be satisfactorily reproduced by xerographic means, photographic prints can be purchased at additional cost and inserted into your xerographic copy. These prints are available upon request from the Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases the best available copy has been filmed.

University Microfilms International
300 N. Zeeb Road
Ann Arbor, MI 48106

8501191

Zappulla, Rosario Anthony

ELECTROPHYSIOLOGIC CONSEQUENCES OF BLOOD-BRAIN BARRIER DISRUPTION

City University of New York PH.D. 1984

University
Microfilms
International 300 N. Zeeb Road, Ann Arbor, MI 48106

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark __√__.

1. Glossy photographs or pages ______
2. Colored illustrations, paper or print ______
3. Photographs with dark background ______
4. Illustrations are poor copy ______
5. Pages with black marks, not original copy ______
6. Print shows through as there is text on both sides of page ______
7. Indistinct, broken or small print on several pages __√__
8. Print exceeds margin requirements ______
9. Tightly bound copy with print lost in spine ______
10. Computer printout pages with indistinct print ______
11. Page(s) ____________ lacking when material received, and not available from school or author.
12. Page(s) ____________ seem to be missing in numbering only as text follows.
13. Two pages numbered ____________. Text follows.
14. Curling and wrinkled pages ______
15. Other ____

University
Microfilms
International

ELECTROPHYSIOLOGIC CONSEQUENCES OF BLOOD-BRAIN BARRIER DISRUPTION

by

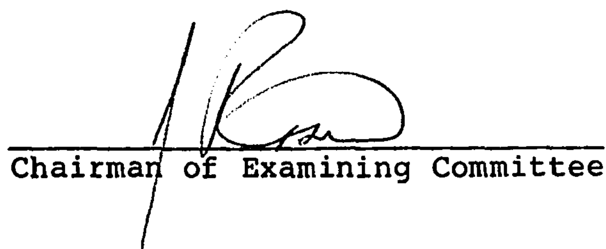
ROSARIO ANTHONY ZAPPULLA

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements of Doctor of Philosophy, The City University of New York.

1984

This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

8/9/84
date



Chairman of Examining Committee

August 13, 1984
date

Herbert D. Saltzstein
Executive Officer

Jeffrey J. Rosen, Ph.D.

Louis Gerstman, Ph.D.

Solomon S. Steiner, Ph.D.

Supervisory Committee

The City University of New York

Abstract

ELECTROPHYSIOLOGIC CONSEQUENCES OF
BLOOD-BRAIN BARRIER DISRUPTION

by

Rosario Anthony Zappulla

Adviser: Professor Jeffrey J. Rosen

This study investigates the acute (up to three hours) and chronic (up to 72 hours) electrophysiologic consequences in rats of blood-brain barrier (BBB) disruption by the intracarotid infusion of the bile salt sodium dehydrocholate. The epileptogenic properties of dehydrocholate and its possible role in barrier disruption were also examined.

Experiment I documents the electroencephalographic (EEG) and visual evoked potential (VEP) changes associated with various grades of disruption for up to three hours following disruption. These changes included slowing and decreased amplitude of the EEG as well as the presence of spike

activity over both the disrupted and non-disrupted hemispheres. The amplitude of the VEPs in these animals decreased following disruption. These electrophysiologic changes tended to be the most severe and persist the longest in those animals with the most extensive disruption.

Experiment II was a replication of Experiment I with the addition of pre-treating the animals with diazepam, an anti-epileptic. While the intensity of the seizure activity was markedly reduced as compared to animals in Experiment I, there was no change in the distribution of the various grades of disruption and their associated EEG and VEP alterations. These findings indicate that the seizures associated with dehydrocholate disruption are a result rather than the cause of disruption.

Experiment III investigated the possiblity that EEG changes may persist when animals are followed for longer time intervals due to a cummulative effect of barrier permeability. The EEG was monitored following BBB disruption and compared to pre-disruption EEG in two groups of animals using visual inspection of the EEG (24 hours following disruption) as well as quantitative spectral analysis (24, 48, 72 hours following disruption). Spectral analysis

of the EEG revealed a significant decrease in alpha activity over both the disrupted and non-disrupted hemisphere and an increase in theta activity over the disrupted hemisphere. Histological examination of the brains of animals in this group was negative for pathological change on light microscopy. These findings indicate that BBB disruption with dehydrocholate is associated with EEG changes in the absence of any pathological abnormality.

The final two experiments detailed the epileptogenic properties of dehydrocholate. Experiment IV demonstrated the epileptogenic effects of dehydrocholate independent of its effects on the BBB. Experiment V investigated the development, configuration and propagation of spike activity following the local application of dehydrocholate to the cortical surface.

Acknowledgments

I would like to express my thanks to my mentor, Jeff Rosen, Ph. D., for the insight, time and experience that he generously shared with me, and to the members of my Thesis Committee as a whole for their valuable criticism. To Leonard Malis, M. D., Chairman of the Department of Neurosurgery, who granted me the time and gave me the encouragement for this project. To Bernie Karmel, Ph.D. for the continuing encouragement in the difficult beginning stages of this work. To Shiela Glennon who gave valuable assistance during the entire endeavor. To Mel Spigelman, M. D., a research colleague and dear friend, whose clear thinking is present throughout this document. To Victoria White, ACSW whose tireless effort, understanding and encouragement in the final stages permitted this thesis to see the light of day. And, finally, to Joyce, Carey and my parents, who once again had to do without me for the duration of this work.

Table of Contents

Acknowledgment	vi
Table of Contents	vii
List of Tables	ix
List of Figures	xi
Introduction	1
Experiment I	18
Method	19
Results	23
Discussion	28
Experiment II	34
Method	37
Results	37
Discussion	40
Experiment III	43
Method	44
Results	46
Discussion	49
Experiment IV	54
Method	57
Results	59
Discussion	60

Experiment V 63

Method 66

Results 69

Discussion 73

General Discussion 78

Appendix 94

Bibliography 134

List of Tables

Table 1 EEG Changes and Course Following Intracarotid Infusion of Dehydrocholate.

Table 2 Spike Activity Following Intracarotid Infusion of Dehydrocholate.

Table 3 EEG Changes and Course Following Intracarotid Infusion of Dehydrocholate in Animals Pre-treated with Diazepam.

Table 4 Spike Activity Following Intracarotid Infusion of Dehydrocholate in Animals Pre-treated with Diazepam.

Table 5 EEG 24 Hours Post-Infusion with Dehydrocholate.

Table 6 Means and Standard Deviations of Relative Spectral Power (Pre- and Post-Disruption).

Table 7 Spike Activity Following Intravenous Dehydrocholate in Animals Disrupted with Mannitol or Etoposide.

Table 8 Spike Activity Over the Disrupted and Non-disrupted Cortex Following the Topical Application of Dehydrocholate.

List of Figures

Figure 1: View of the skull from above depicting bony landmarks of the rat skull and electrode placement for recording the EEG. Electrode labels signify: LF & RF - left and right frontal; LC & RC - left and right central; LP & RP - left and right posterior. A reference electrode (R) is located in the nasal bone, and the ground electrode (Gnd) is located in the posterior bone of the skull. The montages used during the study included a monopolar montage with each electrode referenced to the electrode in the nasal bone (e.g. LF-R) and a bi-polar configuration between hemispheres (e.g. LF-RF). Both montages were recorded simultaneously.

Figure 2: Diagram of the surgical procedure in the left anterior neck for infusion of sodium dehydrocholate into the internal carotid artery of the rat. (A) common carotid artery (B) external carotid artery (C) clip used to secure catheter in the external carotid artery (D) catheter placed in the external carotid artery for infusion (E) internal carotid artery (F) pterygopalatine branch of the internal carotid artery (G) deep branch of the internal carotid artery.

Figure 3: (A) EEG from disrupted (DH) and non-disrupted (NDH) hemispheres during dehydrocholate infusion in an animal with 1+ disruption. Dehydrocholate infusion began three seconds prior to the start of the trace. There is a decrease in the amplitude and an increase in the frequency of the EEG approximately five seconds following the start of the infusion. (B) EEG 30 minutes following the end of the infusion. Bilateral spikes are present in the record. (C) EEG prior to sacrifice, three hours post-infusion. (Calibration in this figure and all subsequent figures: vertical line - 50 microvolts; horizontal line - one second.

Figure 4: EEG five minutes following the end of infusion in a rat with 3+ disurption. There is a marked decrease in the EEG amplitude over the disrupted hemisphere as compared to the non-disrupted hemisphere. Frequent, bilaterally synchronous spikes are present in all leads.

Figure 5: EEG activty at 90 minutes post-infusion in a 3+ disrupted animal that had a flat EEG 15 minutes later. Marked decrease in amplitude over the disrupted hemisphere

and the presence of high voltage slow waves (2-3 c/sec) over the non-disrupted hemisphere.

Figure 6: EEG activty ten seconds into the infusion in an animal with 3+ disruption. Spike activity can be seen starting over the left hemisphere (LF-R) and then quickly spreading to involve both hemispheres diffusely.

Figure 7: Prolonged spike activity occurring 60 minutes following infusion in an animal with 2+ disruption. This is in contrast to the single, bilaterally synchronous activity occurring with 1+ disruption (compare with Figure 3).

Figure 8: Spike activity occurring over the disrupted hemisphere. Amplitude of spike greatest over the left central lead.

Figure 9: Spike isolated over the non-disrupted hemisphere in the frontal lead (closed arrowhead).

Figure 10: The three tracings are the continuous EEG response to photic stimulation in an animal with 3+ disruption.

Bottom channel of each tracing marks the photic stimulus. As photic stimulation increases to 20 c/sec, there is a generalized seizure discharge that subsides as the stimulus rate decreases.

Figure 11: VEPs prior to infusion (A), five minutes (B), 30 minutes (C), and 60 minutes following infusion. Animal with 1+ disruption.

Figure 12: VEPs in an animal with 2+ disruption. Recording conditions the same as in Figure 11.

Figure 13: VEPs in an animal with 3+ disruption. Recording condition the same as in Figure 11.

Figure 14: EEG 30 minutes post-infusion in animal with 1+ disruption. Spike activity over the disrupted hemisphere (closed arrowheads).

Figure 15: EEG seconds following the start of infusion in an animal pre-treated with diazepam. There is the start of a generalized seizure discharge that aborts seconds following

its initiation. This is in contrast to the prolonged generalized seizure activity noted in 2+ and 3+ animals during infusion not treated with diazepam (compare this figure with Figure 6).

Figure 16: Spike activity limited to the disrupted hemisphere (closed arrowheads), 30 minutes following infusion, in an animal pre-treated with diazepam. Animal had a 2+ disruption of the left hemisphere.

Figure 17: Synchronous spike activity in an animal with 2+ disruption, 60 minutes post-infusion. The response is similar to that seen with 1+ disruption in Experiment I (compare with Figure 3).

Figure 18: VEPs prior to infusion (A), five minutes (B), 30 minutes (C), and 60 minutes following infusion. Animal with 2+ disruption.

Figure 19: VEPs in an animal with 3+ disruption. Recording conditions the same as in Figure 18.

Figure 20: Spikes over the disupted and non-disrupted hemisphere 24 hours post-infusion.

Figure 21: Animal with 3+ disruption with mannitol. (A) EEG prior to the intravenous injection of sodium dehydrocholate. (B) The EEG seconds following the intravenous injection of sodium dehydrocholate demonstrates periodic bilateral spikes. (C) EEG three minutes following the intravenous injection of sodium dehydrocholate. There is a continuous spike discharge throughout both the disrupted and non-disrupted hemispheres.

Figure 22: Animal with 1+ disruption over the left hemisphere with etoposide. Spike activity present bilaterally following the intravenous injection of sodium dehydrocholate. The frequency of spike activity less than that with 3+ disruption (compare with Figure 21).

Figure 23: Appartus for the topical application of sodium dehydrocholate. (A) cortex, (B) filter paper overlying cortex to be treated, (C) outer cannula where suction is applied to remove excess dehydrocholate, (D) inner cannula for the administration of dehydrocholate to the cortex, (E)

plastic tubing connected to suction and to outer cannula, and (F) plastic tubing connected to hand held syringe for the administration of dehydrocholate.

Figure 24: Spike activity over the disrupted cortex (RH) following the topical application of sodium dehydrocholate. The contralateral homotopic cortex (LH) is free of spike activity. Calibration in this figure and all subsequent figures: vertical line - 100 microvolts; horizontal line - one second.

Figure 25: Primary spike activity over the disrupted cortex (closed arrowhead) and dependent spike activity in the contralateral cortex (open arrowhead) following the intravenous injection of dehydrocholate.

Figure 26: Dependent spike activity (closed arrowhead) occurring following spikes in the disrupted cortex with a particular configuration.

Figure 27: Dependent (open arrowhead) and independent spike activity (closed arrowhead) over the cortex contralateral to

the primary focus.

Figure 28: (A) Spike activity in the disrupted (open arrowhead) and non-disrupted (closed arrowhead) hemispheres prior to the administration of GABA. (B) Following GABA there is a loss of spike activity in the disrupted hemisphere, but spike activity remains in the non-disrupted (closed arrowhead) hemisphere.

Figure 29: (A) EEG prior to the administration of GABA. Spikes are present over both the disrupted and non-disrupted hemispheres. (B) Seconds following the administration of GABA, there is a decrease in spike activity over both hemispheres. (C) One minute following GABA the spike activity can be seen originating over the non-disrupted hemisphere.

Figure 30: Continuous recording of spike activity from the disrupted cortex prior to and following GABA administration. Reversal of spike polarity can be seen following GABA administration.

Figure 31: (A) Primary spike activity recorded over the disrupted (closed arrowhead) cortex and associated dependent spike activity in the contralateral homotopic (open arrowhead) cortex. (B) Following section of the corpus collosum, there is an absence of dependent spike activity in the homotopic cortex, which is replaced with independent spike activity in both the primary focus (closed arrowhead) and secondary focus (open arrowhead).

INTRODUCTION

General theories of brain-behavior relationships assumes that activity at the neural level can be correlated if not causally related to specific behavioral patterns. That such a relationship exists is supported by behavioral changes associated with various neuropathological conditions such as stroke, head trauma and cerebral tumors. Research has sought to better delineate this relationship by refining the techniques used for altering specific neuronal structures. For example, two well documented techniques include selective ablation and stimulation of specific neuronal populations thought to control specific types of behavior. Although the behavioral consequences of such techniques are dramatic, more subtle alterations in neuronal function that are associated with disturbances in the metabolic milieu of the brain are more difficult to evaluate.

The state of cerebral function depends upon the chemical environment of neurons and their supporting structures. Controlled concentrations of electrolytes and access of needed chemical compounds for the energy requirements of

neuronal metabolism are essential for maintaining these specific neuronal processes. In the maintenance of this metabolic equilibrium, exclusion of circulating compounds from the brain that could be potentially toxic to neuronal function is necessary. The interface between the cerebral vasculature and the brain known as the blood-brain barrier (BBB) helps fulfill these metabolic equilibrium requirements necessary for function. It is generally believed that the BBB is responsible for providing essential compounds to the brain while at the same time selectively preventing access to the central nervous system (CNS) of potentially toxic substances (Bradbury, 1979).

This thesis examines how acute and chronic BBB disruption effects ongoing brain electrical activity as measured by EEG and visual evoked potentials (VEPs). EEG and evoked potentials have been used successfully by investigators as a measure of neuronal function in both clinical and experimental studies. These measurements can be quantitative, and, therefore, ideal for describing the effects of experimental manipulation of variables that affect the neuronal activity, and, thereby, alter electrical activity. Moreover, previous studies have reported that

changes in electrical activity of the nervous system may reflect changes in the state of neuronal activity prior to any behavioral change.

The model used in this thesis is unique in that barrier disruption is prolonged and not associated with neuronal injury (Spigelman, Zappulla, Malis, Holland, Goldsmith and Goldberg, 1983). As a result, the reversible and acute and/or cumulative effects of barrier disruption on cerebral electrical activity can be studied over extended periods of time.

The concept of a blood-brain barrier was proposed from a series of studies that demonstrated a lack of staining of the CNS by systemically administered vital dyes that stained virtually all other tissues of the body (Bradbury, 1979). Numerous studies since that time have confirmed the uniqueness of the cerebral vasculature and its role in homeostatic control of the neuronal environment. Anatomical studies have proposed the presence of tight junctions between the endothelial cells and the lack of a transendothelial transport system as mechanisms for the selective permeability of the BBB (Reese and Kanovsky 1969; Bradbury 1979). Whatever the anatomical substrate, many of the functional

attributes of the BBB have been well described.

Based on the clearance by the brain of systemically administered agents, Oldendorf (1975) demonstrated that the lipid solubility of a compound determines the extent to which it will cross the BBB. The greater the lipid solubility the greater the concentration of the agent in brain. Alternatively, non-electrolyte polar compounds penetrate the BBB poorly and are dependent on their molecular size. This is exemplified by the rapid penetration into the brain and behavioral effects of alcohol, a lipid soluble compound. In contrast, the lack of lipid solubility accounts for the ineffectiveness of various systemically administered agents that are known to have potent effects on neuronal function. For example, gamma amino butryic acid (GABA), a neurotransmitter normally present in the CNS, has been demonstrated to play an important role in the inhibitory neuronal system of the brain and spinal cord. Furthermore, it has been shown that GABA has the ability to lower the threshold for seizures when adequate concentrations of this agent are available in the synaptic pool (Hayashi, T., 1959). However, because of its poor lipid solubility, systemic administration of this agent has limited effects on the CNS

unless GABA is transformed into a lipid soluble agent or the BBB is disturbed (Meldrum & Horton, 1974).

The consequence of barrier disruption has been well described clinically in patients with brain tumors or other destructive lesions of the CNS. In these cases behavioral changes have been associated with the development of edema around the pathological lesion. These changes have been associated with the egress of plasma components through a permeable BBB. The results have included changes in electrolyte concentrations and increased intracranial pressure from an increase in water content. A decrease in the extent of cerebral edema either by medical treatment or spontaneous remission of barrier disruption has been associated with improvement in the patient's condition.

The preceding description represents an extreme case of BBB disruption. Although some of the consequences are due to changes in the chemical constituents of the brain, significant behavioral changes can be associated with the mechanical stresses of increased intracranial pressure. More subtle consequences of BBB disruption have been demonstrated by several researchers who have noted that the systemic administration of several agents normally excluded from the

brain result in behavioral and electrical alterations in the presence of a disrupted BBB. Penicillin, when applied topically to the brain, can result in epileptogenic foci in a matter of hours. However, systemic administration of penicillin in an experimental animal or human with an intact BBB is relatively excluded from the brain by the BBB, and consequently, EEG changes are limited. (Ajmone Marsan, 1969; Gloor, 1969). This has been attributed to its inability to cross the BBB in concentrations great enough to stimulate neuronal tissue (Quesney, Gloor, Kratzenberg & Zumstein, 1977). This mechanism appears to be confirmed by reports demonstrating the establishment of a epileptogenic focus following the systemic administration of penicillin in animals with BBB disruption (Flodmark, 1965b; Remler, 1973: Remler & Marcussen, 1982). Similar results have been demonstrated for the anticholinergic agent neostigmine (Flodmark, 1965a). This agent, when administered systemically in the presence of an intact BBB, fails to alter the EEG since it is poorly lipid soluble and does not cross the BBB. However, when administered to patients with brain tumors, edema and a permeable BBB, slowing of the EEG as well as epileptogenic activity is elicited. These studies

indicate that a permeable barrier can result in behavioral and electrophysiological changes that are associated with systemic agents that are normally prevented access to the CNS. However, these studies fail to demonstrate the CNS effects of a permeable barrier alone, uncontaminated by the effects of exogenous agents that may secondarily gain access to the CNS through a permeable barrier.

The systematic study of the BBB has resulted in the development of several methods for experimental BBB disruption. These methods have provided important information on the anatomical and physiologic characteristics of the BBB. In a recent summary Bradbury (1979) has listed over ten available methods for producing BBB disruption. These methods can be classified by their reversibility and toxic effects on the CNS. In general, those techniques that are reversible and non-destructive usually result in barrier opening for short periods of time (approximately hours). These techniques include hypertension and hyperosmotic perfusion of the carotid artery. Techniques that maintain barrier opening for longer periods (days to weeks) such as radiation or intracarotid infusion of mercuric chloride result in destruction of neural tissue. The difference in

results with these techniques reflect the difference in the extent and type of alterations that occur on the cerebral vasculature and neural tissue with each method. Consequently, the results of barrier disruption must be interpreted with respect to the advantages and disadvantages of each methodology. This is particularly the case with respect to the behavioral and electrophysiologic consequences of barrier disruption. The effects on neural tissue occurring independent of barrier disruption need to be separated from the effects that occur from BBB disruption alone. Therefore, a technique that can disrupt the BBB with little or no concomitant alteration of neural tissue is important in studying the isolated effects of barrier disruption on CNS function.

Experimental disruption of the BBB has demonstrated that BBB disruption results in a series of changes in the EEG that includes slowing and paroxysmal activity (Flodmark & Steinwall, 1962; Flodmark & Steinwall, 1963a,1963b; Bloor, Wrenn & Margolis, 1951). As might be expected, the type and extent of these changes have varied depending upon the technique used for disruption. In most of these studies, disruption was induced in one hemisphere and the electrical

effects of barrier disruption have been determined by comparing the EEG changes between hemispheres over time. Various reports have demonstrated that the iodinated group of agents used for cerebral angiography can result in BBB disruption and EEG changes (Foltz, Thomas & Ward 1951; Bloor et al.,1951). Experimental studies by Broman & Olsson (1949) in animals demonstrated that the intracarotid administration of these compounds can result in disruption of the BBB, and that the extent and duration of disruption is dose dependent. Bloor et al. (1951) in a study relating EEG changes and BBB disruption following intracarotid administration of these contrast agents demonstrated that the extent of EEG slowing and seizure activity was related to the extent of barrier disruption. In addition, pathological changes in the vascular and cellular components of the brain correlated with the extent of BBB disruption and electrical changes. This study, however, did not determine whether the changes in the EEG were due to a permeable barrier or the effect of the contrast agent reaching the neural tissue because of the permeable barrier.

Flodmark & Steinwall (1962,1963b) reported that the carotid administration of low doses of the contrast agent

Urokon produced minor and reversible BBB disruption that was unassociated with EEG changes. They concluded that if barrier disruption is slight and reversible, there is no effect on neural activity. He further demonstrated that an agent used to disrupt the barrier is itself capable of producing EEG changes if it gains access to the brain because of a permeable barrier. Flodmark demonstrated that he could elicit EEG slowing and spike activity by increasing the systemic concentration of Urokon by intravenous injection in those animals whose barrier was disrupted without concomitant EEG changes. They interrpreted these findings as indicating that the EEG changes associated with the increase in systemic concentrations of Urokon were due to the increase in the amount of Urokon reaching the neural tissue through the permeable barrier.

In principle, any agent used to disrupt the barrier may also produce neural effects related to its ability to reach the internal environment of the brain once the barrier is open. Consequently, any neural changes attributed to barrier disruption alone must take into account the possible confounding effects of the technique used to disrupt the BBB. That is not to say that all techniques of experimental

disruption result in changes in the EEG that are a consequence of the above findings. This may be the case with hyperosmotic disruption of the BBB, where disruption of the BBB is a result of the concentration of the agent used rather than the specific compound used. Disruption of the BBB has been produced by perfusion of the carotid artery with compounds such as mannitol and arabinose in concentrations of 1.4 molar (Rapoport, Ohno and Pettigrew, 1980) as well as a 2 molar solution of Urea (greater than two times physiologic osmolarity) (Rapoport, Hori & Klatzo, 1972). Disruption with this technique is reversible (lasting approximately one hour) and has been attributed to transient opening of the tight junctions of the capillary endothelium. The reported epileptic activity (Fieschi, Lenzi, Zanette, Orzi & Passero, 1980)and increase in glucose metabolism (Pappius, Savaki, Fieschi, Rapoport & Sokoloff, 1979) following hyperosmotic disruption has been attributed to changes in the neural environment subsequent to penetration of the BBB of normal blood constituents rather than to any direct effect of the agent used for the disruption. However, studies by Rapoport on non-epinephrin and potassium failed to confirm these agents as the cause for the neural changes seen with

hyperosmotic disruption (Rapoport, London, Fredericks, Dow-Edwards & Mahone, 1981)

Both Flodmark and Fieschi's findings were based on models of BBB disruption that reversed in a matter of hours. In an attempt to study the cumulative effects on neural function of persistent barrier disruption, Flodmark investigated the EEG changes associated with intracarotid mercuric chloride (Flodmark & Steinwall, 1963b). Mercuric chloride, when administered intracarotidly, results in a chronic disruption of the BBB. Flodmark described three stages of EEG following intracarotid administration of mercuric chloride. Stage I occurred during the carotid perfusion and consisted of decreased amplitude and frequency of the EEG on the side of disruption. Flodmark attributed this finding to a transient anoxia that occurs because of a displacement of the cerebral circulation on the side of perfusion. Based on findings in previous studies, these changes were found to reverse minutes following the perfusion (end of Stage I), returning to a symmetrical pattern resembling baseline activity (beginning of Stage II). This period lasted approxiamtely two hours except when disruption was severe as indicated by the extent of

hemispheric staining, and then, Stage II could be as short as ten minutes. Stage II represents a latent period of time before the neuronal effects of the mercuric chloride and the disrupted barrier become manifested in the EEG. As these effects begin to increase, the EEG becomes asymmetrical and may become flat over the disrupted hemisphere (Stage III).

The above findings indicate that persistant barrier disruption with mercuric chloride can lead to severe EEG changes. This is in contrast to the absence or transient changes noted with reversible barrier disruption described with hyperosmotic disruption. It appears that the cumulative effects of an open barrier can result in neuronal dysfunction that is not present when the barrier is reversibly disrupted. However, other studies on barrier disruption have demonstrated that mercuric chloride, beside causing barrier disruption, also inhibits the active transport systems of the BBB as well as having a direct toxic effect on neurons (Chang & Hartmann, 1972). This can then result in neuronal dysfunction that is independent of any effects of an open barrier. Consequently, the conclusions with respect to neuronal dysfunction based on barrier disruption by mercuric chloride must be interpreted in light of the other known

toxic effects of this agent.

The preceding literature review demonstrates that BBB disruption can be employed to investigate the effects on neuronal activity of exposure to systemic agents either produced endogenously or administered systemically. However, such studies must be interpreted with respect to the factors discussed above. A technique that produces prolonged BBB disruption with little or no direct effects on neuronal tissue would be ideal.

Recently, a technique for BBB disruption has been reported that produces prolonged barrier disruption with little or no effect on neural tissue (Spigelman et al., 1983; Levine, Spigelman, Zappulla, Povlishock, Malis & Holland, 1983). Spigelman et al. (1983) reported BBB disruption for as long as 72 hours following the intracarotid administration of the bile salt sodium dehydrocholate. Electron microscopic examination of animals subjected to barrier disruption with dehydrocholate have demonstrated increase pinocytosis in endothelial cells by the horseradish peroxidase technique (Levine et al., 1983). Examination of neurons and supporting cellular structures failed to reveal any injury as a consequence of barrier disruption with

dehydrocholate. The technique is a modification of that initially described by Broman & Lindberg-Broman (1945), who used the more toxic bile salts desoxycholate and glycocholate. This agent was extremely toxic necessitating drainage of the jugular vein to limit systemic toxicity.

Disruption of the BBB with dehydrocholate (Spigelman et al., 1983) was found to be dose dependent over the range employed (5,10,15 and 17.5% solution of sodium dehydrocholate). The extent of disruption was measured using both an ordinal rating of the Evans blue staining as well as quantitatively using hemisphere ratios (disrupted/non-disrupted) of the radioisotope which does not appreciably cross the BBB. Animals disrupted and then followed demonstrated that barrier permeability was greatest at the time of disruption and then gradually decreased over the next 72 hours. The authors concluded that barrier disruption with sodium dehydrocholate can effectively disrupt the barrier, and unlike other reversible techniques, disruption lasts approximately 72 hours.

While the animals disrupted with sodium dehydrocholate demonstrated no untoward toxicity at the time of sacrifice, the investigators did report that some of the animals

demonstrated tonic-clonic movements during and immediately following the injection of sodium dehydrocholate. However, no spontaneous seizures were noted in the animals surviving the acute phase (several hours) till sacrifice 72 hours post disruption.

This model of barrier disruption is particularly adapted for studying the effects on neuronal functioning of barrier permeability. First, the acute phase of disruption appears to be accompanied by seizure activity. Whether this is due to the bile salt or the effects of barrier disruption remains to be determined. Previous published reports have demonstrated that seizures can result in BBB disruption. If the effect on the barrier from dehydrocholate is secondary to its eleptogenic properties, then conversely the extent of barrier disruption should be related to the extent and duration of seizure activity. Similarly, the lateral distribution of seizure activity should correlate with the side of disruption. Moreover, if the preceding is true, then prevention of seizures by an anti-epileptic agent should reduce the extent of disruption.

Second and more importantly, the chronicity of the barrier disruption with dehydrocholic acid makes it ideal for

studying the cumulative effects of barrier disruption on neuronal function as monitored with EEG. Since the barrier remains permeable for days following the disruption, it is possible to investigate the EEG changes isolated from the acute insult of the disruption and the effects of any remaining bile salts that would be in the systemic circulation. In addition, since the barrier has been shown to close gradually over the time course studied, then any changes noted in the EEG can be followed and correlated with barrier closure.

The following investigation consists of five separate experiments dealing with the EEG effects of barrier disruption with intracarotid dehydrocholate. The initial two studies investigate the acute effects of barrier disruption following sodium dehydrocholate and the relationship between EEG changes and the extent of barrier disruption. Also addressed is the relationship between seizure activity and BBB disruption. The third experiment studies the long term EEG effects of BBB disruption using EEG spectral analysis. The final two experiments investigate the central effects of bile salts on neuronal function.

EXPERIMENT I

Experiment I investigated changes in the spontaneous EEG and visual evoked potentials subsequent to BBB disruption by the intracarotid infusion of sodium dehydrocholate. Previous studies on EEG changes following BBB disruption have reported decreased amplitude and increased slowing over the disrupted hemisphere (Bloor et al., 1951; Flodmark et al., 1962; Flodmark et al., 1963a; Flodmark et al., 1963b; Fieschi et al., 1980). Therefore, changes in the amplitude and frequency of the EEG as well as decreased amplitude and increase latency of evoked response components proportional to the extent of disruption might be expected. Based upon previous studies (Bloor et al., 1951; Flodmark et al., 1962; Flodmark et al., 1963b; Fieschi et al., 1980) that have demonstrated seizure activity following barrier disruption from a variety of techniques as well as the finding of behavioral seizures following dehydrocholate disruption, one might expect seizure activity following dehydrocholate BBB disruption, especially if photic stimulation were used.

METHODS

Female Sprague-Dawley rats (250-350 grams), having free access to pellet food and water, were anesthetized with xylazine (Rompun-Cutter Laboratories, Shawnee, Kansas), 12 mg/kg intramuscularly and ketamine (Ketalar-Parke Davis, Morris Plains, N.J.), 30 mg/kg intraperitoneally. Each animal also received atropine, 0.15 mg/kg subcutaneously.

Eight stainless steel screws were placed in the calvarium at the following locations: the reference lead--in the nasal bone along the sagittal suture; the ground--in the posterior aspect of the skull along the midline; and two frontal, medial and parietal leads, symmetrically placed, 4 mm from the sagittal suture in the parietal bone (Figure 1). The screws were fixed with plastic dental cement, the skin incision was closed, and the rats were placed back in their cages. After 24 hours the rats were used in the following experiments.

Under ether anesthesia 28 rats were intubated with an endotracheal tube and ventilated with a mixture of 70% nitrous oxide and 30% oxygen at the rate of 200 ml per minute. A polyethylene catheter was inserted in the left

femoral vein and clamped in place. An intravenous injection of tubocurarine chloride (E.R. Squibb Sons, Inc., Princton, N.J.), 1 mg/kg was given. A rectal temperature probe was inserted, and the animals temperature was maintained at 36.8 C by an external heat source. Blood pressure and heart rate were monitored throughout the procedure.

Following isolation of the left external carotid artery, a polyethylene catheter was placed in a retrograde manner to the bifurcation of the common carotid as described by Rapoport, (1978) (Figure 2). A 2% solution of Evans blue (.5cc) was then injected into the femoral vein. Then 1 ml of saline (control group N=4) or a 17.5% solution of sodium dehydrocholate (experimental group N=20) (Decholin-Miles Pharmaceuticals, West Haven, Ct.) was infused into the internal carotid artery at a constant rate of 1 ml per minute by an infusion pump (Harvard pump). During the infusion, the pterygopalantine branch of the internal carotid was occluded with an aneurysm clip. This branch does not supply the intracranial structures and so was occluded during infusion to insure maximal flow of the dehydrocholate into the brain. The infusion was microscopically observed to insure that the sodium dehydrocholate passed upward into the brain.

A separate group of four animals were injected with 1 cc of 17% dehydrocholate intravenously to determine the effects of systemic dehydrocholate alone. These animals did not undergo carotid infusion with dehydrocholate.

EEG activity was recorded with a ten channel Grass EEG machine (Model 8-10). A baseline EEG was recorded just prior to infusion, during infusion and for up to three hours following infusion at approximately 15 minute intervals. The EEG recorded from the disrupted and non-disrupted hemispheres was inspected for changes in background frequency and amplitude. Amplitude was graded within minutes following disruption according to the per cent change from the EEG amplitude recorded prior to disruption in the following manner:

<u>Grade I</u> Less than 50% change in amplitude

<u>Grade II</u> Greater than 50% change in amplitude

The background frequency of the spontaneous EEG was evaluated by noting any shift to lower frequencies. A shift to delta activity for more than ten seconds of spontaneous EEG was

considered positive for slowing in the EEG. The EEG records were also reviewed for the presence of spikes or generalized seizures. Particular attention was paid to changes in the EEG over time to determine if just prior to sacrifice (at 3 hours post infusion) there was a worsening (as determine by an increase of delta activity) or an improvement (return to baseline EEG).

EEG reponse to photic stimulation was measured in 14 animals following infusion using various flash rates from 1 to 30 Hz. Photic stimulation was considered positive if either spike activity was initiated or if there was an increase in seizure activity that was already present. In the latter case, comparison of the EEG activity prior to and following photic stimulation was compared to EEG activity during photic stimulation to determine the epileptogenic effect of photic stimulation.

Flash visual evoked potentials (VEPs) were measured at 2 c/s stimulation rate preceeding and following disruption. In order to collect the VEPs uncontaminated by seizure activity, the EEG was monitored simultaneously as the VEP was collected. VEPs were measured at approximately 30 minute intervals following disruption until sacrifice or death. The

VEPs were evaluated on the basis of amplitude and latency changes in VEP components.

At sacrifice (three hours post-infusion) or death, the brains were removed and visually inspected for the presence of Evans blue indicator dye. All surfaces and areas of the brain including cerebellum, pineal and brain stem were examined. In addition, sagittal and coronal sections were made and visually inspected. The staining of each hemisphere was graded as follows: grade 0 - no stain; grade 1+ -just noticeable staining; grade 2+ -moderate staining; and, grade 3+ -dark staining.

RESULTS

The brains of all four rats infused intracarotidly with 1 cc of normal saline were negative for the presence of barrier disruption as evidenced by the lack of Evans blue on inspection. There were no changes in the EEG in any of the saline animals during or after infusion. Similarly, those animals injected intravenously with 17% dehydrocholate failed to demonstrate any change in their EEG or hemispheric

staining.

Tables 1 & 2 are a summary of the extent of disruption and the associated EEG changes for the 20 animals infused with intracarotid dehydrocholate. The distribution of Evans blue staining of the infused hemispheres included four animals with 1+, eight animals with 2+ and eight animals with 3+ disruption. Eleven animals also had staining of the thalamus on the side of perfusion. In addition, seven of ten animals with 3+ disruption had some staining of the contralateral hemisphere. In all cases this was limited to the medial surface of the right hemisphere and was never greater than 1+.

For 1+ disruption there was a decrease in amplitude and an increase in the frequency of the EEG over both hemispheres during the infusion (Figure 3a). Following infusion, there was a decrease in amplitude over the disrupted hemisphere in all four animals (Table 1). The decrease in amplitude was grade I for three animals and grade II for one animal. In addition, two animals demonstrated a decrease in amplitude over the non-disrupted hemisphere. Delta activity was present in only one animal with 1+ disruption. Over the course of the observation period, these changes progressively

reversed and at the time of sacrificed had returned to levels present prior to disruption (Figure 3c).

In three of the four animals with 1+ disruption, spike activity was noted during the observation period (Table 2). In two of the four animals, spike activity was present bilaterally during the infusion. This activity persisted to sacrifice in both animals (Figure 3b). In the third animal spike activity developed minutes following the infusion but had remitted at the time of sacrifice.

The EEG changes with 2+ and 3 + disruption were more pronounced than those previously described with 1+ disruption. The decrease in amplitude that occurred within minutes following disruption was greater than 50% (Grade II) in 13 animals over the disrupted hemisphere. Unlike animals with 1+ and 2+ disruption, the EEG amplitude in animals with 3 + disruption progressively deteriorated during the observation period with seven of eight animals having a flat EEG bilaterally prior to the end of the observation period (Table 1). When this is compared to EEG outcome in animals with 1+ (all four animals had EEG at sacrifice) and 2+ (one animal of eight had a flat EEG at sacrifice) disruption, animals with 3+ disruption had a statistically higher risk of

developing a flat EEG within the three hour observation period (Fischer Exact Test $p<.05$). Animals whose EEG activity ceased prior to sacrifice had the most marked decreases in amplitude on the side of disruption from infusion (Figure 4). In addition, the EEG from the non-disrupted hemisphere progressively deteriorated with high voltage delta activity prior to becoming flat (Figure 5). Of eight animals with 2+ and 3+ BBB disruption that had EEG at the time of sacrifice, the EEG amplitude over the disrupted hemisphere in three animals remained below baseline levels, while the EEG oin the remaning five animals had reverted to baseline levels at the time of sacrifice. Delta activity was present over the disrupted hemisphere in 11 of 16 animals in this group. The slowing of the spontaneous EEG progressed bilaterally in those animals that subsequently lost their EEG prior to sacrifice. In two animals (13 & 16), the EEG was flat immediately following disruption and frequency changes could not be determined.

With greater barrier disruption, spike activity occurred seconds following the start of the infusion (Figure 6). Thirteen of the 16 animals with 2+ to 3+ disruption developed spike activity during infusion. In most animals the spikes

originated over the disrupted hemisphere and then immediately became bilaterally synchronous. Spike actitvity persisted following the infusion and was more frequent and prolonged than that occuring with 1 + disruption (Figure 7). Although the majority of spike activity was bilaterally snychronus, independent spikes arising from both the disrupted and non-disrupted hemisphere were present (Figures 8 & 9).

Photic stimulation was performed in 11 of the animals and provoked seizures in three. The animals responding to photic stimulation were distributed among all grades of disruption. The response to photic stimulation ranged from the induction of spikes on the disrupted hemisphere to generalized seizures (Figure 10 a,b,c).

Visual evoked potentials were obtained in six animals, two having a grade of 1+, two having a grade of 2+ and the remaining two, 3+. There was a marked change in the amplitude of the evoked potential depending upon the extent of disruption. For grade I disruption there was no noticable change in the VEPs immediately following or after the disruption for up to three hours (Figure 11). In those animals with 2+ disruption, there was a transient decrease in the amplitude of the VEPs recorded over both the left and

right hemispheres immediately following disruption (Figure 12). In contrast, the amplitude of the VEPs in the two animals with 3+ disruption were dramatically effected following disruption (Figure 13). There was a marked decrease in amplitude as well as a loss in waveform configuration of the VEP over both hemispheres in the two animals. In both animals the VEPs from the non-disrupted hemisphere progressively deteriorated during the course of observation. Similar to the EEG in these animals, the VEPs were unobtainable prior to the termination of the observation period.

DISCUSSION

In summary, this experiment demonstrated that the intracarotid administation of the bile salt dehydrocholate results in BBB disruption and thus replicated the findings of Spigelman et al. (1983). The dose of dehydrocholate used in this study produced all three grades of disruption in animals which also concurred with the previous report by Spigelman et al. (1983). Consequently, the electrophysiologic effects of

varying degrees of disruption as measured here could be examined.

The EEG reflected the extent of disruption in all animals as evidenced by the presence of spike activity and slowing of the background activity. Although seizure activity was present for all three grades of disruption, the frequency of seizure activity increased as the extent of disruption increased. Therefore, seizure activity reflected the extent of BBB disruption and might be considered a non-invasive and potential indicator of barrier disruption.

Previous reports have demonstrated that desoxycholate, a more toxic bile salt, can produce seizure activity when the agent is applied directly to the pial surface or cerebrospinal fluid (CSF). The seizure activity observed in the present study most likely resulted from access of dehydrocholate into the neuronal enviornment through the permeable barrier. This is substantiated by the failure of any control animals to develop seizure activity following the intravenous injection of dehydrocholate in the absence of BBB disruption. Moreover, the development of seizures in some animals minutes into the infusion of dehydrocholate suggests that the barrier disruption with intracarotid dehydrocholate

was immediate. The persistence of some seizure activity in nine of 12 animals with EEG at the end of the observation period may be attributed to the continued recirculation and penetration through the disrupted barrier by the residual dehydrocholate in the systemic circulation.

Bilateral seizure activity was the predominant pattern in animals undergoing unilateral BBB disruption. This was true despite the fact that there was no evidence of BBB disruption in the hemisphere contralateral to the carotid infusion of dehydrocholate. Therefore, it seems unlikely that the seizure activity observed on the non-disrupted hemisphere was a direct effect of bile salt on neurons of the non-disrupted hemisphere. The bilateral synchronous spike activity observed suggests transcallosal propagation of spike activity from the primary seizure focus (disrupted hemisphere) to the non-disrupted hemisphere, resulting in dependent spike activity. The occasional independent spike recorded in some animals over the non-disrupted hemisphere suggests the establishment of an independent focus in the non-disrupted hemisphere. The presence of dependent and independent spike activity in the hemisphere contralateral to a primary seizure focus has been well described for other

seizure models. An alternative explanation for the bilateral seizure activity is the development of a seizure focus in subcortical structures with subsequent activation of cortical neurons bilaterally. However, although in some animals staining of the thalamus was present on the infused side, this was not present in all animals that demonstrated bilateral seizure activity.

The shift to lower frequencies in the EEG following disruption indicated a neuronal disturbance associated with BBB disruption with dehydrocholate. It is unlikely that seizures contributed significantly to the slowing since seizure activity was present bilaterally while slowing of the EEG for animals with Grade I and II disruption was, for the most part, limited to the disrupted hemisphere. Although slowing of the EEG reflected neuronal disturbance following BBB disruption, it was difficult to attribute the slowing solely to the effects of a permeable barrier or the egress of bile salt into the brain since both occured simultaneously. The disturbance of neuronal function, however, was transient despite persistant barrier opening, since most of the animals with Grade I and II disruption reversed during the observation period. In contrast, all of the animals with

grade III disruption and several of the animals with grade II disruption developed slowing of the EEG over the non-disrupted hemisphere before becoming silent bilaterally prior to the end of the observation period. These EEG changes may have reflected a diffuse disturbance of the brain arising from BBB disruption.

The alterations in the VEPs for each grade of disruption resembled those observed for the EEG. Specifically, there was a transient change in the amplitude of the VEPs following disruption. The alterations were more pronounced as the extent of disruption increased, and in some animals the VEPs were affected over both the disrupted and non-disrupted hemispheres. In those animals whose EEG reversed at the end of the observation period, the VEPs also reversed. In contrast, those animals whose EEG became silent during the observation period, the VEPs also deteriorated and were not obtainable at the time that the EEG was flat.

In conclusion, the BBB disruption with dehydrocholate resulted in changes in the electrophysiology of the brain that can be associated with the extent of disruption. These changes consisted of epileptiform activity that was marked by the presence of bilateral seizures despite unilateral

disruption. In addition, associated with barrier disruption is a decrese in the frequency of the EEG over the disrupted hemisphere. However, with an increase in BBB disruption, EEG changes occur over both the disrupted and non-disrupted hemispheres and suggest some diffuse brain disturbance which might be attributed to an increase in intracrainial pressure. The cortical response to specific sensory stimulation as measured by the visual evoked response was also affected by barrier disruption, and like the EEG, reflects the neuronal disturbance associated with BBB disruption with dehydrocholate. These findings suggested that spontaneous and elicited brain electrical activity in the form of EEG patterns and VEP component alterations can be used reliably as a non-invasive indicator of BBB disruption.

EXPERIMENT II

This experiment investigated the potential for the seizure activity associated with intracarotid dehydrocholate to produce BBB disruption. The results from the previous experiment demonstrated the association of epileptic activity with barrier disruption following the intracarotid administration of dehydrocholate. Although seizure activity occurred with all grades of disruption, the most severe and prolonged electrical irritability occurred in those animals with the most extensive disruption. Similarly, the most marked and prolonged slowing of the EEG occurred in those animals with the most extensive disruption of the BBB. These findings indicated that the EEG is a non-invasive indicator of the extent of BBB disruption, and as such, changes in the electrical excitability or background EEG reflect physiologic changes at the neuronal level.

The association of the brain's electrical activity with the extent of barrier disruption, however, raised the question of causality with respect to these findings. Previously published reports have demonstrated that seizure

activity can in and of itself result in BBB disruption (Lee & Olszewski, 1961; Lorenzo, Shirahige, Liang & Barlow, 1972; Lorenzo, Hedley-White, Eisenberg & Hsu, 1975). Lee et al. (1961) has suggested that an increase in metabolic end products resulting from intense neuronal activity during seizures can have a toxic effect on the BBB. Other workers, however, have demonstrated that the BBB disruption associated with seizure activity can be linked to systemic physiologic changes and not a result of local toxic products (Bradbury, 1979; Johansson & Nilsson, 1977). These authors noted both that circulatory and ventilatory changes occured during seizures. These systemic changes included hypertension and increase serum levels of carbon dioxide both of which have been associated with BBB disruption. Furthermore, control of systemic blood pressure and ventilation during seizures has resulted in the prevention of disruption of the barrier during seizure activity.

In Experiment I, the lack of an increase in systemic blood pressure following disruption and the control of ventilation during the experiment suggested that barrier disruption was not a consequence of the systemic effects of seizures described above. Similarly, the findings in

Experiment I of bilateral seizure activity and the lack of cortical staining in the hemisphere contralateral to infusion in animals with 1+ and 2+ disruption argues against seizure induced metabolic changes as a cause of barrier disruption. This is supported by the studies of Pappius et al. (1979) and Fieschiet et al. (1980), who demonstrated a decrease in glucose utilization and seizure activity respectively without a concomitant effect on barrier disruption in animals treated with an anti-epileptic agent (diazepam) prior to hypertonic BBB disruption.

In an attempt to determine if barrier disruption from dehydrocholate was a result of the seizure activity generated by this technique, BBB disruption was perfomed in Experiment II in the same manner as Experiment I except with the addition of an anti-epileptic agent (diazepam) prior to and at intervals following disruption. Administration of diazepam should abort or decrease the seizures associated with dehydrocholate disruption and allow observation of the extent of disruption and the EEG changes subsequent to disruption in the absence of seizure activity. Thus, in the absence of seizures any EEG changes associated with disruption should represent the effect of disruption alone on

neural activity.

METHODS

Ten rats were used in the experimental group. The rats were disrupted by the same technique as in Experiment I with the addition of the administration of diazepam .35cc intravenously five minutes before carotid infusion with dehydrocholate and then at hourly intervals until sacrifice or death. Dosage level was based on the studies of Pappius et al. (1979) and Fieschi et al. (1980) who successfully decreased the incidence of seizure activity associated with hyperosmotic disruption. Electrophysiological testing included EEG monitoring, photic stimulation and visual evoked potentials as described in Experiment I.

RESULTS

The extent of disruption was distributed similar to Experiment I (Tables 1 & 3) (Fischer Exact Test p=1.4).

Intravenous injection of diazepam resulted in an increase in frequency and a decrease in amplitude of the EEG prior to disruption.

In the two animals with 1+ disruption, the spontaneous EEG was unchanged following disruption except for a brief period of decrease in amplitude (Grade I) over the disrupted hemisphere in one animal and a decrease in frequency in both animals (Table 3). Seizure activity was absent throughout the entire record in one animal, and in the other was limited to spikes over the disrupted hemisphere (Table 4) (Figure 14). This was in contrast to the bilateral periodic spikes that developed in three of four with 1+ disruption in Experiment 1.

Animals with 2+ and 3+ disruption demonstrated EEG findings similar to those in Experiment I except that the severity of the changes were reduced. In rats treated with diazepam, five of eight developed spikes during infusion; however, their intensity and duration were aborted when compared to similar animals in Experiment I (compare Figure 5 with Figure 15). The seizures following infusion in animals treated with diazepam tended to be restricted to the disrupted hemisphere with occasional periodic bilaterally

synchronous spikes (Figure 16 & 17). This was in contrast to the periods of bilateral prolonged discharges observed in 2+ and 3+ disrupted animals in Experiment I. In most instances, the seizure activity in 2+ and 3+ disrupted animals treated with diazepam resembled that seen with 1+ disrupted animals not treated with diazepam. For all grades of disruption, three of six rats with EEG at the time of sacrifice still demonstrated occasional spikes as compared to nine of 12 in Experiment I.

Changes in the spontaneous EEG tended to be less severe in 2+ and 3+ (N=8) disrupted animals as compared to similarly disrupted animals in Experiment I. As in Experiment I, there was a shift to lower frequencies and decreased amplitude over the disrupted hemisphere but to a much lesser extent and for a shorter period of time (Table 3). In addition, in all four animals that had EEG at the time of sacrifice, the amplitude had returned to baseline levels in contrast to six of ten animals in Experiment I. Of four animals whose EEG became flat prior to sacrifice, one had 2+ and three had 3+ disruption. This reflected the severity of the EEG changes associated with grade III disruption demonstrated in Experiment I.

Photic stimulation was performed in all animals and provoked seizures in only one animal with 3+ disruption. This is in comparison to Experiment I where three of 11 animals seized in response to photic stimulation.

VEPs were measured in five animals in this group. In two animals with 1+ disruption, there was no change in the VEP characteristics following disruption. These findings were similar to those in Experiement I. Two animals with 2+ disruption also failed to demonstrate any changes in their VEPs following disruption (Figure 18). This was in contrast to the findings in Experiment I, where the animals with 2+ disruption revealed a transient decrease in all component amplitudes following disruption. The one diazepam treated animal with 3+ disruption showed a marked decrease in the amplitude of the VEP over the disrupted hemisphere that progressively deteriorated over both hemispheres (Figure 19). This was similar to the VEP changes observed in Experiment I for 3+ disruption.

DISCUSSION

The results of the present experiment demonstrated that

although seizures occur subsequent to barrier disruption with dehydrocholate, their reduction by the administration of an anti-epileptic was not associated with changes in the extent of barrier disruption. All animals treated with diazepam despite the grade of disruption had a reduction of seizure activity compared with animals with similar disruptions in Experiment I. In addition, there was a preferential effect of diazepam on the non-disrupted hemisphere as evidenced by the decrease in the frequency of spike activity in this hemisphere as compared to the disrupted hemisphere. This suggested that treatment with diazepam had a more profound effect on the seizure threshold in the non-disrupted as compared to the disrupted hemisphere.

The decrease in amplitude and frequency over the disrupted hemisphere in diazepam treated animals followed the same pattern as those animals not treated with diazepam in Experiment I. However, in those animals in Experiment II whose EEG returned to baseline levels at the time of sacrifice, the decrease amplitude and slowing of the EEG was less severe than similar animlals in Experiment I. In contrast, in those animals with extensive disruption (3+), the EEG, although initally better than similar animals in

Experiment I, progressively deteriorated.. This followed the pattern observed in Experiment I where animals with extensive disruption had flat EEGs prior to the termination of the observation period. Thus, the ultimate deterioration in the EEG seen with extensive disruption was independent of seizure activity and was a function of some other mechanism associated with disruption. As discussed in Experiment I, the deterioration in the EEG was diffuse involving not only the disrupted but also the non-disrupted hemisphere.

The results of the present study indicated that although seizures are a manifestation of dehydrocholate disruption, their presence does not account for the BBB disruption but rather may be a consequence of disruption. It may well be the case that the disruption of the BBB by dehydrocholate is necessary for the penetration of the bile salt into the neuronal environment with the subsequent development of seizure activity. Alternatively, intracarotid dehydrocholate in exclusion of barrier disruption may produce metabolic changes that result in the lowering of the seizure threshold without direct contact with neuronal tissue.

EXPERIMENT III

This experiment examines EEG changes for up to 72 hours following BBB disruption with dehydrocholate. The two previous experiments detailed the electrophysiological changes occurring within three hours following BBB disruption with dehydrocholate. These changes consisted of cortical irritability manifested by seizure activity, the severity of which was directly related to the extent of barrier disruption, with some animals demonstrating spike activity at the end of the observation period. Slowing of the background EEG was associated with cortical irritability. In the case of grade I disruption and the majority of animals with grade II disruption, these changes reversed within the period of observation. However, in some of the animals with grade II disruption, the EEG remained slow at the end of the observation period, while in the majority of animals with grade III barrier disruption, the EEG progressively deteriorated. In the worse case, the EEG became flat within minutes of injection while in others the progression was slower with the EEG becoming silent two hours following

disruption. This deterioration in EEG occurred in grade III animals despite pre-treated with an anti-epileptic to prevent or reduce seizures.

Since disruption with dehydrocholate persists for a period of 72 hours, it is possible that the cumulative effects of barrier disruption may be manifested in the EEG at a later time than that previously studied. Therefore, whether the EEG will progressively deteriorate, or, after a period of time, will improve with restoration of the EEG to pre-disruption levels was not clear. In order to determine the long term effects of chronic BBB disruption on neuronal activity, Experiment III investigated EEG changes longitudinally for up to 72 hours following the intracarotid infusion of dehydrocolate. Histologic examination of the brains in a group of animals subsequent to disruption was performed.

METHODS

Recording electrodes were placed using the same technique described in Experiment I. Twenty-four hours following the application of electrodes, EEG recordings were

obtained in awake animals prior to disruption. In 12 animals (group 1), recordings were obtained using a standard polygraph. During the collection of data, the animals were observed closely for movements that could contaminate the recording, and appropriate logging of the artifacts were made on the EEG record. In a separate group of 12 animals (group 2), the EEG was recorded using a Cordis Neurosurgical Monitor, and a spectral analysis was obtained on one minute of artifact-free EEG activity in order to more specifically quantify any changes in the EEG over time. The artifact levels were set for each recording session by obtaining samples of two seconds of EEG activity immediately prior to the collection of data for spectral analysis . If by visual examination the recording was free of contamination, then the parameters of the acquisition system were set so that all subsequent EEG data above the voltage amplitude levels of the EEG sample were rejected. The EEG spectral analysis was reported as relative power for delta, theta, alpha and beta frequency bands.

Following collection of the baseline EEG, the animals were anesthesized with Rompum and Ketamine and their BBBs disrupted with intracarotid dehydrocholate (17.5%) as

previously described. During disruption and for a period of one hour following disruption, the EEG was monitored. The animals were then placed in cages to recover from the anesthesia.

EEG recordings were collected at 24 hours post-disruption in group 1 (N=12). Approximately 15 minutes of artifact-free EEG were recorded. The EEG of group 2 animals (N=14) was collected as described above in all animals at 24 hour post-disruption and in up to 72 hours post-infusion in those animals that survived this time period. At the time of sacrifice or death, the brains were removed and inspected for the presence of Evans blue and graded for BBB disruption as previously described. In addition, light microscopic examination of the brains was performed, without the reviewer having information on the extent of disruption.

Visual evoked potential and photic stimulation were not performed in this study due to the inability to adequately control the stimulus in awake and free moving animals.

RESULTS

Group 1

Table 5 lists the grades of disruption for all animals in group 1. Of the 12 animals, three died within 24 hours of disruption, and consequently, no post-disruption EEG was obtained. The remaining nine animals were distributed between 1+ and 2+ disruption. Two animals failed to demonstrate any Evans blue staining 24 hours after disruption.

The acute EEG changes associated with dehydrocholate disruption described in Experiments I and II were present in this group of animals, i.e., seizures with slowing and decreased amplitude of the EEG over the disrupted hemisphere. As in the previous experiments, these findings were more pronounced at the higher grades of disruption. At 24 hours, the background EEG was unchanged from pre-disruption recordings in all animals except one. In that animal the voltage of the EEG was lower over the disrupted hemisphere as compared to the non-disrupted hemisphere.

Two animals demonstrated spike activity 24 hours post-disruption. Spikes were present in the spontaneous EEG record predominantly over the disrupted hemisphere but occassionally ocurring bilaterally (Fig 20).

Group 2

Ten of 12 animals demonstrated Evans blue staining of the infused hemisphere at the time of sacrifice or death. Two animals had no staining of the brain at the time of disruption. Half the animals (six) were found to have 1+ disruption, while three animals had 2+ and one animal 3+ disruption. Five animals died prior to the end of the 72 hour observation period. These animals were distributed between 1+ and 3+ disruption.

Table 6 lists the means and standard deviations of the relative power for all four frequency bands for the pre and post-infusion conditions. Over 50% of the EEG power resided in the theta and alpha bands during the pre-break recordings. A repeated measures analysis was performed for each hemisphere across the four recording sessions. Significant effects were obtained for alpha activity for both the left ($F=4.96$; $df=3,33$; $p<.01$) and right ($F=3.38$; $df=3,33$; $p<.01$) hemispheres as well as theta activity recorded over the left hemisphere ($F=2.88$; $df=3,33$; $p<.05$). Tukey post-hoc comparisons of the means of all four conditions revealed a

significant decrease in alpha activity over both hemispheres for all post-disruption conditions compared to pre-disruption levels ($p<.05$). There was a trend toward a return to pre-disruption alpha activity at 72 hours post-infusion. Theta activity increased over the recordng sessions with a significant difference between pre-infusion and 72 hours post-infusion.

Light microscopic examination of the brains failed to reveal any pathological changes in the disurpted or non-disrupted hemispheres.

DISCUSSION

The EEG findings presented in this experiment (group 2) are an extension of those described in the previous studies. The presence of spike activity in two animals 24 hours following disruption may represent the residual of the seizure activity that was observed acutely following BBB disruption (Experiments I & II). Whether the spike activity present at 24 hours represents a chronic seizure focus like

that produced by penicillin or cobalt is not answered by this study.

The lack of significant changes on visual inspection of the background EEG at 24 hours in the group 1 animals (all animals except one that survived had normal EEGs at 24 hours) suggests as in the initial experiments that the slowing and decrease in amplitude associated with dehydrocholate disruption are reversible. Quantitative analysis of the relative spectral power in group 2 animals revealed subtle changes in the EEG that were not present on visual inspection in group 1 animals. These changes represent alterations secondary to the BBB disruption, and at least for the decrease in alpha activity, tended to normalize over 72 hours as the barrier was reconstituted. The slight but significant increase in theta activity over the disrupted hemisphere represents a shift to lower frequencies which tend to occur with pathological processes that effect neural activity. Of even more interest is the lack of more dramatic changes in the EEG in light of the EEG changes known to occur acutely with dehydrocholate disruption (Experiment I). This indicates that BBB disruption with dehydrocholate is compatible with relativlely normal neuronal function despite

the persistence of barrier permeability for up to 72 hours post-infusion. This conclusion is supported by the lack of neuronal or glial damage three hours following carotid infusion with dehydrocholate (Levine et al., 1983) as well as the lack of neuropathological findings in animals in this study.

Similar findings have been reported for behavioral measures in monkeys, who underwent BBB disruption with hyperosmotic carotid perfusion (Rapoport & Thompson, 1973; Rapoport, Matthews & Thompson, 1976; Rapoport, Matthews, Thompson & Pettigrew, 1977). Seven animals with 2+ disruption failed to show any neurologic signs or edema two days following disruption, while one animal with 3+ disruption had a hemiparesis contralateral to the disrupted hemisphere (Rapoport et al., 1977). In a second study (Rapoport et al., 1973) 11 of 12 animals disrupted with hypertonic urea were neurologically normal five to ten days following disruption. In addition, a decrease in amplitude of the EEG in these animals 24 hours following disruption reverted to normal at the time of sacrifice five to ten days following disruption. Histological study of the neurologically normal animals failed to reveal any

pathological changes, although the brains were diffuesly stained with Evans blue (the grade of Evans blue staining was not reported).

The results of the present study not only confirm previous reports of a lack of long term neurologic effects of BBB disruption, but also extend these findings. Specifically, disruption of the BBB with dehydrocholate has been demonstrated to last approximately 72 hours (Spigelman et al., 1983). This is in contrast to the permeability period for hyperosmotic disruption, where the duration of barrier permeability is a maximum of several hours. Therefore, for the period studied by Rapoport (up to ten days) the barrier had already regained its normal state of selective permeability and any neurologic changes associated with barrier opening were presumably a consequence of the short period of permeability. The minor changes in the EEG over the prolonged period of BBB disruption (72 hours) with intracarotid dehydrocholate in this study suggest that brain function as measured by EEG need not be adversely affected by continued disturbances of the BBB. Indeed, the decrease in alpha activity in both hemispheres following disruption was normalizing during the time the barrier was still permeable.

Whether this is also the case at a cellular or metabolic level remains to be determined.

The pattern of EEG changes reported here permits this model to be used in the assessment of the effects of various compounds on the EEG in a situation where access to the brain is unhindered by the presence of a BBB. Specifically, the effects on the CNS of potential neurotransmitter substances normally excluded from the brain by the BBB may be evaluated by means of EEG analysis after being injected systemically during the period of barrier dysfunction.

Experiment IV

This experiment was designed to investigate the effects of sodium dehydrocholate on neuronal activity in isolation from its effects on the BBB.

The three previous experiments substantiated the reported barrier disruptive effects of intracarotid dehydrocholate (Spigelman et al., 1983). In addition, these studies demonstrated that disruption of the barrier with dehydrocholate is associated with changes in the frequency and amplitude of the spontaneous EEG recorded during disruption. These changes reflected the extent of disruption and in most cases, where disruption is mild to moderate, reversed or improved over the first 24 hours. Spike activity was regularly observed following BBB disruption with dehydrocholate and was present for all grades of disruption. It was proposed in Experiment I that following barrier disruption dehydrocholate permeates the BBB, thus gaining access to neuronal tissue, and consequently provoking seizure activity. That dehydrocholate has potential epileptogenic properties is supported by previous published studies that

have shown that desoxycholate, a more toxic bile salt than dehydrocholate, can cause seizure activity when applied directly to the brain or CSF (Horral, 1938).

Consequently, it is difficult to conclusively attribute the seizure activity following dehydrocholate infusion to either the effects of permeable BBB alone or the neuronal effects of the dehydrocholate that has penetrated the disrupted barrier. A number of other studies have also reported seizure activity associated with a variety of techniques of barrier disruption (Flodmark et al., 1962, 1963b; Fieschi et al., 1980). As in the present study, some of these studies have attributed seizure activity to neuronal activation by agents used to disrupt the BBB. As a result, the contribution of a permeable barrier to seizure activity is confounded by the potential and real epileptogenic properties of the agents used to disrupt the barrier.

We conducted pilot investigations into other agents capable of opening the BBB but without the concomitant seizure activity. We found one such agent etoposide, an anti-neoplastic agent, when infused into the carotid artery was capable of reversibly disrupting the BBB for up to 72 hours. Subsequent to disruption, the EEG in animals

disrupted with etoposide, demonstrated a slowing of the EEG that reverses in approximately five hours and was free of any seizure activity. Similarly, hyperosmotic disruption of the BBB with the intracarotid perfusion of mannitol resulted in slowing of the EEG. Although Fieschi et al. (1980) have reported spike activity occurring subsequent to barrier disruption with mannitol perfusion, this researher was unable to observe this effect. The barrier-disruptive properties of these agents, along with the lack of associated seizure activity, permit investigation of the potential epileptogenic properties of agents normally prevented from gaining access to the CNS due to the BBB.

In the present study, the EEG effects of intravenously administered dehydrocholate were investigated subsequent to barrier disruption with etoposide or mannitol. This model permits the study of the CNS effects of dehydrocholate in isolation from its effects on the BBB, since it has been demonstrated in Experiment I that the intravenous injection of dehydrocholate is without barrier or EEG effects. In addition, this study addresses the effects of systemic dehydrocholate on the CNS in the presence of a permeable barrier. In Experiment I the dose of dehydrocholate used to

disrupt the barrier continues to be available to the brain through the disrupted barrier via its recirculation in the systemic circulation. Consequently, the changes noted in the EEG subsequent to barrier disruption may be accounted for in some part by the continued entry into the brain through the permeable barrier of the residual systemic bile salt. The findings of the present study will indicate if systemic dehydrocholate in the doses used is capable of producing EEG effects that occur subsequent to the intracarotid administration of this agent.

METHODS

Electrode caps were placed on the skulls of 12 rats as described in Experiment I. Twenty-four hours following placement of the electrodes the animals were anesthetized and ventilated with 70% Nitrous oxide. Arterial and venous lines were inserted. EKG, blood pressure and temperature were monitored throughout the procedure.

The BBBs were disrupted in eight animals (experimental group) with either intracarotid etoposide (N=4) or mannitol

(N=4). The control group was made up of the remaining four animals that under-went the entire procedure except for the intracarotid infusion of etoposide or mannitol. The technique of etoposide disruption consists of the constant infusion through the left internal carotid artery of etoposide (15 mg/kg) over 25 minutes. The barrier is disrupted immediately and remains open for approximately 72 hours. The remaining four animals were disrupted with a hyperosmotic perfusion of 3.5cc of mannitol (25%) over 30 seconds through the left internal carotid artery (Rapoport S., 1978).

The EEG was monitored prior to, during and following disruption. Thirty minutes following disruption, a 17.5% solution of dehydrocholate was administered intravenously. The animals in the control group also received intravenous dehydrocholate 30 minutes following the induction of anesthesia. The EEG was observed for the presence of spike activity related to the injection of the dehydrocholate for one hour at 15 minutes intervals.

Following the observation period, the animals were sacrificed and the brains examined and graded for the presence of Evans blue as described in Experiment I.

RESULTS

All eight animals were disrupted as evidenced by the presence of Evans blue at sacrifice (Table 7). All the animals with 3+ (N=3) disruption were in animals disrupted with intracarotid mannitol, while three of the four animals with 1+ disruption were in the etoposide group. All four of the control animals were free of Evans blue staining.

BBB disruption with either etoposide or mannitol resulted in a decrease in amplitude and slowing of the EEG over the disrupted hemisphere (Figure 21 A). The changes in the EEG were initiated during infusion and persisted into the post-infusion period. No seizure activity was noted in any of the EEG records following disruption with either etoposide or mannitol.

Following the intravenous injection of dehydrocholate, seven of eight experimental animals developed spike activity within seconds following the intravenous injection of dehydrocholate (Table 7) (FIgure 21 B). Spike activity was predominantly over the disrupted hemisphere, but in all animals it quickly became bilaterally synchronous. In the

animals with 3+ disruption, the spike activity occurred in bursts lasting for approximately seconds (Figure 21 C) . In contrast, in those animals with 1+ disruption the spikes occurred singly and the interspike interval tended to be longer than that seen in animals with 3+ disruption (Figure 22). The spike activity persisted for the entire one hour period of observation. The frequency of firing and the duration of spike trains were greater immediately following the dehydrocholate injection and then tapered, occurring singly approximately every five seconds till the end of the record).

None of the animals in the control group demonstrated seizure activity subsequent to the dehydrocholate injection.

DISCUSSION

The preceding results indicate that dehydrocholate is a potent epileptogenic agent. In addition to its ability to disrupt the BBB, systemic dehydrocholate can, in the presence of a barrier disrupted from other techniques, initiate

seizure activity. The fact that the control group was free from any seizure activity demonstrates that the BBB must be disrupted for dehydrocholate to penetrate into the neural tissue and exert its epileptogenic properties. This finding concurs with previous studies that have shown that epileptic agents such as penicillin and Urokon can, in the presence of a disrupted barrier, result in seizure activity.

These findings also strongly suggest that the seizures resulting from dehydrocholate disruption may be more a function of access of the agent into the brain substance rather than simply a consequence of barrier disruption. This is suggested by the lack of seizures with both etoposide and mannitol in the presence of a disrupted barrier.

The results of Experiment I demonstrated that in a certain number of animals seizures were still present at the end of the observation period (three hours) despite the fact that in some animals the amplitude and frequency of the EEG had returned to baseline levels. The findings in Experiment IV that dehydrocholate can produce seizures in the presence of an open barrier when administered intravenously, but not produce seizures in the presence of an intact BBB, indicate that the seizure activity associated with dehydrocholate

disruption may be potentiated by the recirculation of this agent and continued penetration into the brain after its administration intracarotidly. Consequently, the duration of seizure activity may be related to levels of dehydrocholate in the systemic circulation.

Finally, the present study emphasizes the role of the BBB as a protective mechanism to potentially toxic substances that may alter neural activity. It further suggests that normally circulating substances such as bile salts can be of potential harm to the brain except for the fact that the BBB maintains a relative barrier between the systemic circulation and the brain.

EXPERIMENT V

Experiment V studied the effects on the BBB and EEG of the cortical application of sodium dehydrocholate. The generation, nature and duration of seizure activity of both the treated cortex and the homotopic cortex were investigated. Gamma-amino butyric acid (GABA), a neurotransmitter and inhibitor of seizure activity, is normally excluded from the brain by the BBB when given systemically. This agent was given systemically following the topical application of dehydrocholate and was used as a marker of BBB permeability by measuring its effects on seizure activity generated by the topical application of dehydrocholate.

The association of seizure activity with BBB disruption subsequent to the intracarotid infusion of dehydrocholate has been demonstrated in the previous experiments. The cortical irritability recorded over the disrupted hemisphere can be attributed to bile salt penetration through the BBB and its subsequent neuronal activation. The mechanism of seizure activity recorded over the non-disrupted hemisphere is less clear. The lack of Evans blue staining of the non-disrupted

hemisphere in some animals with bilateral seizure activity argues against a direct action of dehydrocholate on neuronal structures of the non-disrupted hemisphere and suggests that activation of the non-disrupted hemisphere may be due to propagated activity from the disrupted hemisphere.

The bilateral synchronous spike activity that dominated the records of animals with all grades of disruption in Experiment I suggests possible subcortical or interhemispheric spread of seizure activity from the disrupted to the non-disrupted hemisphere. This mechanism of seizure spread has been recently reviewed by Torres and Jacome (1982). The spike activity recorded from the cortex contralateral to the primary focus has been classified into dependent and independent. In the former case spike activity in the hemisphere contralateral to the primary focus occurs in the homotopic area and is dependent on propagated activity from the primary focus. That is the spikes generated in the dependent focus occur within milliseconds of the primary discharge and result from activation of the secondary focus by the primary focus discharge. In contrast, independent spike activity arising in the secondary focus occurs independent of discharges in the primary focus and indicates

the establishment of an independent focus in the cortex contralateral to the primary focus. This occurs after a variable period of time of dependent discharges which presumably lowers the threshold of neurons in the secondary focus resulting in the establishment of an independent spike focus. This has been substantiated by metabolic changes that have been observed in cortical areas contralateral to a primary focus that have developed into secondary seizure foci (Westmoreland, Hanna & Bass, 1972). The presence of both dependent and independent spikes in Experiment I indicate that spike activity in the non-disrupted hemisphere may be a consequence of activation of the non-disrupted hemisphere due to propagated activity from the disrupted hemisphere.

In order to determine if propagation of activity from the disrupted hemisphere accounts for the seizure activity over the non-disrupted hemisphere, bile salts were applied topically to one hemisphere (primary focus) and recordings were obtained from the experimental seizure focus and the homotopic area of the opposite hemisphere (secondary focus). The nature and time course of independent and dependent spike activity in both the treated hemisphere and the hemisphere opposite the experimental focus was evaluated.

A group of animals in the present study was also treated with intravenous GABA subsequent to the initiation of seizure activity by the topical application of dehydrocholate. This agent is known to raise the seizure threshold when in contact with neuronal tissue (Purpura, Girado, Smith & Gomez, 1958). However, GABA does not penetrate the BBB, and, consequently, its anti-epileptic activity is limited in the presence of an intact BBB (van Gelder & Elliott, 1958; Oldendorf, 1971). The potential effects of permeability changes secondary to the application of dehydrocholate were tested in these animals by noting the effect of systemic GABA on established seizure activity in the primary and secondary foci.

METHODS

Eight rats were induced with ether and then tracheostomized and ventilated with 70% nitrous oxide. Femoral arterial and venous lines were inserted. Each animal was given atropine 15 mg/kg subcutaneously and tubocuarine chloride 1 mg/kg intravenously. Temperature was maintained at 36.8 degrees by an external heating source. Heart rate

and blood pressure were monitored throughout the procedure as previous described in experiment I-IV.

The animals were placed in a sterotaxic instrument, and the skull was exposed through a midline skin incision. Under a surgical microscope with 25 magnification, a 7mm in diameter circular area of bone was removed bilaterally between the coronal and lamboid sutures with a 2mm dental drill. A segment of bone 3mm wide was left between the two craniectomies over the saggital sinus. The dura and arachnoid were removed over the exposed right and left cortical surfaces under magnification. To insure that no damage to the BBB occurred during the cortical exposure, Evans blue was administered (1cc of a 2% solution) intravenously prior to cortical application of bile salt. If the cortex stained within 15 minutes, the animal was not included in the study. The Evans blue also aided as a marker for barrier disruption from the topical application of bile salt in the study.

Application of bile salt to the right cortex was carried out by a technique described by Reichenthal et al. The technique has the advantage of limiting the spread of the applied agent beyond the area of application. The technique

involves the placement of filter paper over the cortex to be treated and then applying over the filter paper a glass tube (5mm in diameter) by which a solution of bile salt is delivered to the filter paper (Figure 23). Also within the glass tube is a separate tube that extends to within 3mm of the surface of the glass tube that rests against the filter paper. Suction is then applied to the inner tube to remove the bile solution from the filter paper to insure that none of the bile salt leaks out beyond the area defined by the glass tube. Using this procedure 10cc of 20% dehydrocholate was then applied to the right cortical surface over a ten minute period.

Following the application of bile salt to the right cortex (primary focus), bi-polar ball electrodes (2mm separation between electrodes) were placed on the perfused right cortex and over the left cortical homotopic area. Recordings were obtained from the right and left exposed cortices for approximately three hours following application of bile salt. After a period of 30 minutes following the application of bile salt in all animals, an injection of 17.5% bile salt was administered intravenously to enhance the seizure activity of the primary seizure focus presumably by

penetrating the disrupted BBB in the area where bile was applied topically.

Following the establishment of seizures in both the primary and secondary foci, electrodes were moved within the exposed cortices to deliniate the seizure foci. In addition, in several animals an attempt was made to examine the activity of the secondary focus in the absence of the influences of the primary focus. This was accomplished by the intravenous administration of GABA, a neurotransmitter that has been demonstrated to be an anti-epileptic when it reaches sufficient levels in brain tissue. Under normal circumstances, GABA is unable to penetrate the BBB and so has limited or no effect in the presence of an intact BBB. Consequently, since the barrier in the primary foci should be disrupted from the application of bile salt, GABA should reach levels high enough to alter activity in the primary foci without having a direct effect on the secondary foci where the BBB is intact.

RESULTS

Minutes following the end of the bile salt application,

Evans blue could be seen extravasating around cortical vessels and ultimately spreading to stain the surrounding cortex. The stain was 3+ in all animals as assesed by the procedure in Experiment I and was limited to the area where the bile salt was topically applied. The area demarcated by the Evans blue was a guide for placing the electrodes over the perfused cortex. Electrode recordings were confined to this area on the right hemisphere and to the homotopic area of the left cortex.

Immediately following the termination of the cortical application of dehydrocholate, spike activity was present in the primary focus in three of eight animals (Table 8)(Figure 24). Dependent spikes were recorded over the contralateral hemisphere in only one animal. Over the next 30 minutes the spike activity remitted in two animals and was limited to infrequent spikes in the third (Table 8).

Subsequent to the intravenous injection of 1cc of 17.5% dehydrocholate, spike activity was recorded from the disrupted hemisphere in all animals. In two animals spikes occurred during the intravenous injection of dehydrocholate and in the remaining animals were observed within eight minutes of the injection (Table 8). The activity in the

primary focus consisted of spikes and after discharges (Figure 25) that varied in frequency among the experimental animals.

After a variable period of time (5-30 minutes) following the intravenous dehydrocholate, spike activity occurred in the cortex contralateral (secondary focus) to the prefused hemisphere in all animals (Table 8)(Figure 25). These spikes (dependent) occurred regularly after discharges of the primary focus. However, the dependent spikes did not follow every discharge of the primary focus but tended to follow spikes that had a particular configuration (Figure 26).

Six of eight animals developed spike activity in the secondary focus independent of activity in the primary focus (Table 8) (Figure 27). Except for one animal (#2) where this activity developed within minutes of the development of dependent spikes, the presence of independent spike activity occurred from 30 minutes to 90 minutes following the development of dependent spike activity.

GABA was administered intravenously in five rats with active discharges in the primary and secondary foci. Spike activity in the primary focus and and dependent discharges in the secondary focus were inhibited in two animals (Figure

28). In both of these animals a previously established independent discharge focus was not affected by the administration of GABA (Figure 28 & 29). In a third animal the spike activity in the primary focus and the dependent discharges in the secondary focus were decreased following the administration of GABA. Of the two remaining animals, one animal demonstrated a reversal of spike polarity in the primary focus following the administration of GABA (Figure 30). One animal had no change in spike activity following GABA administration.

In one animal (# 3) with spike activity in the primary and secondary focus (dependent and independent spikes), the corpus collosum was sectioned by a right parasaggital incision through the cortex extending medially through the collosum. Immediately following the collosal section, the dependent spikes in the secondary focus remitted while spike activity remained in the primary focus (Figure 31). In addition, although dependent spike activity was abolished with the collosal section, the independent spikes in the secondary focus remained. Collosal section was confirmed at autopsy to include the entire collosum except for the anterior and posterior commisures.

DISCUSSION

From Experiments I-V it was apparant that dehydrocholate is affective in disrupting the BBB whether it is applied to the luminal or abluminal surface of the cerebral vasculature. Whether the mechanism of disruption is the same in both the carotid administration and the topical application of dehydrocholate is as yet to be determined.

In Experiment V the type and time course of the cortical irritability initiated by dehydrocholate was further defined to indicate that the the topical application of dehydrocholate like the intracarotid administration results in seizure activity presenting as spikes over the disrupted area. However, it would appear that the intensity and duration of seizure activity with intracarotid administration are greater than that elicited with the topical application of dehydrocholate alone. This difference in intensity and duration may be due to the fact that with local application the available dehydrocholate within the epileptogenic area is limited compared to the supply of dehydrocholate that is

continually recirculating in the systemic circulation following its intracarotid administration. In the latter condition, since the barrier is disrupted, the bile salt can continue to penetrate the BBB reinforcing the seizure focus as in Experiment I or initiating the spike focus as demonstrated in Experiment IV. This conclusion is supported by the electrophysiologic activity following the intravenous injection of dehydrocholate in the present Experiment V in that spike activity that had abated or stopped after a period of time following topical application was enhanced following the intravenous injection of dehydrocholate. The frequency of spike activity following intravenous dehydrocholate closely resembled that observed in Experiment I.

Furthermore, the seizure activity that occurs with intracarotid dehydrocholate is marked by seizure activity over the disrupted and non-disrupted hemisphere and thus bilateral in nature. Although, as discussed in Experiment I, various mechanisms can account for the seizures over the non-disrupted hemisphere, Experiment V supports the theory of an inter-hemispheric propagation of seizure activity from the disrupted to the non-disrupted hemisphere. Inter-hemisheric propagation of seizure activity has been proposed in other

studies that have demonstrated the presence of seizure activity in an area of homotopic cortex opposite a previously established primary seizure focus. The nature of the seizure activity in the non-disrupted hemisphere as measured in Experiment V closely resembles that reported to occur in secondary foci, namely the presence of dependent spike activity in the non-disrupted hemisphere as a result of transcollosal propagation of seizure activity from the primary focus. The dependent spikes observed would then be the result of activation of cortex to which the primary focus projects. In five of ten animals in this experiment, independent spikes were observed thereby indicating the establishment of an independent spike focus in the non-disrupted hemisphere. Thus it appears from the presence of these independent spikes that the continued excitation of the homotopic cortex from propagated activity in the primary focus (disrupted hemisphere) results over time in the establishment of an independent seizure focus in the homotopic cortex. A conclusion that corroborates previously published studies (Torres et al., 1982).

The changes in seizure activity with the intravenous administration of GABA support the nature of the dependent

and independent properties of the secondary focus in the non-disrupted hemisphere. Since GABA, a demonstrated anti-epileptic agent, is unable to penetrate the BBB (van Gelder et al., 1958; Oldendorf, 1971), then access of the drug should be limited to the primary focus where the BBB is disrupted. Therefore, the effects of GABA should be limited to seizures arising from and dependent upon the primary focus. This was the case in Experiment V in three animals where GABA was able to decrease or stop the seizure activity in the primary focus as well as the dependent spike activity in the secondary focus that depended on the activity of the primary focus. However, in two animals that were demonstrating independent spikes, GABA was not able to alter this activity. This was presumably due to the fact that the BBB in the secondary focus was intact, hence impeding the passage and the effects of GABA on the secondary focus. The change in spike polarity in one animal concurs with a previous report of a decrease in the surface negative response by GABA due to an inhibition of the depolarizing post-synaptic potentials (Purpura et al., 1958).

Following section of the corpus collosum, seizure activity in the primary focus was unaltered, while the

dependent spike activity ceased. These results emphasize the collosal pathway in the generation of dependent spike activity in the non-disrupted hemisphere. Propagation along this pathway probably explains the bilateral seizures observed during unilateral BBB disruption in Experiments I and II. Although a possible subcortical mechanism has not been entirely ruled out, the findings presented in this study indicate that seizures originating over the non-disrupted hemisphere are due totally or in large part by propagation from the disrupted hemisphere to the non-disrupted hemisphere by inter-hemispheric commissures.

GENERAL DISCUSSION

The results of the present investigation corroborate and extend the conclusion that disturbances in neuronal function accompany barrier disruption with dehydrocholate. Alterations in the EEG were noted in all animals subjected to BBB disruption. Electroencephalographic consequences of BBB disruption have also been the subject of a series of reports investigating the effects of enhanced BBB permeability on neuronal function. These effects have included slowing and decreased amplitude of the spontaneous EEG as well as the development of epileptiform activity (Bloor et al, 1951; Flodmark et al., 1962, 1963a, 1963b; Fieschi et al., 1980); findings similar to those reported in the present investigation.

The type and extent of EEG changes reported in the literature have varied both with the technique used for disruption and the degree of disruption. In an investigation of EEG changes associated with barrier disruption from the intracarotid administration of various contrast agents used for angiography, Bloor et al. (1951) reported an increase in slowing of the EEG as well as an increase in spike activity

as disruption increased. Flodmark et al. (1962) reported that reversible disruption of the BBB with a lower dose of contrast agent than that used by Bloor et al. (1951) was unaccompanied by EEG changes. Fieschi et al. (1980), however, reported tardive paroxysmal activity subsequent to reversible barrier disruption with hyperosmotic perfusion through the internal carotid artery. In contrast, reversible disruption of the BBB by the intracarotid administration of mercuric chloride resulted in a progressive decrease in amplitude and an increase in slowing of the EEG over the disrupted hemisphere (Flodmark et al., 1963a).

The results of the present thesis substantiate the EEG findings from the previous studies as well as the description by Spigelman et al. (1983) of behavioral seizures following barrier disruption with sodium dehydrocholate. Barrier disruption with dehydrocholate results in slowing of the spontaneous EEG as well as the development of seizure activity. Although seizure activity was present for all grades of disruption, the frequency of seizure activity increased as the extent of disruption increased. The ability of photic stimulation to increase spike activity in some of the animals further indicates a lowering of the seizure

threshold following BBB disruption with sodium dehydrocholate. The development of seizures in some animals after only a few seconds of the dehydrocholate infusion suggests that barrier disruption is immediate. Therefore, seizure activity reflected the time and extent of BBB disruption and deserves study as a potential non-invasive indicator of barrier disruption.

Previous reports have demonstrated that desoxycholate, a more toxic bile salt, can produce seizure activity when the agent is applied directly to the pial surface or introduced into the CSF. Similarly, the seizure activity observed in the present thesis most likely resulted from access of dehydrocholate into the neuronal environment through the permeable barrier. This is supported indirectly in Experiment I by the failure of any of the control animals to develop seizure activity following the intravenous injection of dehydrocholate in the absence of BBB disruption. Direct evidence for the epileptogenic properties of dehydrocholate is presented in the results of Experiments IV and V. The development of seizures subsequent to the intravenous injection of dehydrocholate in animals whose barriers were previously disrupted (Experiment IV) implies that for

dehydrocholate to activate neuronal tissue, access to the neuronal enviornment is essential. This is further supported by the production of seizure activity following the topical application of dehydrocholate, where there is no barrier between the agent and the neuronal tissue.

The finding that circulating dehydrocholate can result in seizure activity in the presence of a permeable barrier may account for the persistance of spike activity in 9 of 12 animals with EEG at the end of the observation period (three hours) in Experiment I. This may be attributed to the continued recirculation and penetration through the disrupted barrier by the residual dehydrocholate in the systemic circulation subsequent to the carotid infusion. The same explanation has been suggested by Flodmark et al. (1962), who reported that reversible BBB disruption with low dose carotid perfusion with the contrast agent Urokon is unaccompanied by any EEG changes. However, intravenous injection of Urokon during barrier disruption resulted in EEG changes which included spike activity. Flodmark attributed this to the penetration through the BBB of the circulating contrast agent with subsequent alterations of neuronal function. Although recircualtion and penetration of dehydrocholate may account

for the persistence of seizure activity in the three hours following BBB disruption, this mechanism is unlikely to account for the persistance of seizures in two animals 24 hours following disruption (Experiment III) when dehydrocholate has been cleared from the systemic circulation. Rather, the occurrance of spike activity 24 hours following BBB disruption with dehydrocholate may result from the establishment of an epileptic focus, whose presence depends upon the persistence of altered neural activity generated shortly after disruption.

While the the epileptic activity recorded during and following BBB disruption is due to the effects of dehydrocholate on neuronal tissue, the mechanism for the shift to slower activity and decrease in amplitude of the EEG associated with dehydrocholate disruption is less certain. Although the changes in the spontaneous EEG can result in part from the direct action of dehydrocholate on neuronal tissue or indirectly from neuronal dysfunction secondary to seizure activity, slowing of the EEG as well as decreases in amplitude have been described following BBB disruption using a variety of other techniques. In addition, slowing of the EEG has been reported to occur in the absence of any seizure

activity. This was also the case in Experiment IV, where BBB disruption with etoposide and mannitol resulted in slowing of the EEG and decreased amplitude over the disrupted hemisphere in the absence of seizure activity. Slowing and amplitude changes in the EEG have been observed in response to a variety of clinical conditions that affect brain function (e.g. brain tumors, ischemia and metabolic encephalopathy). In the absence of any known ischemic changes with dehydrocholate disruption (Levine et al., 1983), the slowing and amplitude alterations seen in the present study may be in part a direct consequence of barrier permeability. Specifically, because of the break down in the barrier between the vascular and extracellular compartments of the brain, changes in the electrolyte and metabolic milieu of the extracellular and cellular spaces of the brain are possible. These changes can lead to alterations in neuronal function that would be manifested by alterations in the EEG. One would expect that if this is the case, then the greater the disruption in the BBB, the more marked the changes in the EEG. This was the case in Experiment I, where the decrease in amplitude and prevalence of slowing was greater for animals with more extensive disruption (Experiment I, Table

1).

Despite the marked changes in the EEG that occurred immediately following disruption, the EEG in the majority of animals with 1+ and 2+ disruption (9 of 12) reverted to pre-disruption levels (Table 1). This was also the case in experiment III, where on visual inspection (Group 1) the spontaneous EEG recorded 24 hours following the intracarotid infusion of dehydrocholate was unchanged from that recorded prior to disruption in six of nine animals with 1+ and 2+ disruption. This was substantiated quantitatively in that the spectral components of the EEG did not change significantly from pre-disruption values for up to 72 hours following disruption. These results indicate that whatever electrophysiologic changes occur acutely with dehydrocholate disruption, they tend to normalize in time. These findings also support work by Rapoport et al., (1973; 1976; 1977), who demonstrated a lack of permanent behavioral and electrophysiological changes subsequent to reversible BBB disruption. However, similar findings in this study support a more general claim with respect to the effect of barrier disruption on brain function. Namely, since dehydrocholate results in disruption that persists up to 72 hours, while the

disruption arising from hyperosmotic disruption is a matter of hours at the most, the normalization of the EEG within the 72 hour window of disruption in this investigation, supports the claim that with at least 1+ and 2+ disruption, neuronal activity as measured by EEG is unaffected by barrier permeability.

This conclusion, suggests that whatever distrubances of neuronal function occur in the initial stages of disruption, they are compensated for in the presence of a permeable barrier. Whether this compensation is complete at a metabolic or cellular level cannot be determined from the studies reported in this thesis. However, the electrophysiologic findings in the present study does indicate that the presence of a disrupted barrier does not adversely affect the EEG in most animals with 1+ and 2+ disruption three hours following disruption.

The above discussion, although important with respect to the physiologic question of the effect of the BBB on neuronal function, suggests that controlled BBB disruption as demonstrated in this thesis may have implications for investigating the effects on the brain of systemically administered agents that have limited access to the CNS.

Agents that may have potential effects on neuronal function, but are limited in action because of their inability to penetrate the BBB, may be tested in this model during the period of EEG normalization and increased barrier permeability. The normalization of the EEG during the 72 hours of disruption permits evaluation of the EEG effects of these agents free from the any confounding of the EEG by the disruption itself. This conclusion is exemplified in Experiment V, where the limited acces of GABA into the brain because of its inability to penetrate the BBB could be overcome by altering the barrier permeability through the topical application of dehydrocholate. An additional application of a procedure that permits prolonged reversible BBB opening includes potential therapeutic manipulation of the BBB. The effectiveness of certain agents on CNS neoplasms is limited by their inability to penetrate the BBB. Initial studies have been performed with hyperosmotic disruption of the BBB in humans in an attempt to increase the tumorcidal dose of chemotherapy delivered to the brain (Neuwelt & Frankel, 1980). The use of dehydrocholate disruption with its longer duration of BBB permeability would offer a wider window for drug administration.

The uniformly severe and progressive EEG changes associated with 3+ disruption in the present investigation indicate that the effect of BBB disruption on neural activity may have a critical threshold for reversal. The absence of EEG bilaterally in seven of eight animals with 3+ disruption, some within minutes of disruption, suggests an insult to the CNS of significant proportion. Although the changes in these animals were initially greater over the infused hemisphere, there was a rapid progression which then involved the non-disrupted hemisphere. Although in most of these animals there was some staining of the non-infused hemisphere, involvement was always limited to midline cortex and never greater than 1+ and could not account for the marked changes over the non-infused hemisphere that were recorded following disruption.

The dramatic alterations in the EEG for animals with 3+ disruption in Experiments I and II suggest a different mechanism for changes in neuronal function with this grade of disruption. Unlike animals with a lesser degree of disruption, most of the 3+ animals in Experiment I developed marked changes in the spontaneous EEG on the disrupted hemisphere, and, in all but one animal in this group. the EEG

was flat over both hemispheres prior to the end of the observation period. Similarly, in Experiment II all three animals with 3+ disruption progressed to bilaterally flat EEGs prior to the end of the observation period. These findings suggest that the changes noted following BBB disruption resulted from a generalized process affecting the EEG bilaterally. This is further supported by the progressive deterioration of the the VEPs from the non-disrupted hemisphere in the same group of animals. Previous studies by Rapoport (1976; 1977) on the effect of the extent of disruption on fluid and electrolyte composition of the brain revealed that animals with 2+ disruption had no differences in water or salt content between the disrupted and non-disrupted hemispheres 48 hours following disruption. In contrast, animals with 3+ disruption demonstrated an increase cerebral water and salt content on the side of disruption indicating the presence of cerebral edema. Rapoport et al. (1977) proposed that the lack of edema with 2+ disruption is secondary to a compensatory mechanism that adjusts the osmotic, oncotic and hydrolic factors necessary to decrease the amount of water entering the brain through the disrupted barrier. Whereas with 3+ disruption, the

intensity and duration of the barrier opening results in fluid egress into the brain that cannot be overcome by the brains compensatory system (Rapoport, 1976).

In the present study the occurrence of a severe degree of disruption (3+) in those animals with progressive deterioration of the EEG and VEPs bilaterally suggests as in Rapoport's studies that the electrophysiologic changes may be a result of an increase in salt and water content within the disrupted hemisphere with a concomitant increase in intracranial pressure. The rise in intracranial pressure could result in neuronal disturbances in both the disrupted and non-disrupted hemisphere. As the amount of fluid entering the brain's extravascular space increased, the level of intracranial pressure would reach critical levels resulting in marked abnormalities of the EEG and VEP's recorded from both hemispheres.

Besides the bilateral changes in EEG associated with 3+ disruption, bilateral abnormalities of the EEG in the form of seizures were present with lesser grades of disruption. Bilateral seizure activity was the predominant pattern even in animals with unilateral BBB disruption with dehydrocholate. This has been reported in other studies

following unilateral BBB disruption (Flodmark et al., 1963b; Fieschi et al., 1980). In the present study bilaterally synchronous spikes were present even in those animals with 1+ and 2+ disruption, who failed to show any disruption of the hemisphere contralateral to the carotid infusion of dehydrocholate. The predominance of bilateral synchronous spike activity suggests propagation of spike activity from the primary focus (disrupted hemisphere) to the non-disrupted hemisphere. Inter-hemispheric spread of seizure activity has been demonstrated electrically during the topical application of various epileptogenic agents (Torres et al., 1982). The anatomic spread of seizures depends on the site of the cortical discharge as well as the strength and duration of the discharge. There is a preference for spread to the contralateral homotopic cortex through the corpus collosum as well as subcortical structures (Collins, Kennedy, Sokoloff & Plum, 1976).

The bilateral synchronous activity observed in Experiments I and II resembled the activity recorded in Experiment V during the topical application of dehydrocholate. This pattern was consistent with previously described primary and dependent spike activity observed with

other models of focal epilepsy and represents the spread of discharges to the contralateral homotopic cortex through inter-hemispheric pathways. The results in the one animal, where section of the corpus collosum resulted in the abolishment of the dependent spike activity confirms, the corpus collosum as the predominant pathway for inter-hemispheric spread. Therefore, the the seizure activity recorded from the non-disrupted hemisphere following disruption in the present investigation reflects propagated activity from the disrupted hemisphere. The finding that pre-treatment with the anti-epileptic diazepam results in spike activity confined mostly to the disrupted hemisphere suggests that diazepam was more effective in decreasing seizure propagation. This may indicate that the seizure threshold for propagated spike activity is higher than that in the primary focus, and hence, more adequately controlled by anti-epileptics.

The fact that the seizures recorded in the contralateral hemisphere were unassociated with any disruption of that hemisphere argues against seizures as the cause of BBB disruption. This conclusion is in opposition to previous studies which have associated the presence of seizure

activity with disruption of the BBB (Lee et al,1961; Lorenzo et al., 1972; Lorenzo et al., 1975). Although early studies attributed BBB disruption during seizures to local metabolic changes subsequent to seizure activity (Lee et al., 1961), more recent studies have linked the disruption of the BBB to increases in systemic blood pressure that can accompany seizure activity (Bradbury, 1979; Johansson, 1977). In the present thesis the lack of disruption in the hemisphere contralateral to the dehydrocholate infusion even in the presence of significant seizure activity argues against local metabolic changes resulting from seizures as a cause for barrier disruption. Moreover, the presence of BBB disruption in Experiment II even when seizure activity was abolished or significantly diminished by pre-treatment with anti-epileptics corroborates the above findings. In addition, the lack of significant blood pressure changes during or following dehydrocholate infusion fails to explain the observed disruption. Presumably, therefore, the seizures observed with dehydrocholate infusion follow rather than cause disruption.

Although the predominant pattern of seizure discharge following dehydrocholate disruption included spikes over the

disrupted hemisphere in conjuction with synchronous discharges of the non-disrupted hemisphere, occasional independent spike activity was recorded in some animals over the non-disrupted hemisphere. These independent spikes occurred after a period of dependent spike activity during both carotid infusion and topical application of dehydrocholate and is indicative of the establishment of an independent spike focus in the non-disrupted hemisphere. The presence of dependent and independent spike activity in the hemisphere contralateral to a primary seizure focus has been well described for other seizure models (Torres et al., 1982).

In summary, BBB disruption with dehydrocholate is accompanied by seizure activity that most likely results from the effects of the bile salt on neuronal tissue. The EEG and VEP changes associated with BBB disruption with sodium dehydrocholate are a reflection of the extent of disruption. In addition, these electrophysiologic changes usually reverse in approximately 24 hours despite the persistence of barrier disruption for approximately 72 hours and may be a useful, non-invasive indicator of barrier disruption.

Table 1

EEG Changes and Course Following
Intracarotid Infusion of Dehydrocholate

ANIMAL	EVANS BLUE STAINING		DELTA ACTIVITY		EEG AMPLITUDE		EEG OUTCOME
	Cortex	Thalamus	Left	Right	Left	Right	
1	1+	-	-	-	Grade II	Grade I	R (1)
2	1+	-	-	-	Grade I	Grade I	R
3	1+	1+	-	-	Grade I	NC	R
4	1+	-	+	-	Grade I	NC	R
5	2+	-	+	-	Grade II	Grade I	R
6	2+	1+	+	+	Grade II	NC	NR (2)
7	2+	1+	+	+	Grade II	NC	(100) (3)
8	2+	-	-	-	Grade II	NC	R
9	2+	1+	+	-	Grade II	Grade II	NR
10	2+	-	+	-	Grade I	NC	NR
11	2+	1+	-	-	NC	NC	R
12	2+	1+	-	-	Grade II	NC	R
13	3+	2+	-	+	Grade II	Grade I	(90)
14	3+	2+	-	+	Grade II	Grade II	(60)
15	3+	2+	+	+	Grade II	Grade I	(20)
16	3+	-	+	+	Grade II	Grade II	(50)
17	3+	-	+	-	NC	NC	R
18	3+	1+	+	+	Grade II	Grade I	(10)
19	3+	-	+	+	Grade II	Grade I	(90)
20	3+	2+	+	+	Grade II	Grade I	(120)

KEY

(1) R = EEG Return to Pre-Infusion Levels
(2) NR = EEG Failed to Return to Pre-Infusion Levels
(3) () = Minutes from Infusion to When EEG Becomes Flat Bilaterally
(4) NC = No Change

Table 2

Spike Activity Following Intracarotid Infusion of Dehydrocholate

ANIMAL	INFUSION	POST-INFUSION	AT SACRAFICE
1	+	+	+
2	+	+	+
3	-	+	-
4	-	-	-
5	+	+	+
6	-	+	+
7	+	+	D
8	+	+	+
9	-	+	+
10	+	+	+
11	+	+	+
12	+	+	-
13	+	+	D
14	+	+	D
15	+	+	D
16	+	+	D
17	-	+	+
18	+	+	D
19	+	+	D
20	+	+	D

KEY: + = Presence of Spikes D = Died Prior to End of Experiment
- = Absence of Spikes

Table 3

EEG Changes and Course Following Intracarotid Infusion of Dehydrocholate in Animals Pre-treated with Diazepam

ANIMAL	EVANS BLUE STAINING		DELTA ACTIVITY		EEG AMPLITUDE		EEG OUTCOME
	CORTEX	THALAMUS	LEFT	RIGHT	LEFT	RIGHT	
1	1+	-	+	-	Grade I	NC	R
2	1+	-	+	+	NC	NC	R
3	2+	-	+	+	Grade II	NC	R
4	2+	1+	+	+	NC	NC	R
5	2+	-	-	-	Grade I	NC	R
6	2+	-	+	+	Grade I	Grade I	R
7	2+	3+	+	+	Grade II	Grade I	(60) Flat
8	3+	2+	+	+	Grade II	NC	(60) Flat
9	3+	2+	+	+	Grade I	Grade I	(60) Flat
10	3+	2+	+	+	Grade II	Grade II	(120) Flat

KEY

+ = Presence of Spikes
- = Absence of Spikes
R = Return to Baseline
NC = No Change

Grade I = Less than 50% Change
Grade II = More than 50% Change
Flat = No EEG Present Bilaterally
( ) = Time of Death from Infusion (in minutes)

Table 4

Spike Activity Following Intracarotid Infusion
of Dehydrocholate in Animals Pre-treated with Diazepam

ANIMAL	INFUSION	SPIKE ACTIVITY POST-INFUSION	AT SACRAFICE
1	+	+	+
2	-	-	-
3	+	+	+
4	+	+	-
5	-	-	-
6	-	+	+
7	+	+	
8	-	+	
9	+	+	
10	+	+	

KEY

+ = Presence of Spikes

- = Absence of Spikes

TABLE 5

EEG 24 Hours Post-Infusion with Dehydrocholate

ANIMAL	GRADE	SPIKES	EEG
1	0	-	Normal
2	0	-	Normal
3	1+	-	Normal
4	1+	-	Normal
5	1+	-	Normal
6	1+	+	Normal
7	1+		Died
8	2+	+	Normal
9	2+	-	Abnormal[1]
10	2+	-	Normal
11	2+		Died
12	3+		Died

[1]Decreased amplitude over disrupted hemisphere

Table 6

Means and Standard Deviation of Relative Spectral Power
(Pre- and Post-Disruption)

	DELTA		THETA		ALPHA		BETA	
	X	S. D.	X	S. D.	X	S. D.	X	S. D.
Pre-break (N=12)								
L Hem	16	3.2	37	3.3	26	4.0	18	4.3
R Hem	16	2.9	37	2.9	25	3.9	17	3.5
24 Hours (N=12)								
L Hem	19	6.7	41	6.6	21	4.1	16	5.0
R Hem	17	5.7	39	7.3	21	3.8	19	6.0
48 Hours (N=8)								
L Hem	18	5.4	40	1.7	22	4.3	17	3.1
R Hem	16	3.6	38	3.0	23	4.6	19	3.7
72 Hours (N=7)								
L Hem	17	3.9	42	2.8	21	3.3	17	2.1
R Hem	15	3.4	39	4.7	24	2.6	19	2.7

Table 7

Spike Activity Following Intravenous Dehydrocholate in Animals Disrupted with Mannitol or Etoposide

ANIMAL	TECHNIQUE	SPIKES	GRADE
1	E	+	1+
2	E	+	1+
3	E	-	1+
4	E	+	2+
5	M	+	1+
6	M	+	2+
7	M	+	3+
8	M	+	3+

KEY

E = Etoposide
M = Mannitol
+ = Presence of Spikes

Table 8

Spike Activity Over the Disrupted and Non-disrupted Cortex Following the Topical Application of Dehydrocholate

	After Topical Bile		After Bile Injection		
ANIMAL	DH	NDH	DH	NDH	GABA
1	-	-	+ (3)	D/IS 5/90	NC
2	-	-	+ (8)	D/IS 10/12	NC
3	-	-	+ (6)	D/IS 30/60	ND
4	+	D	+ (1)	D/IS 5/23	NC
5	-	-	+ (2)	D/IS 4/60	+
6	+	-	+ I	D/IS 5/60	ND
7	+	-	+ (3)	D (20)	ND
8	-	-	+ I	D (3)	+

KEY

D	=	Dependent Spikes
IS	=	Independent Spikes
NC	=	No Change
ND	=	Not Done
DH	=	Disrupted Hemisphere
NDH	=	Non-disrupted Hemisphere
I	=	During Infusion
( )	=	Minutes to Spike Activity from Bile Injection
/	=	Minutes to Independent/Dependent Spike Activity

Table 3

EEG Changes and Course Following Intracarotid Infusion of Dehydrocholate in Animals Pre-treated with Diazepam

ANIMAL	EVANS BLUE STAINING		DELTA ACTIVITY		EEG AMPLITUDE		EEG OUTCOME
	CORTEX	THALAMUS	LEFT	RIGHT	LEFT	RIGHT	
1	1+	-	+	-	Grade I	NC	R
2	1+	-	+	+	NC	NC	R
3	2+	-	+	+	Grade II	NC	R
4	2+	1+	+	+	NC	NC	R
5	2+	-	-	-	Grade I	NC	R
6	2+	-	+	+	Grade I	Grade I	R
7	2+	3+	+	+	Grade II	Grade I	(60) Flat
8	3+	2+	+	+	Grade II	NC	(60) Flat
9	3+	2+	+	+	Grade I	Grade I	(60) Flat
10	3+	2+	+	+	Grade II	Grade II	(120) Flat

KEY

+ = Presence of Spikes
- = Absence of Spikes
R = Return to Baseline
NC = No Change

Grade I = Less than 50% Change
Grade II = More than 50% Change
Flat = No EEG Present Bilaterally
( ) = Time of Death from Infusion (in minutes)

Figure 1

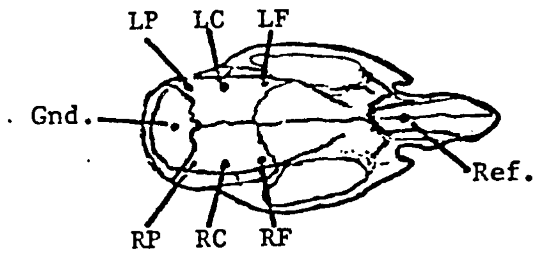


Figure 2

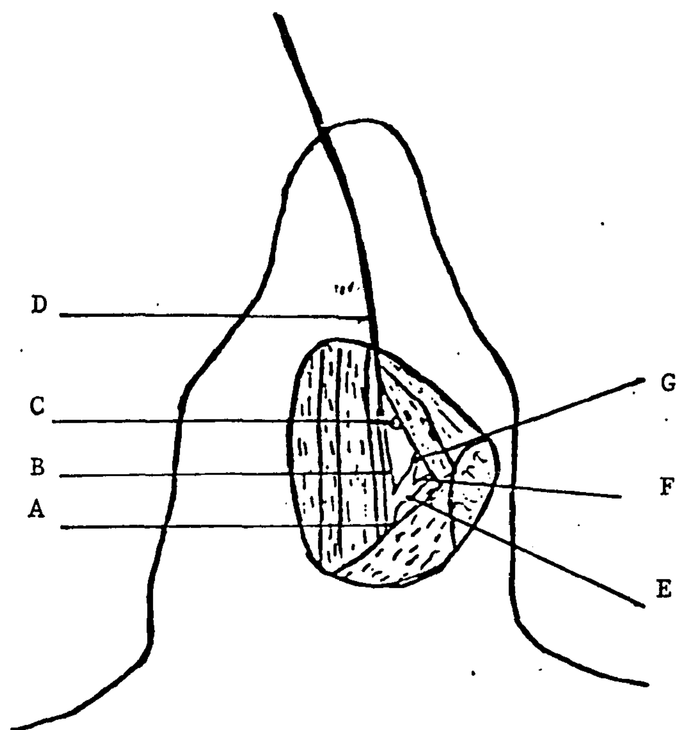
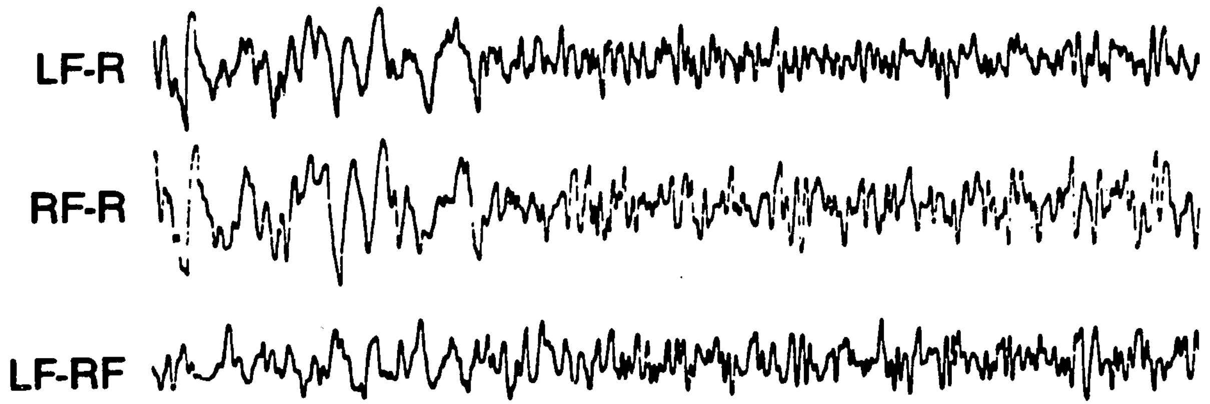
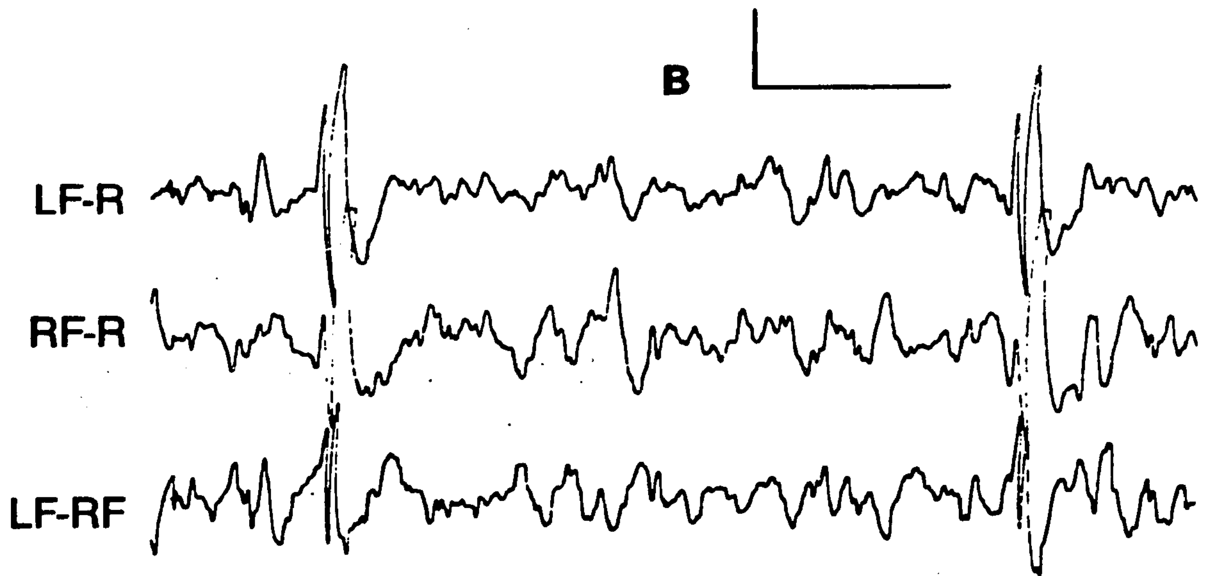


Figure 3





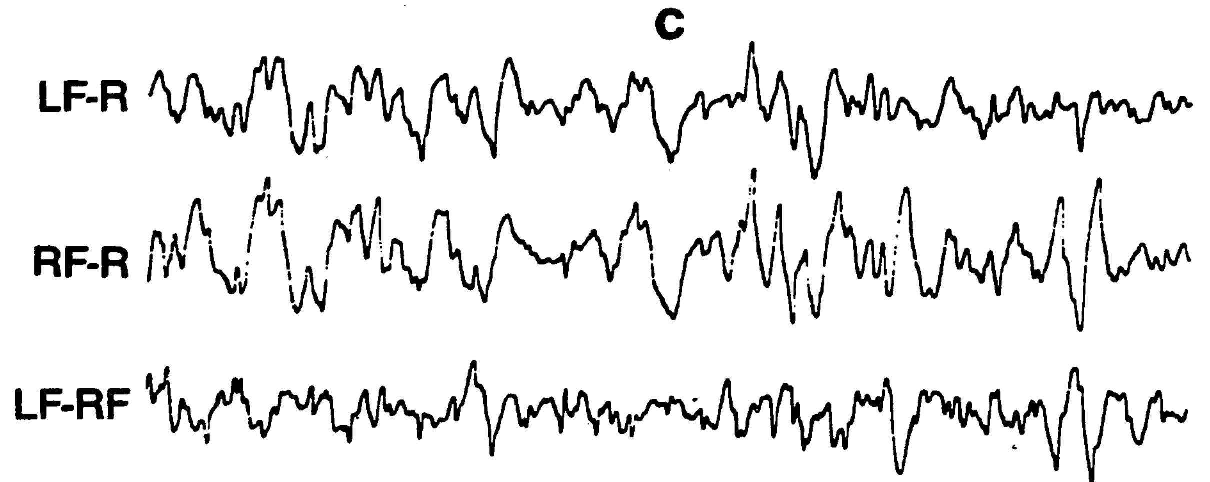


Figure 4

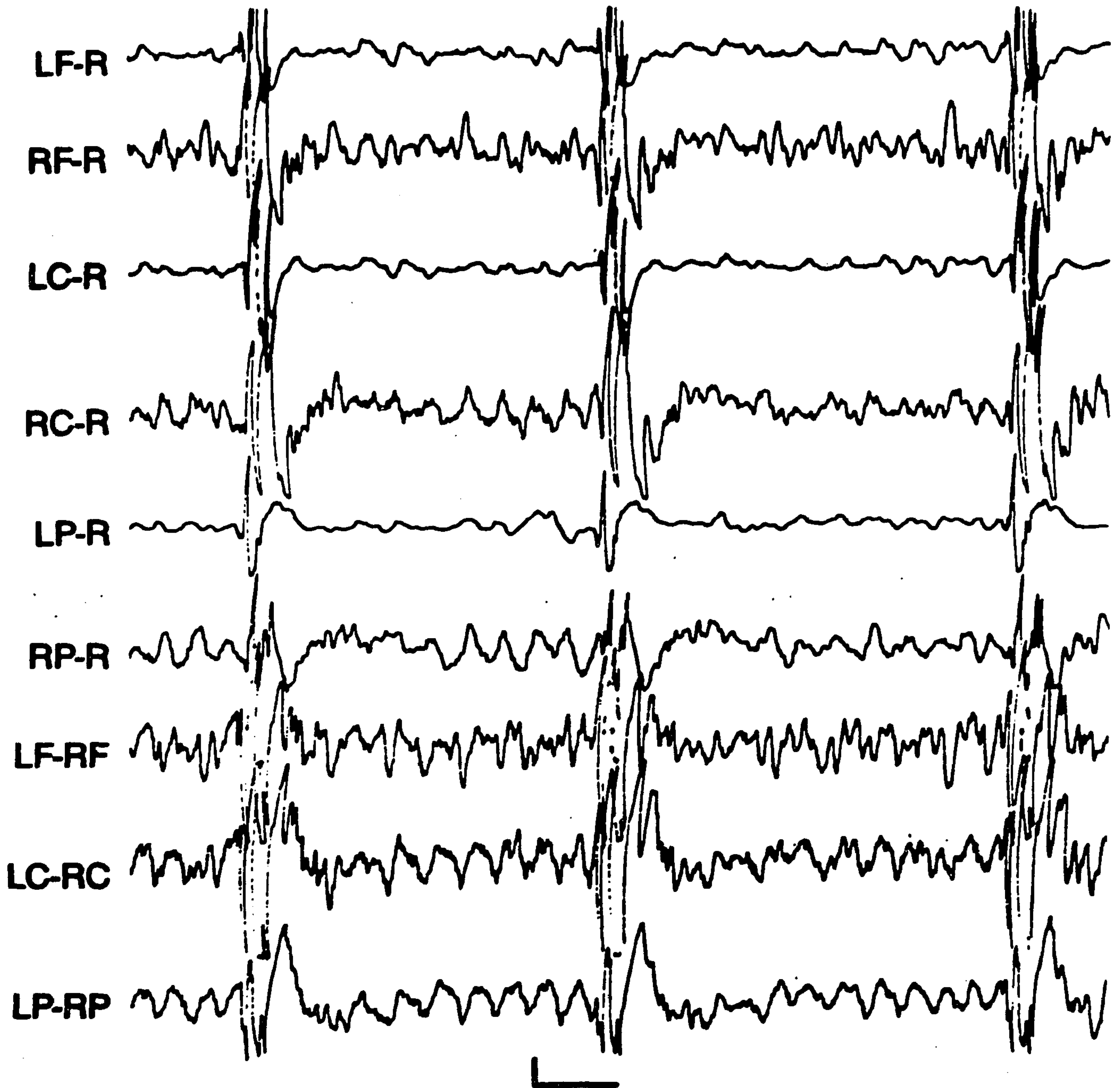


Figure 5

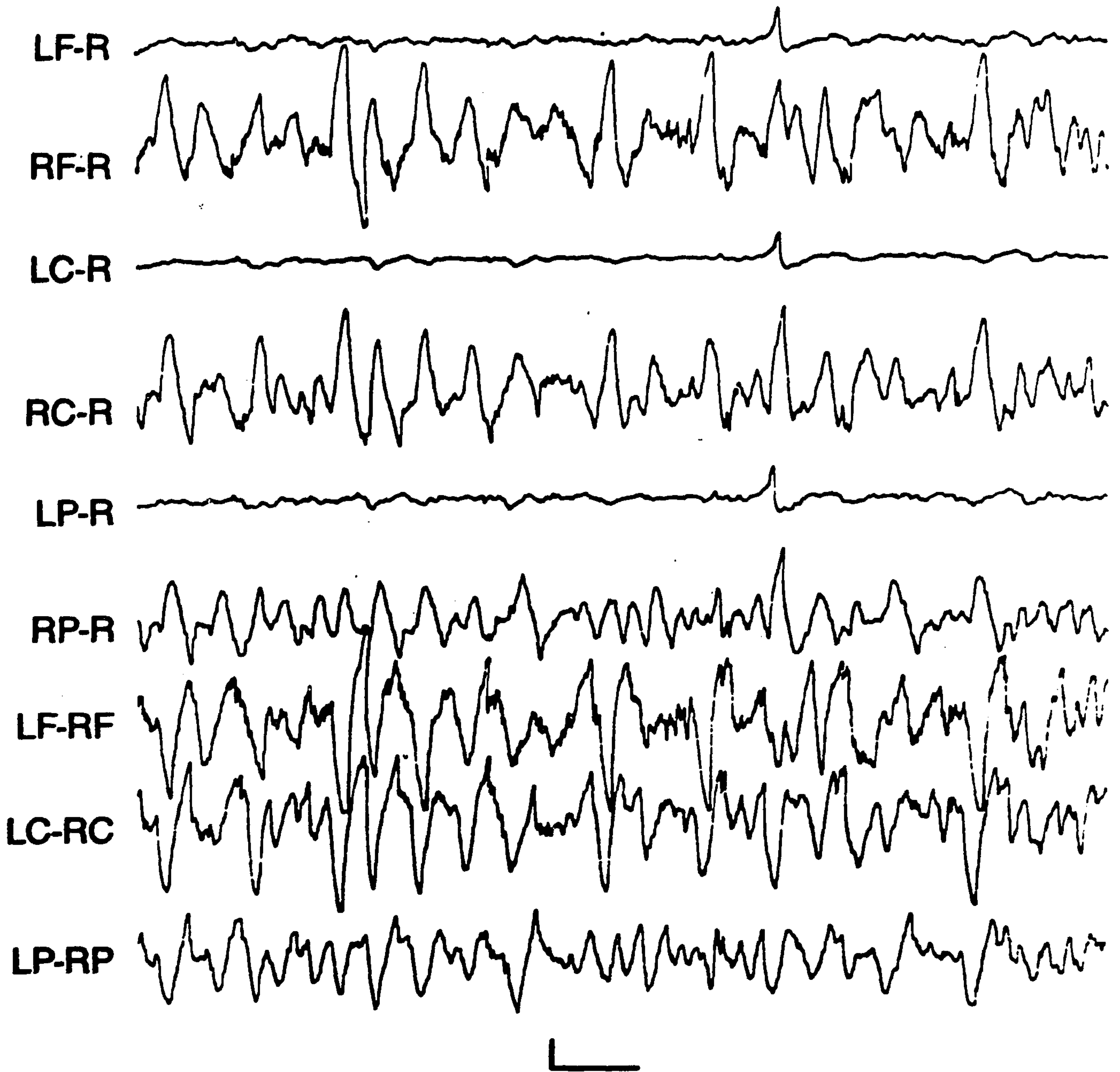


Figure 6

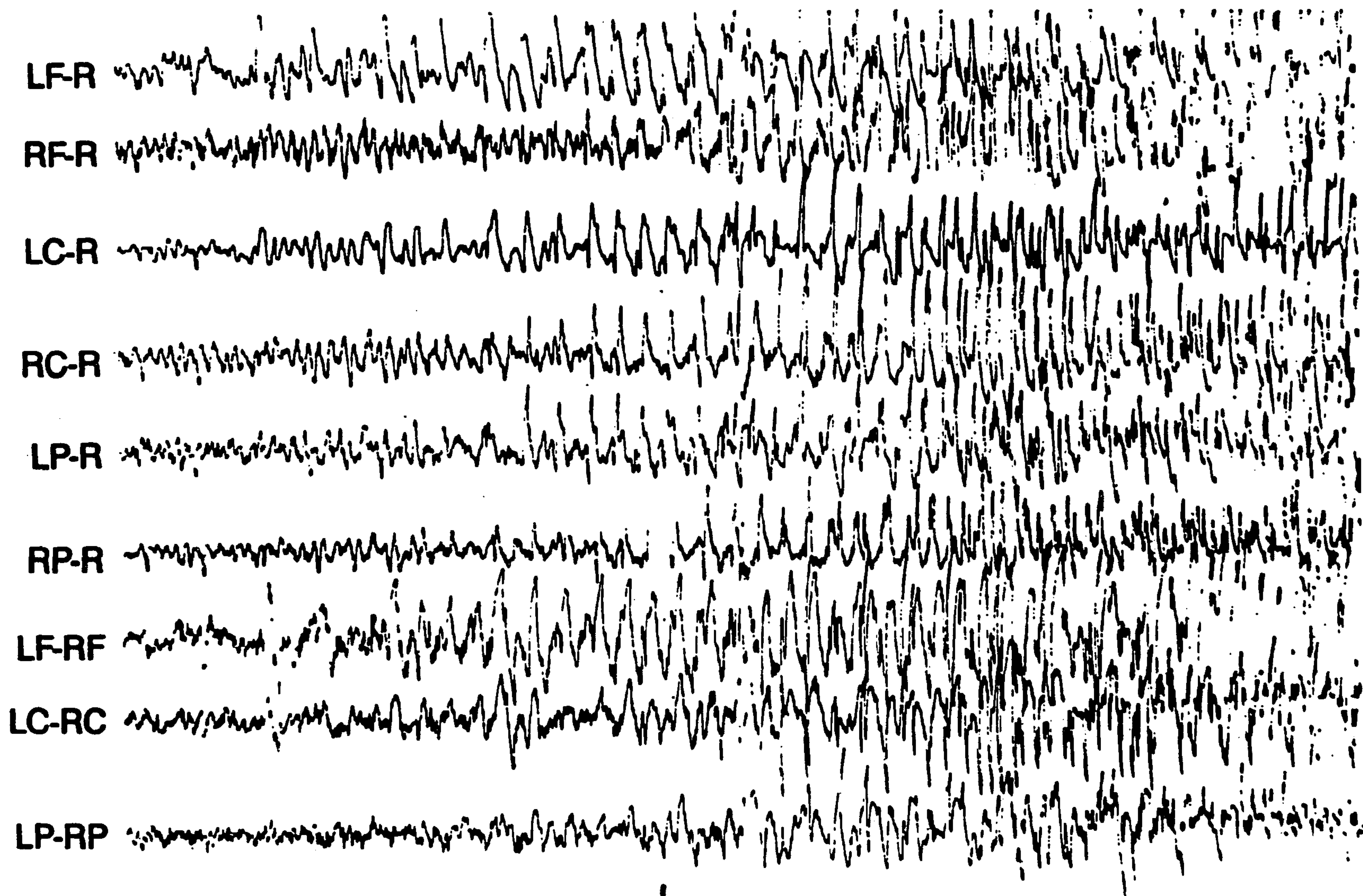


Figure 7

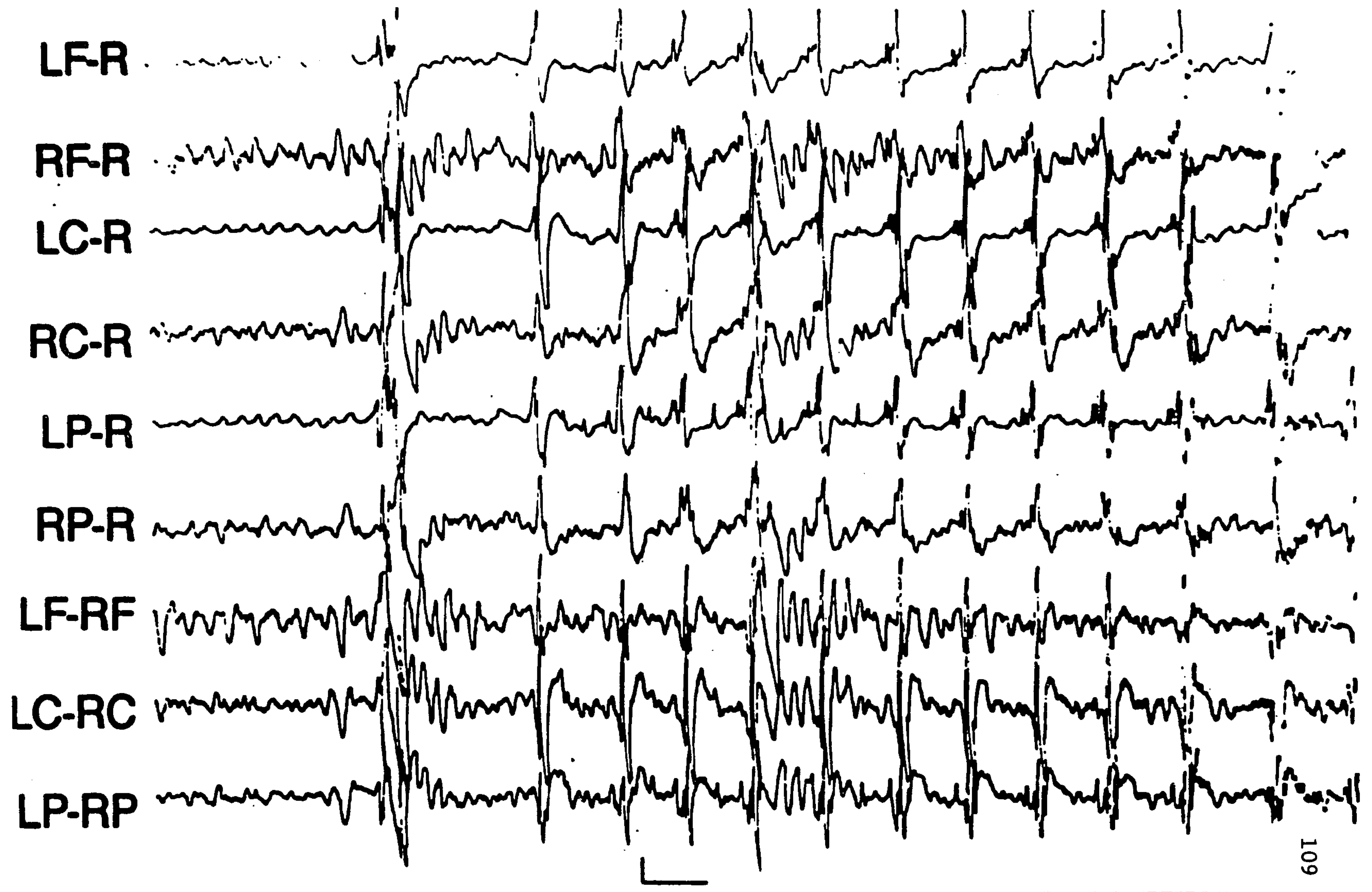


Figure 8

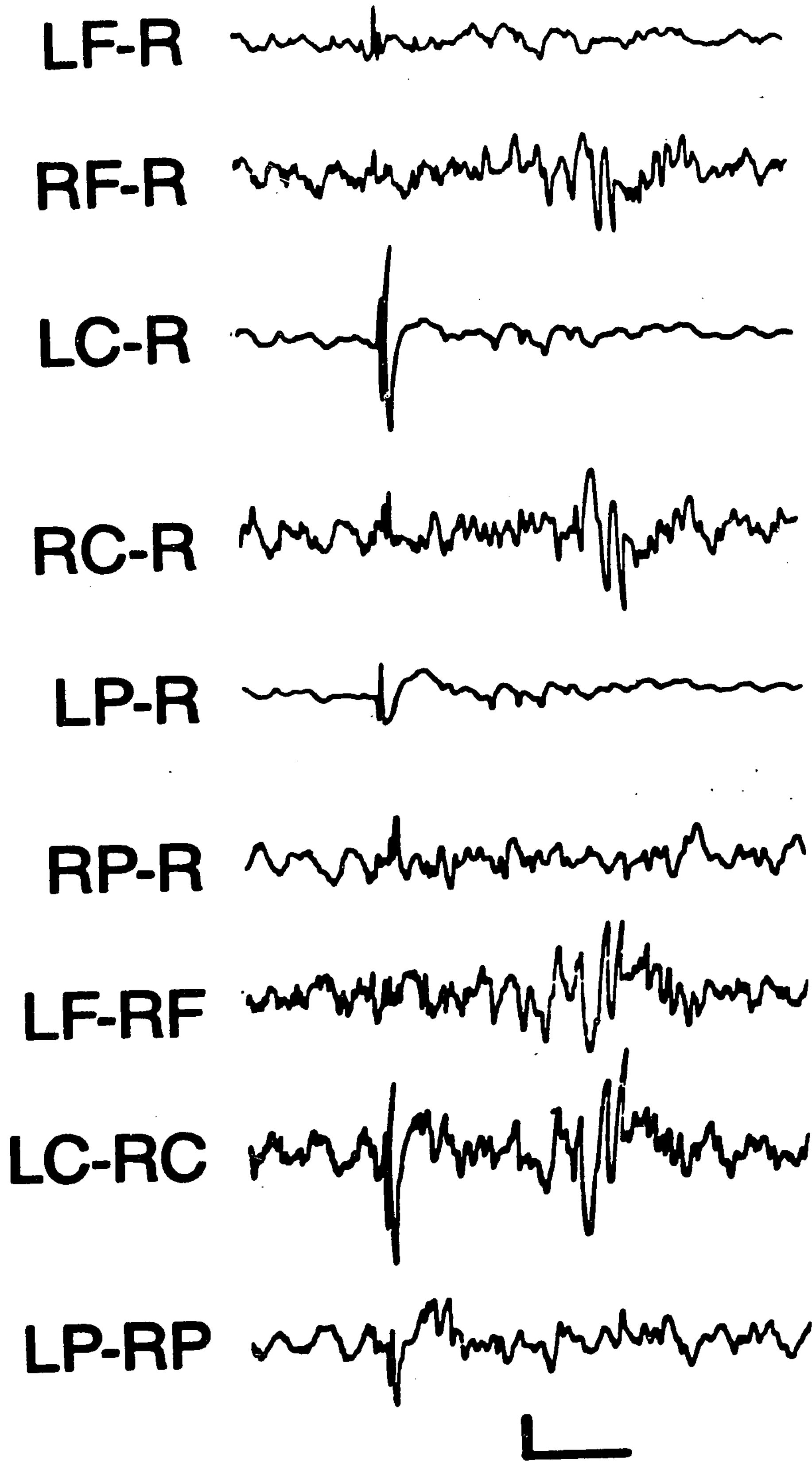


Figure 9

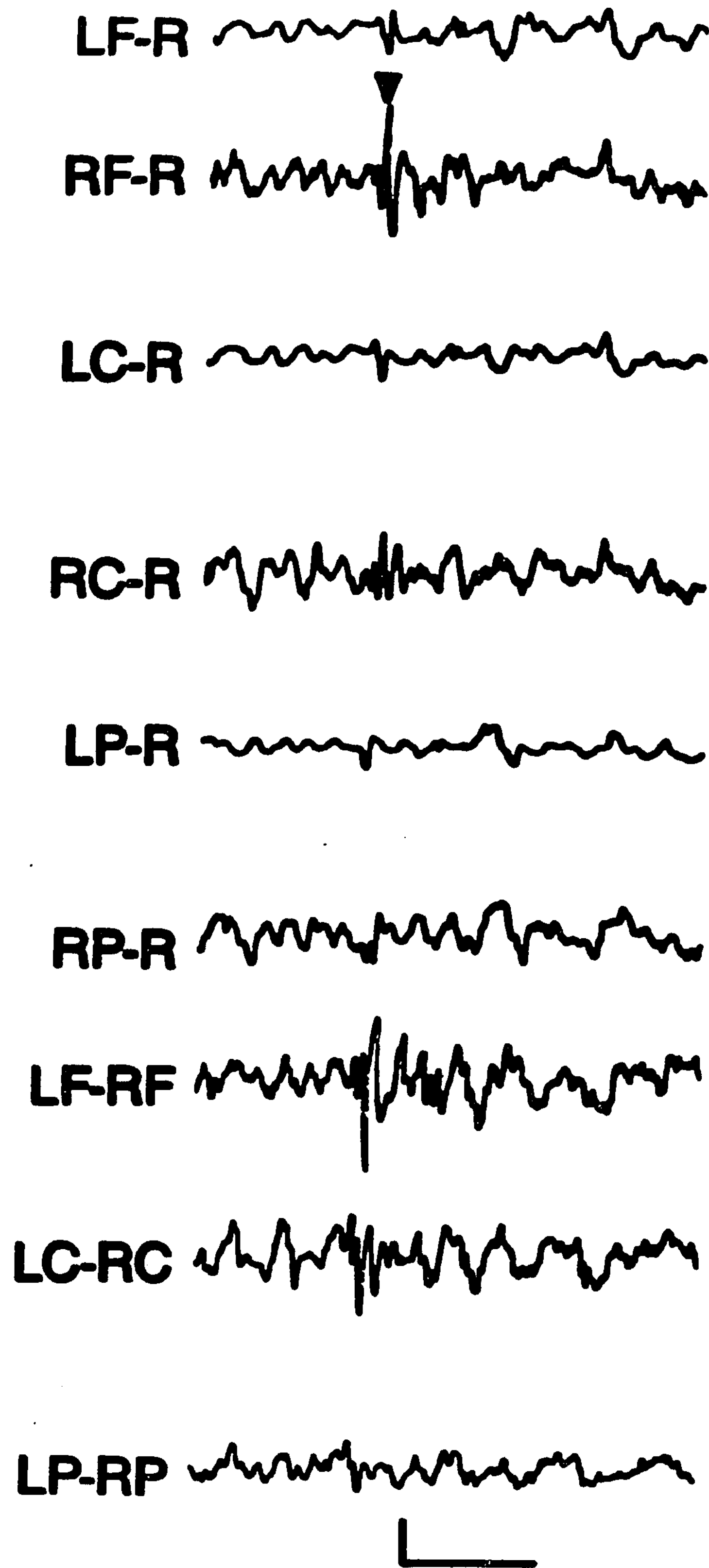
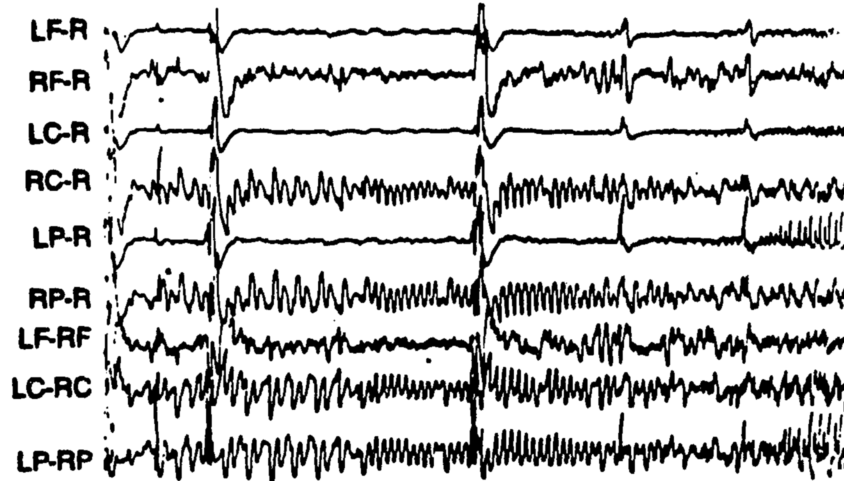
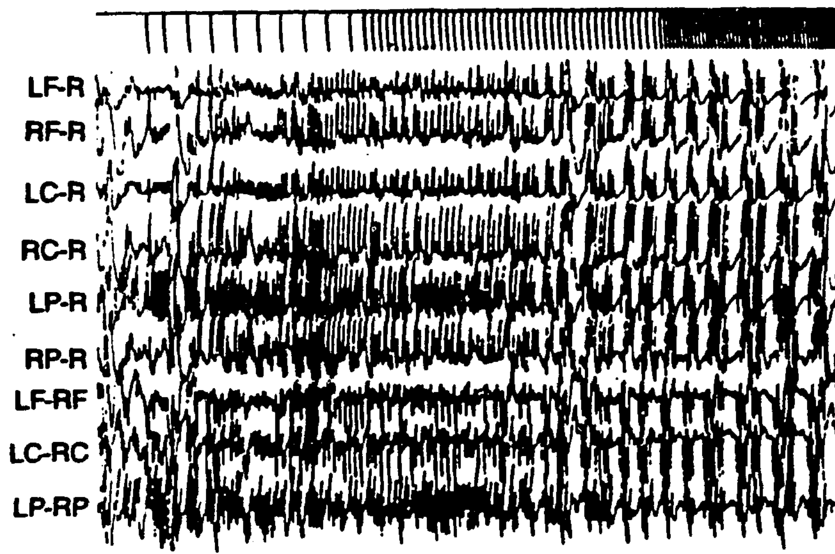


Figure 10





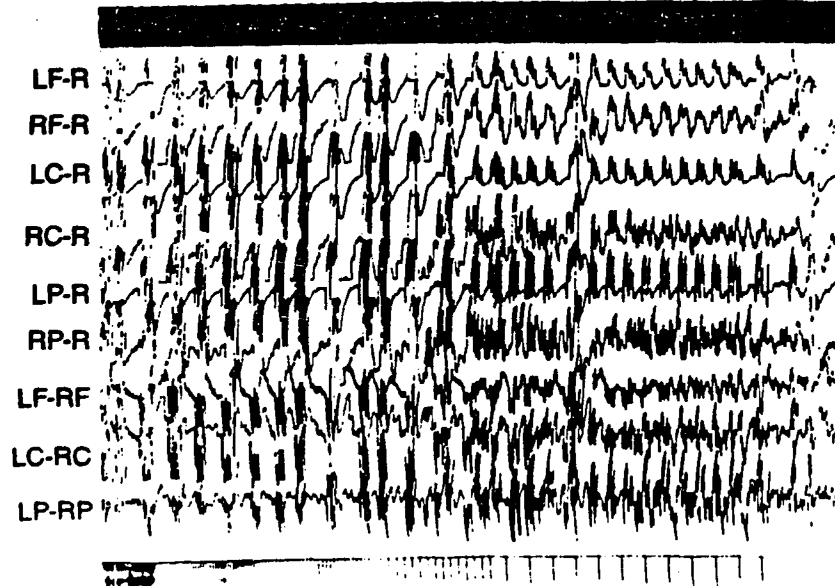


Figure 11

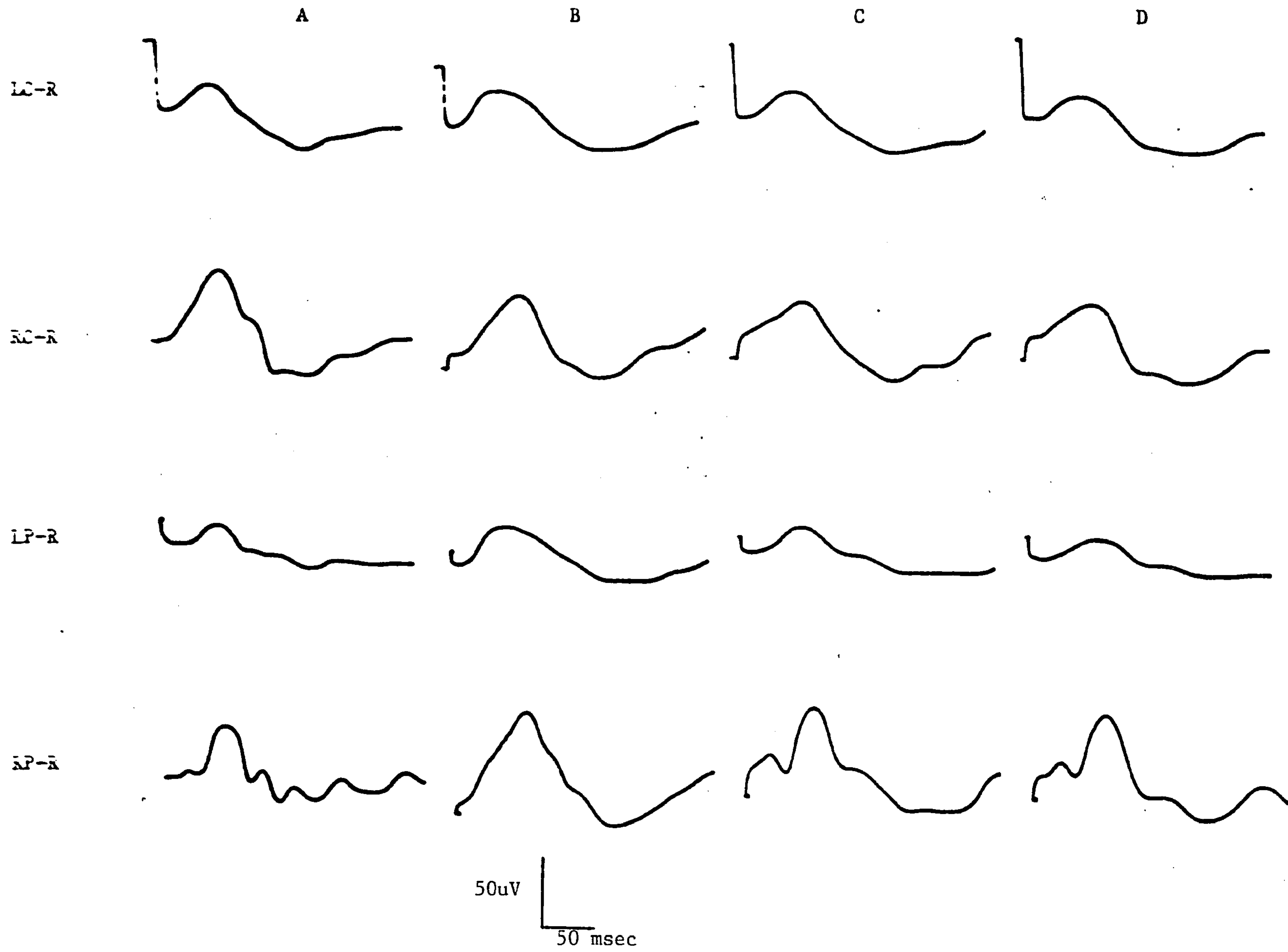
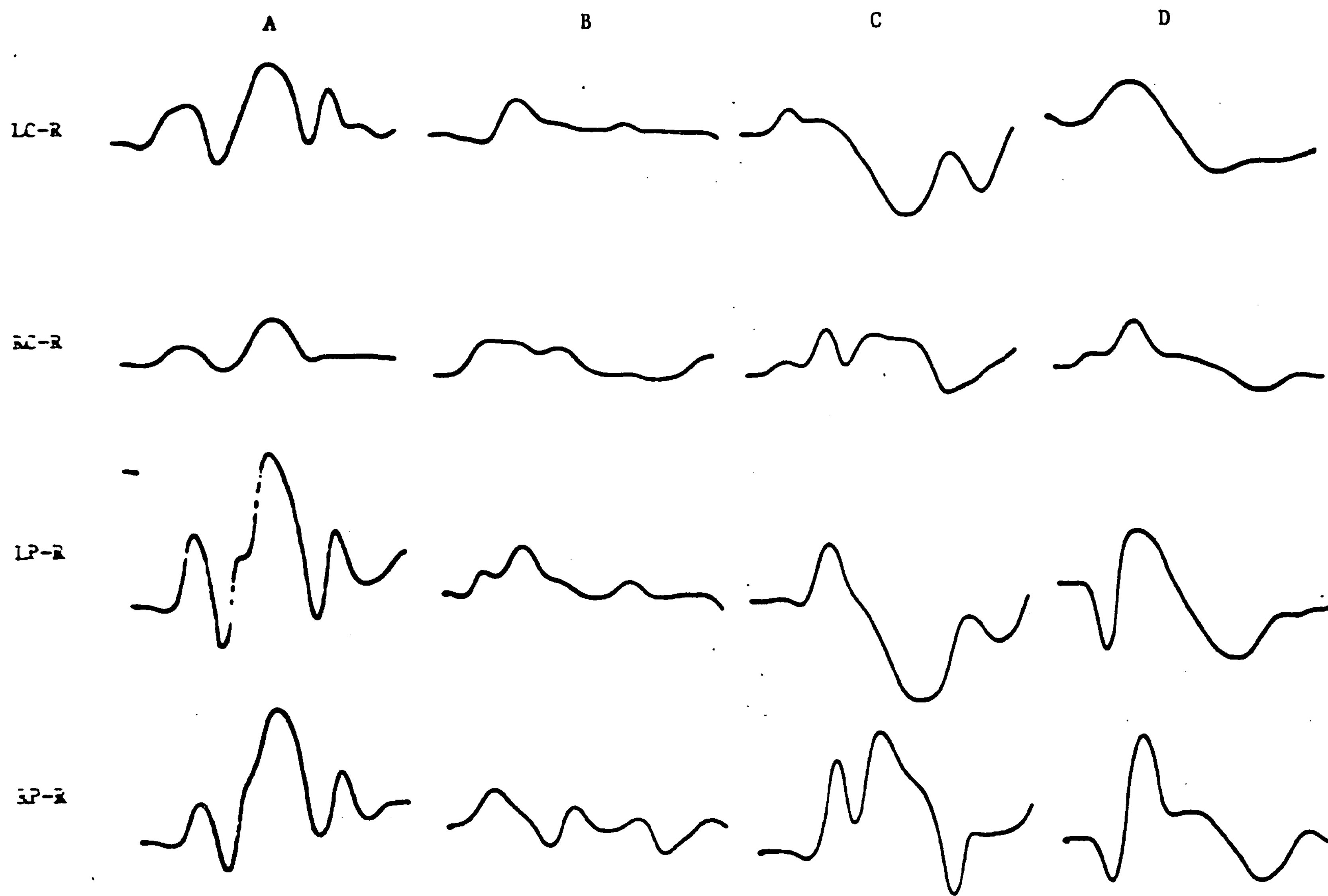


Figure 12



50uV
50 msec

Figure 13

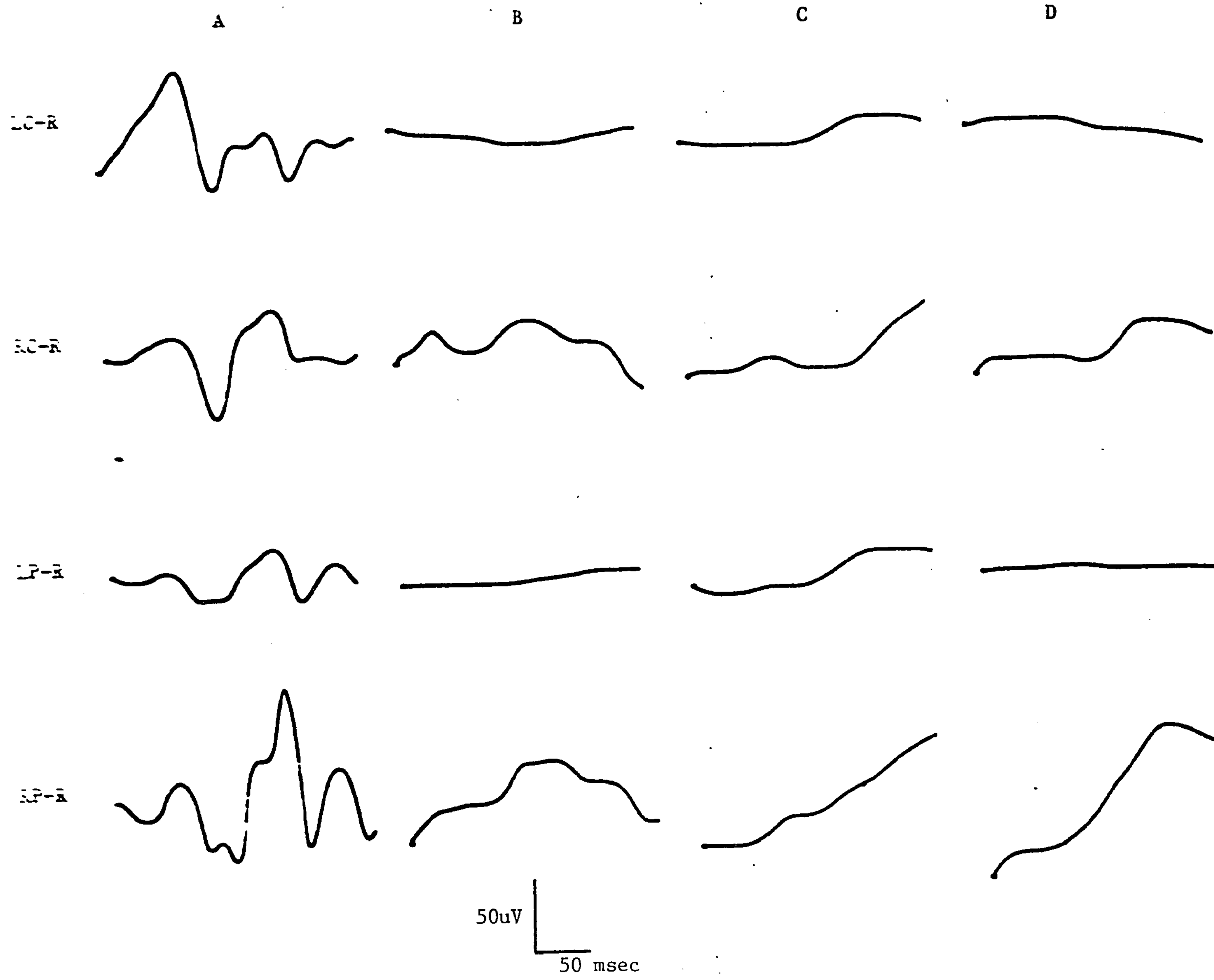
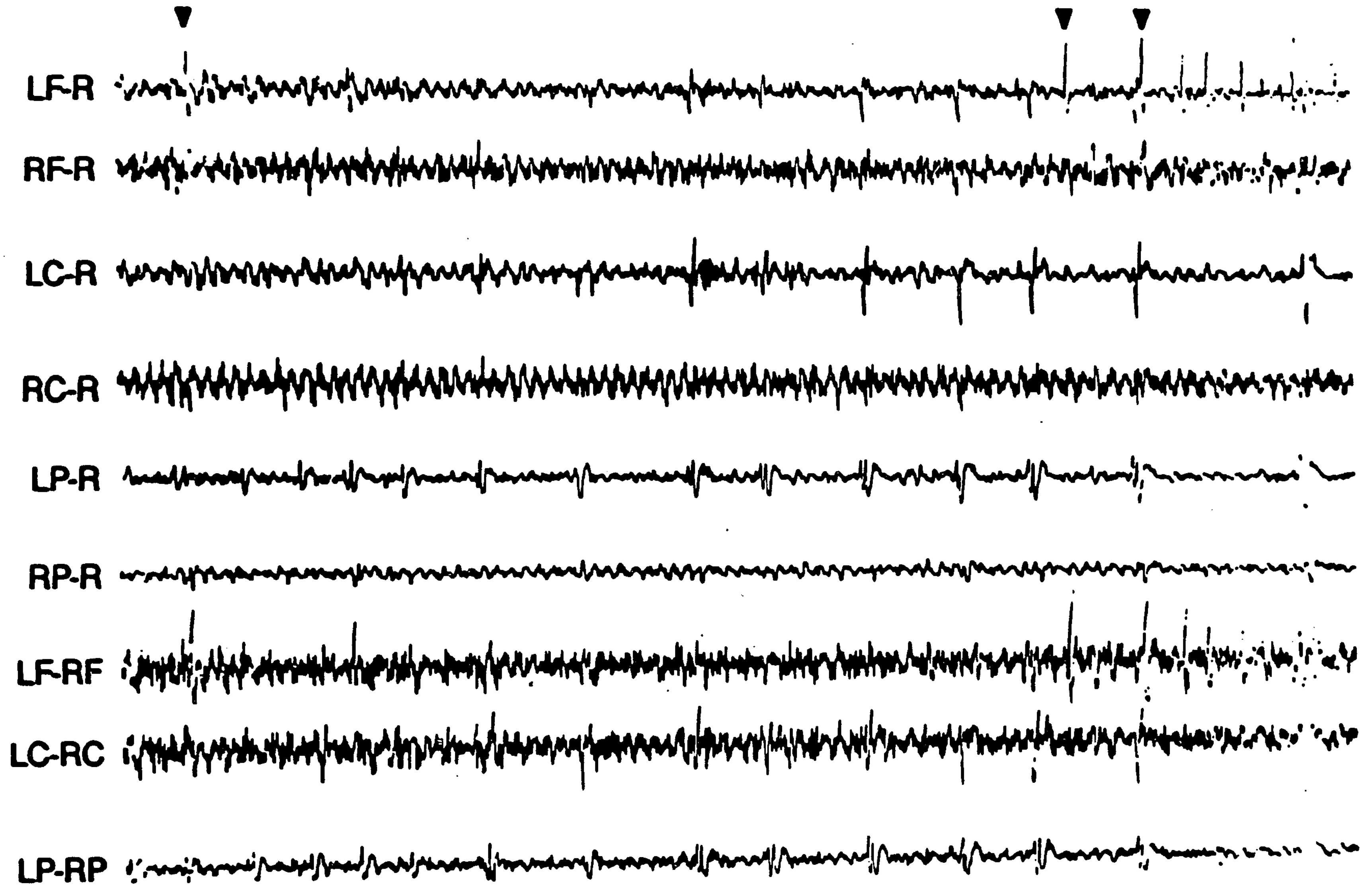


Figure 14



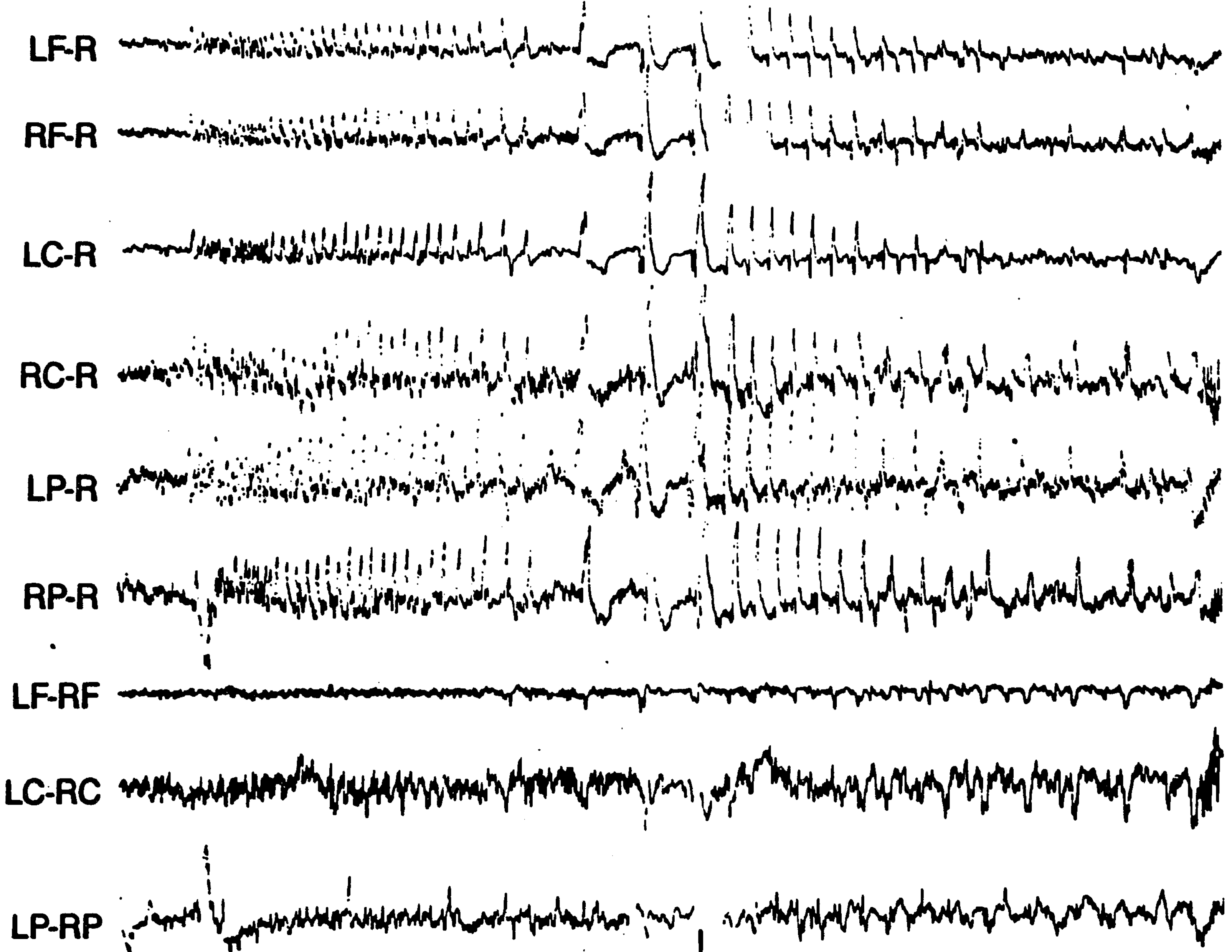
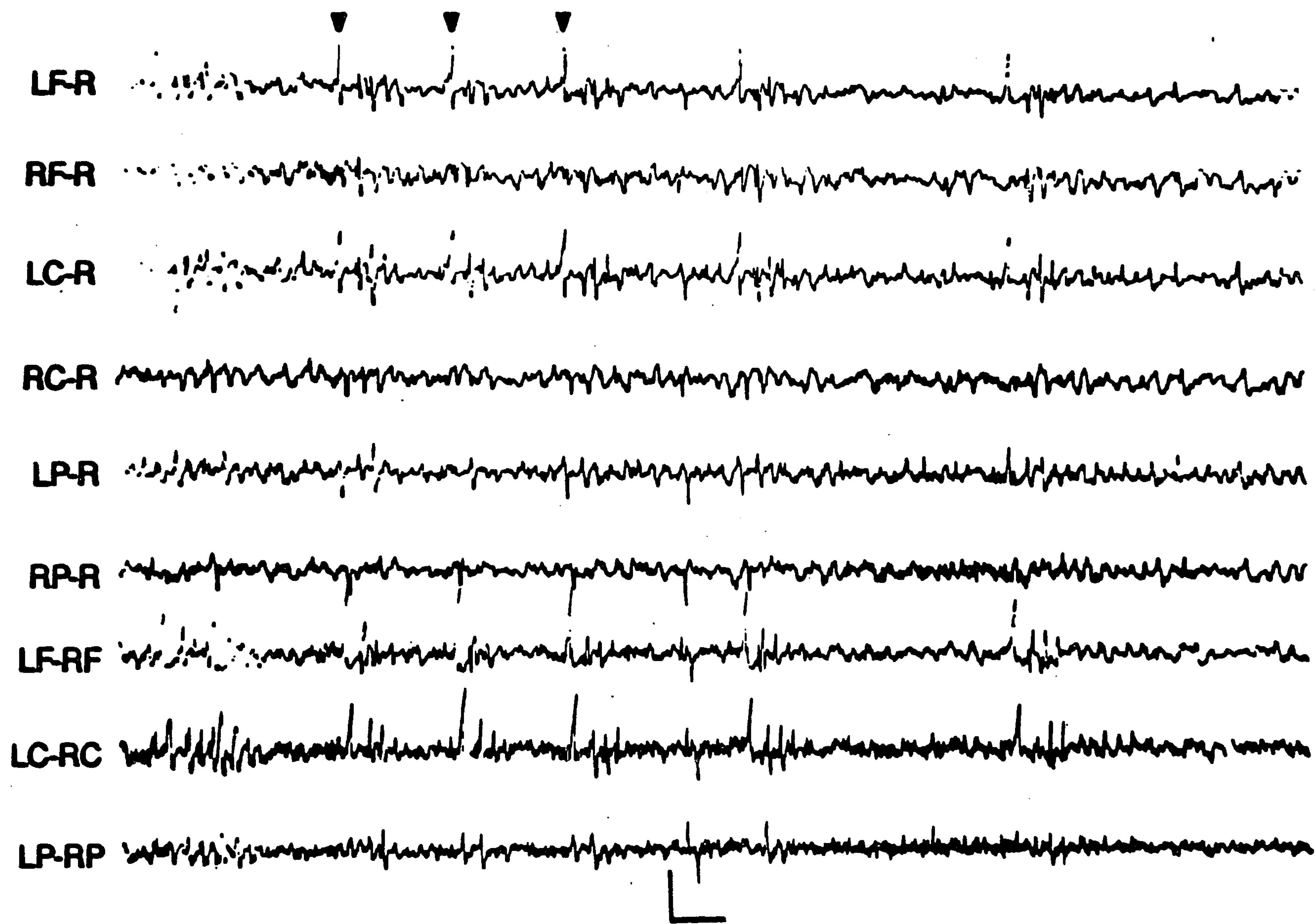

LF-R
RF-R
LC-R
RC-R
LP-R
RP-R
LF-RF
LC-RC
LP-RP

Figure 16



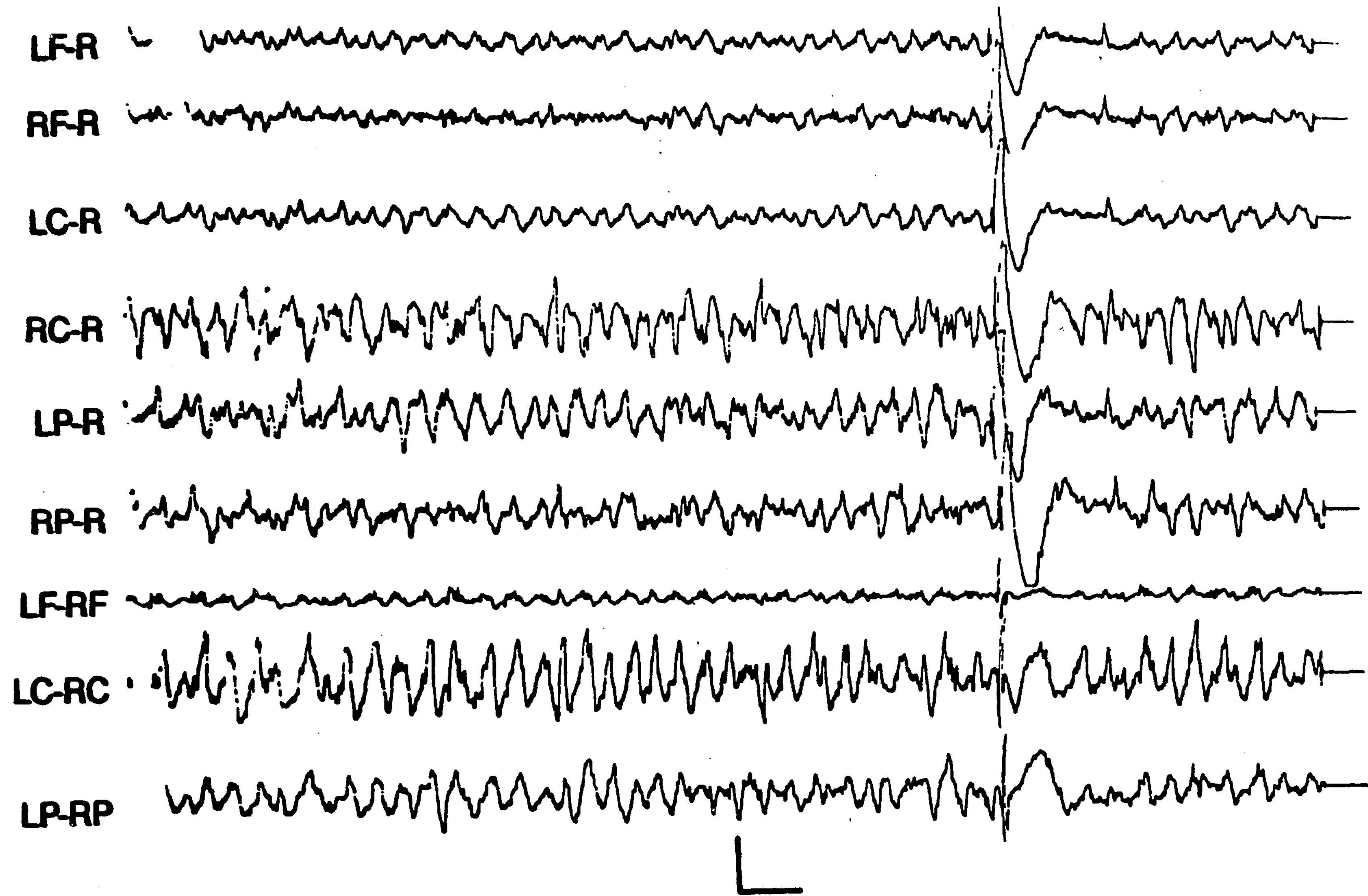

LF-R
RF-R
LC-R
RC-R
LP-R
RP-R
LF-RF
LC-RC
LP-RP

Figure 18

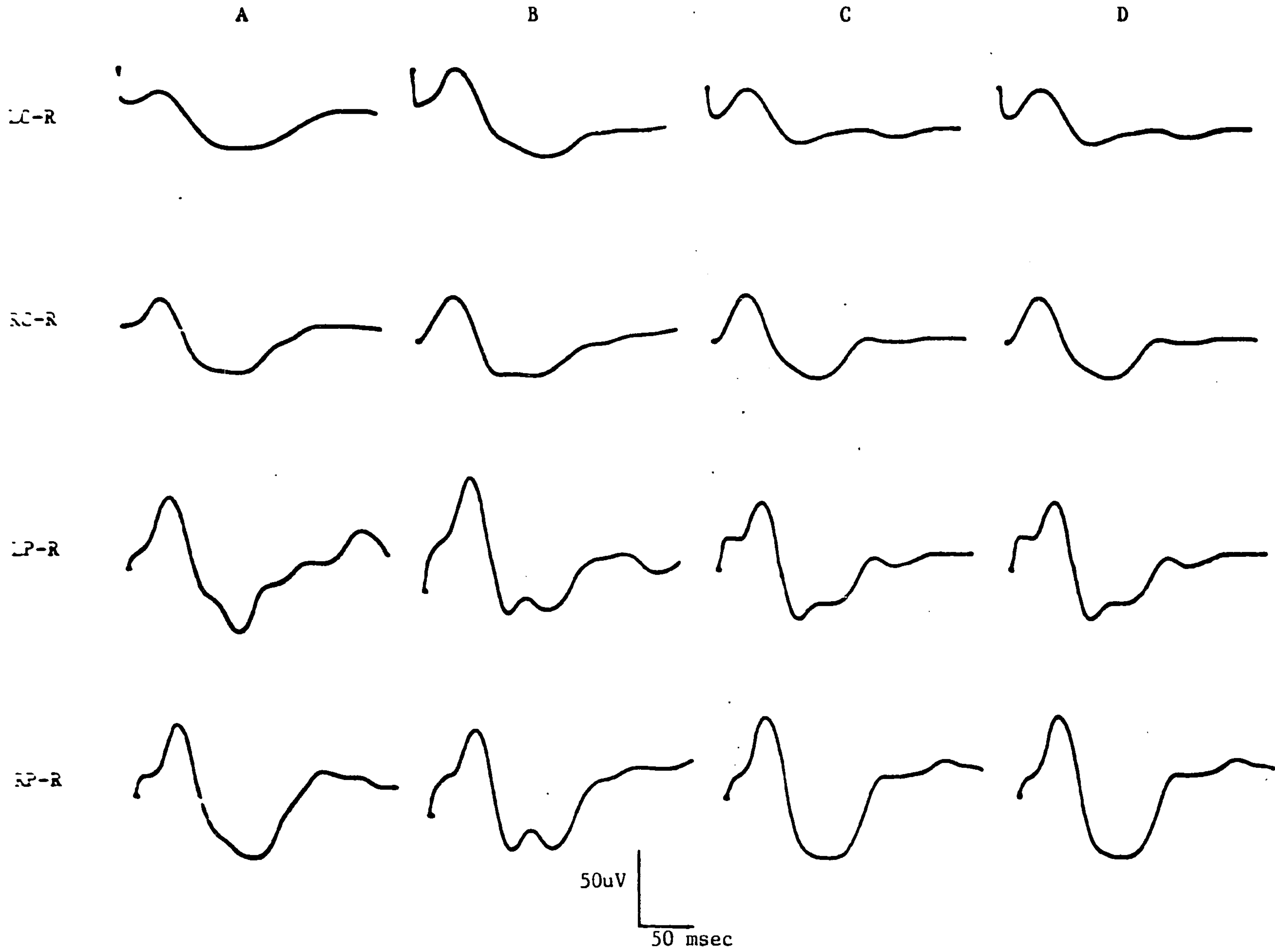


Figure 19

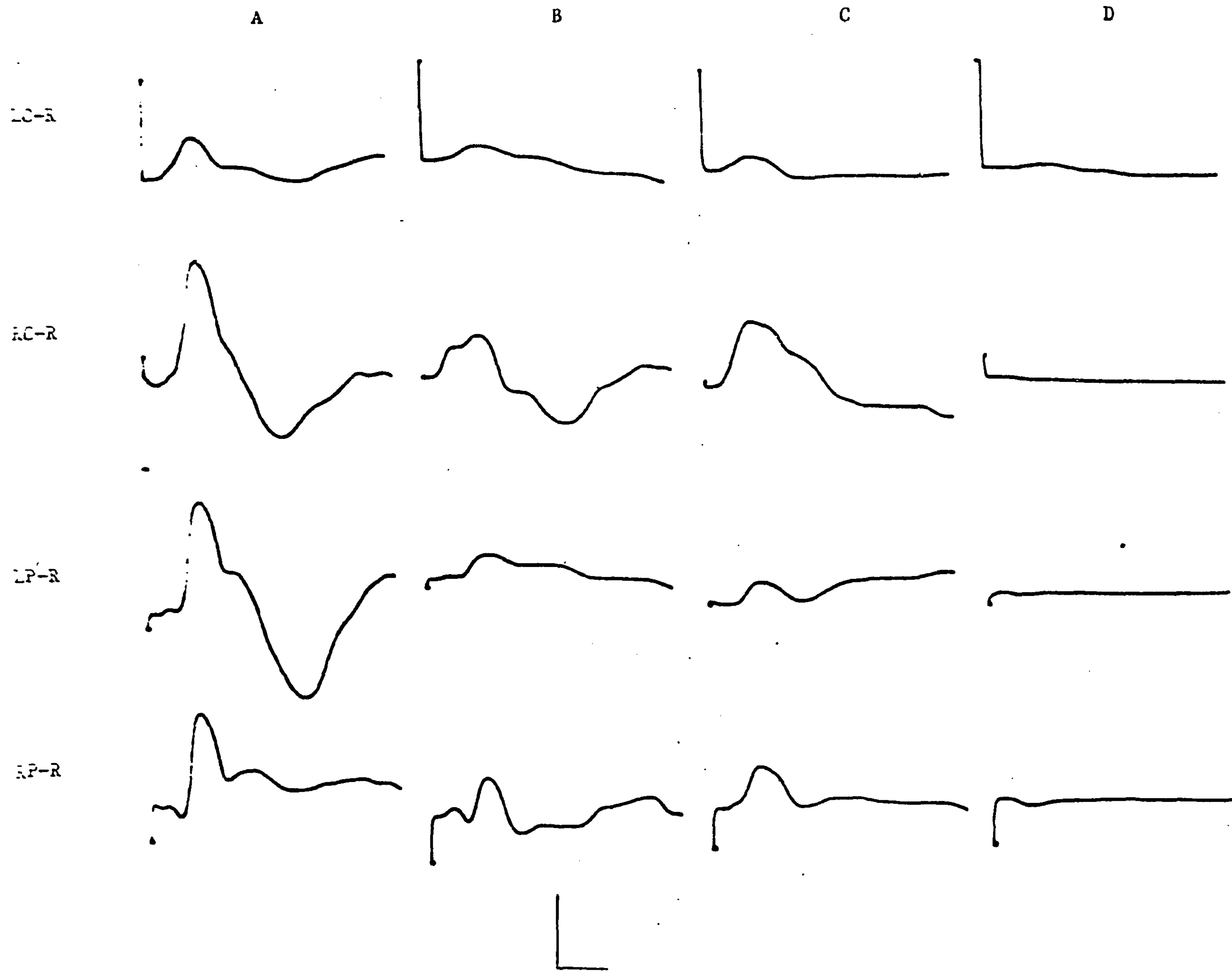


Figure 20

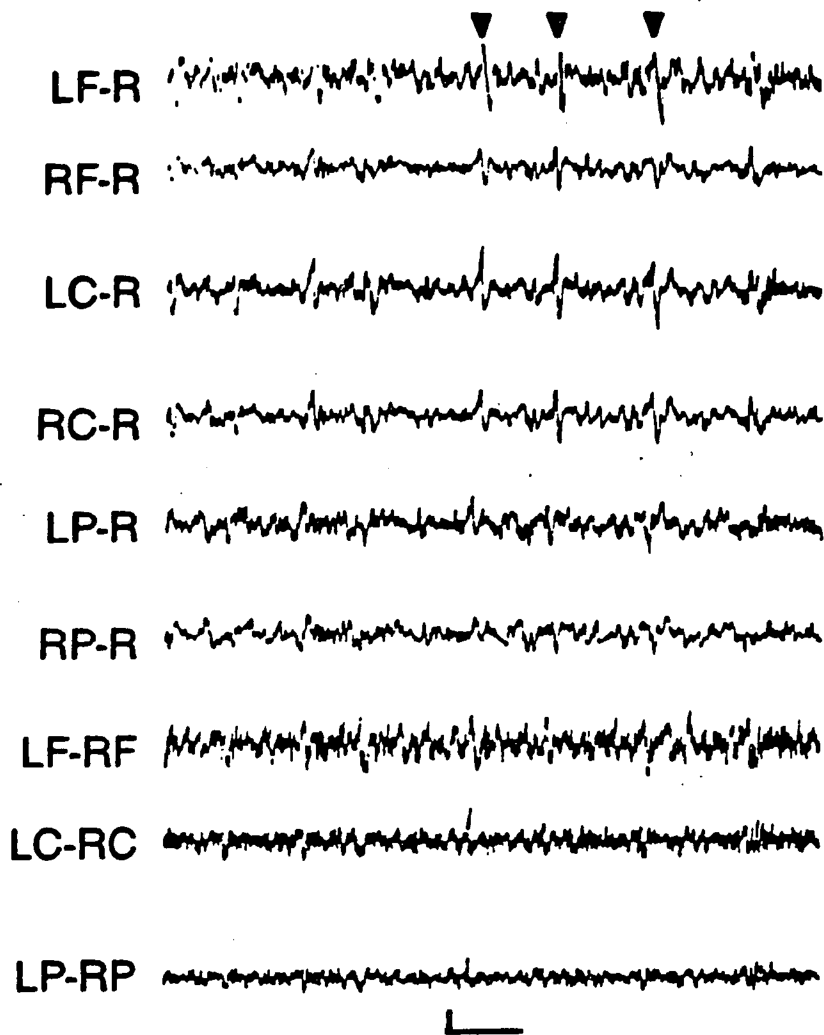
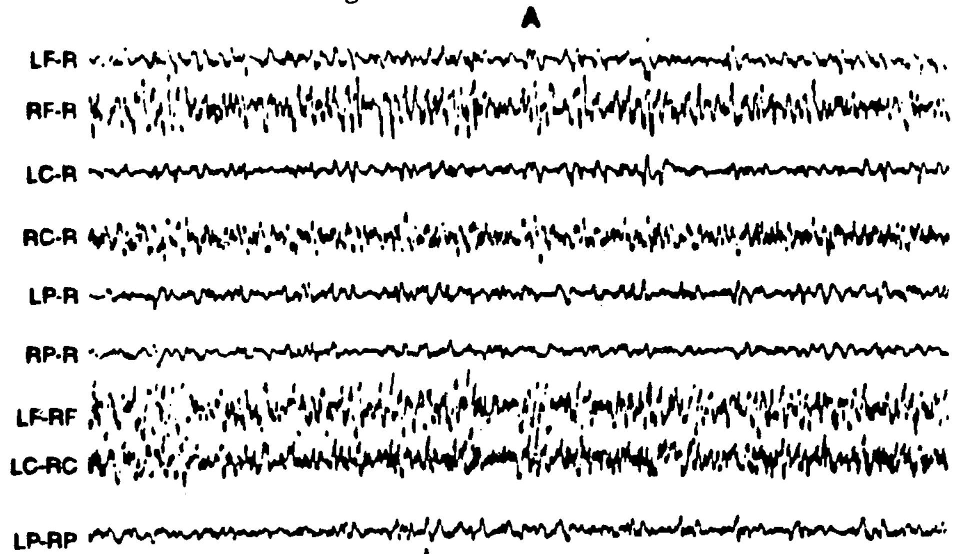
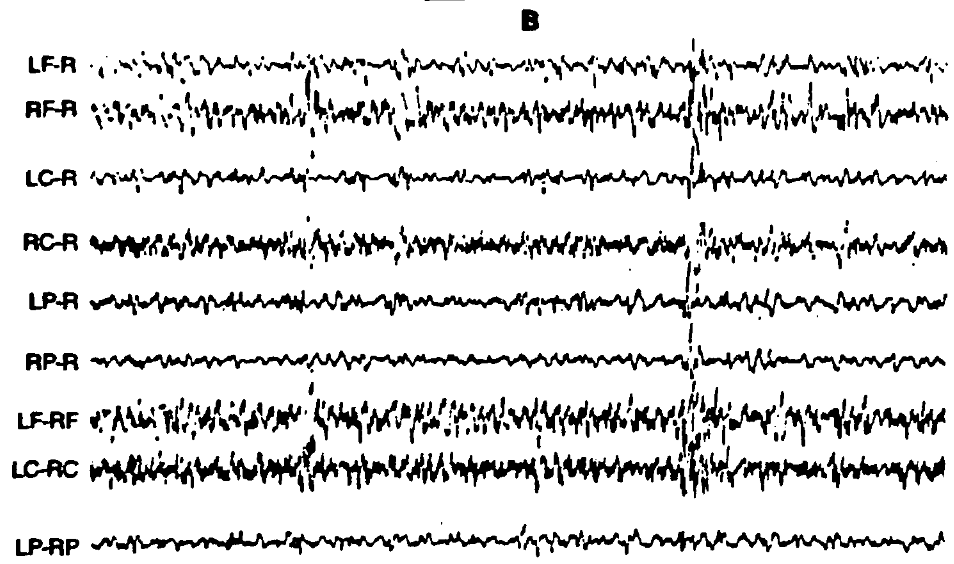


Figure 21





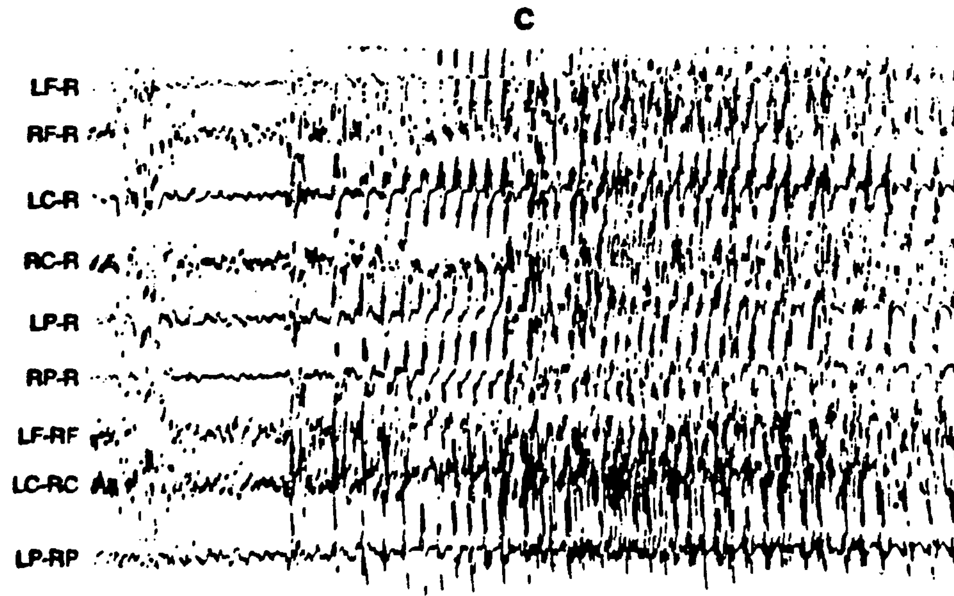


Figure 22

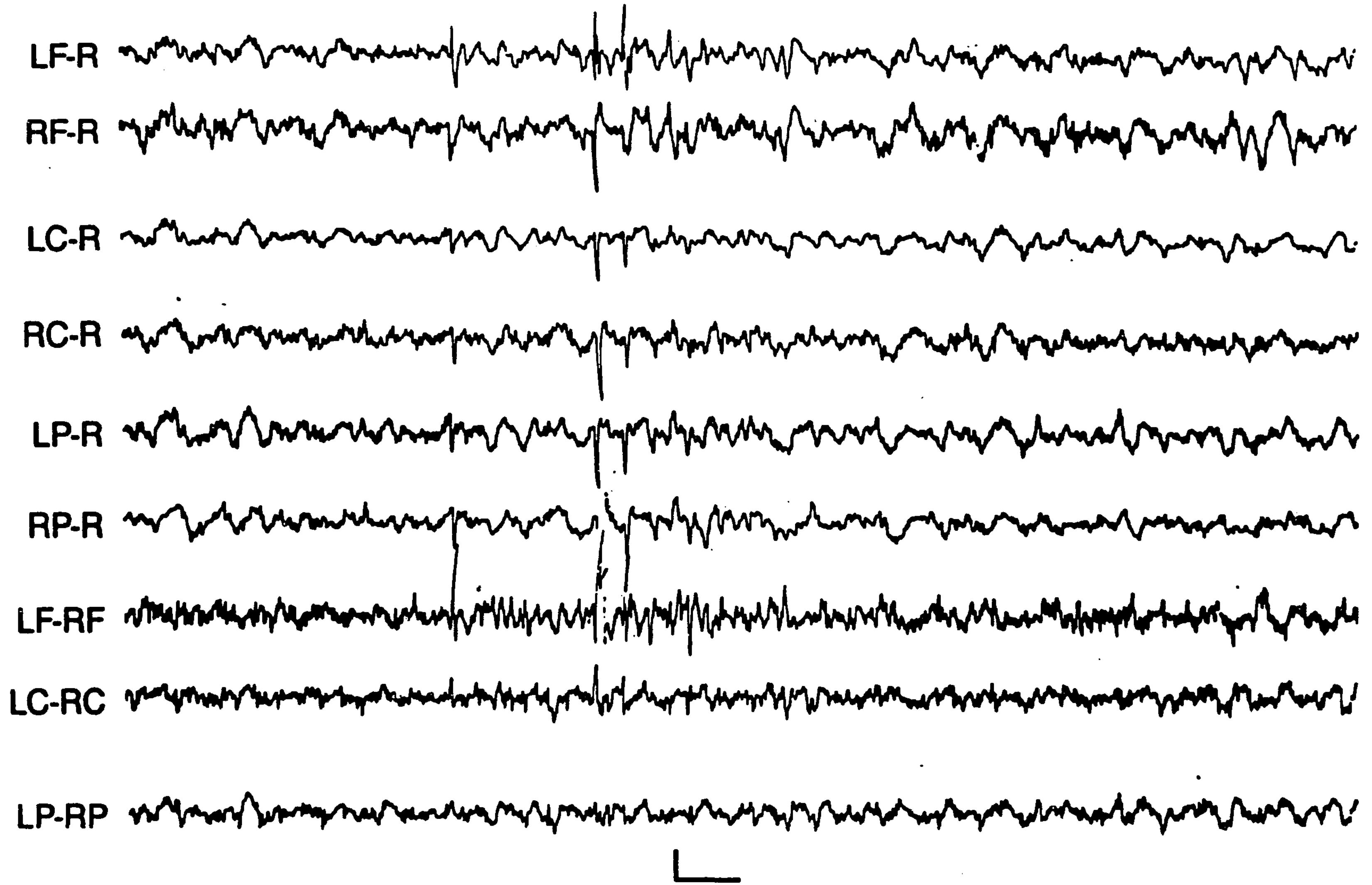


Figure 23

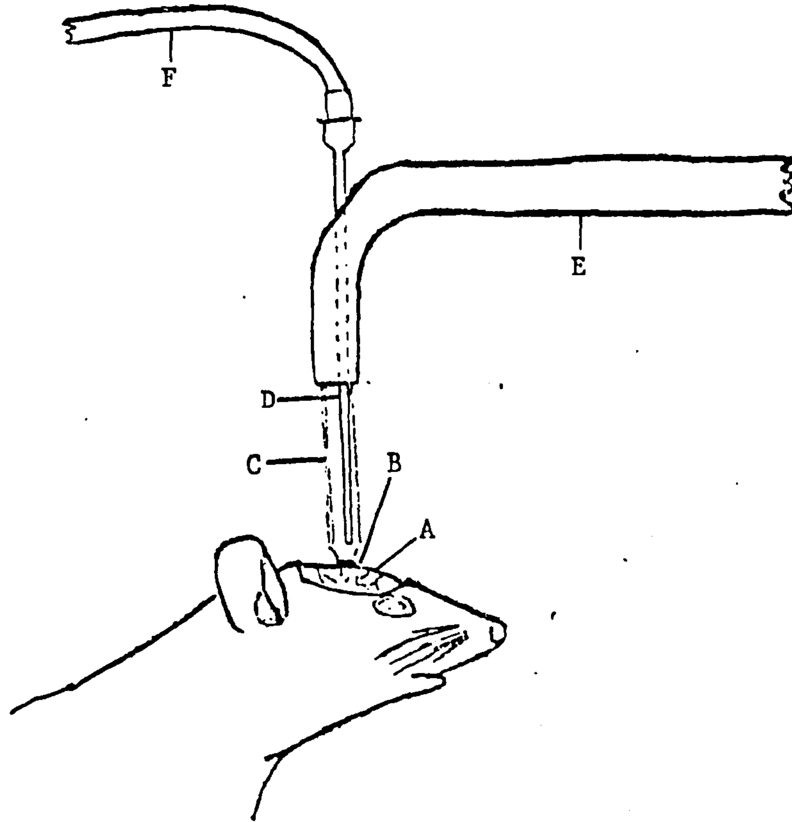


Figure 24

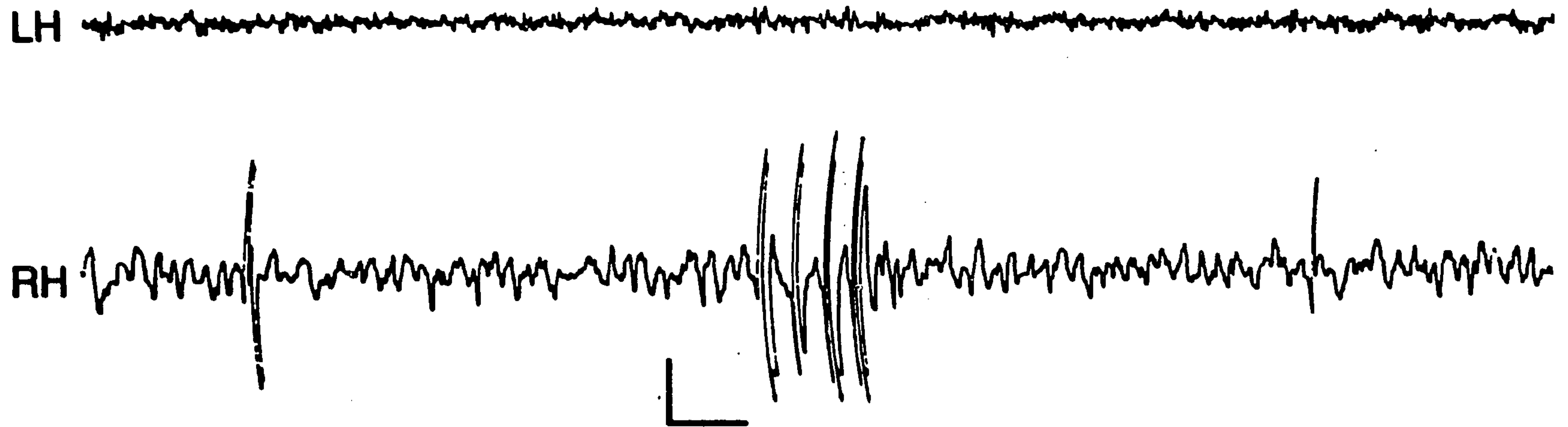


Figure 25

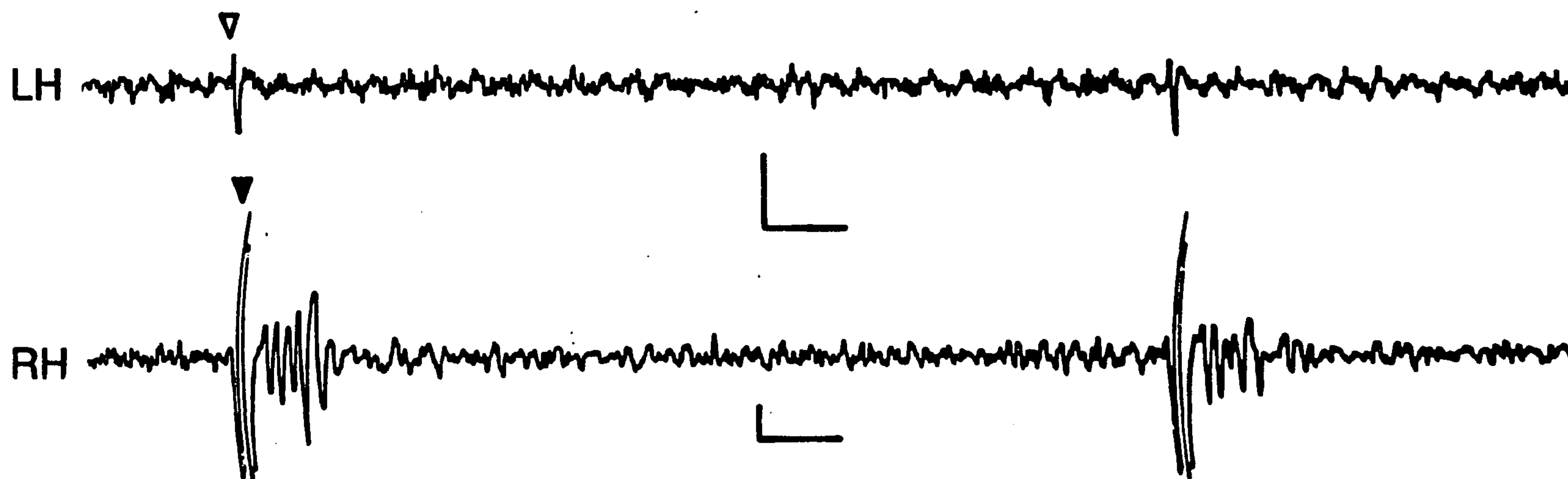


Figure 26

LH

RH

Figure 27

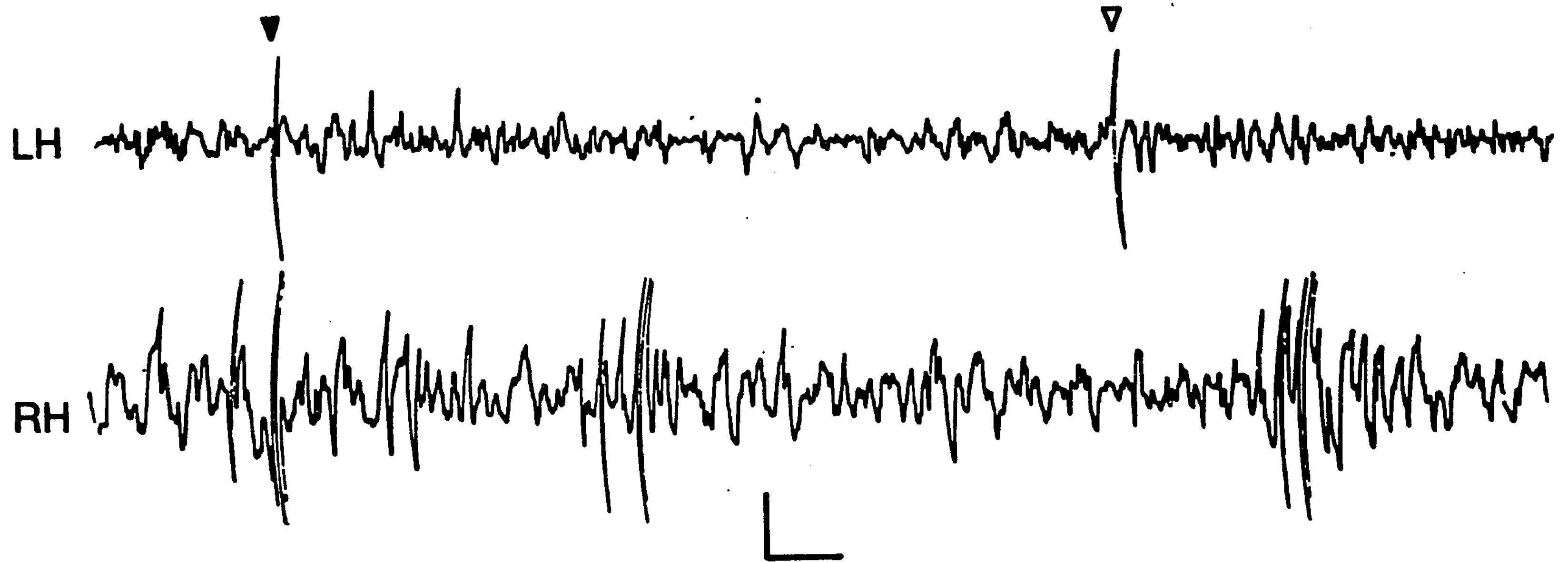


Figure 28

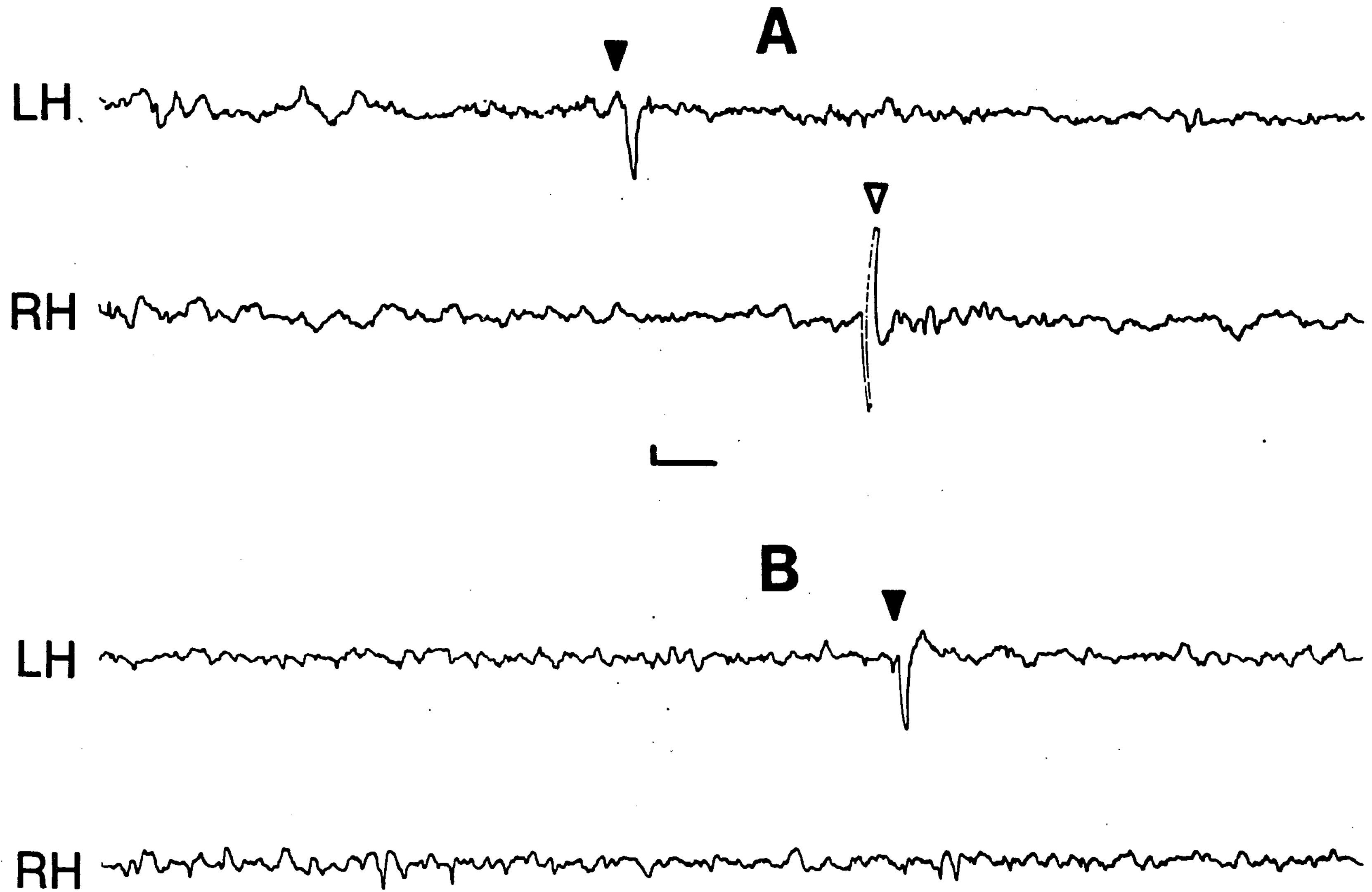


Figure 29

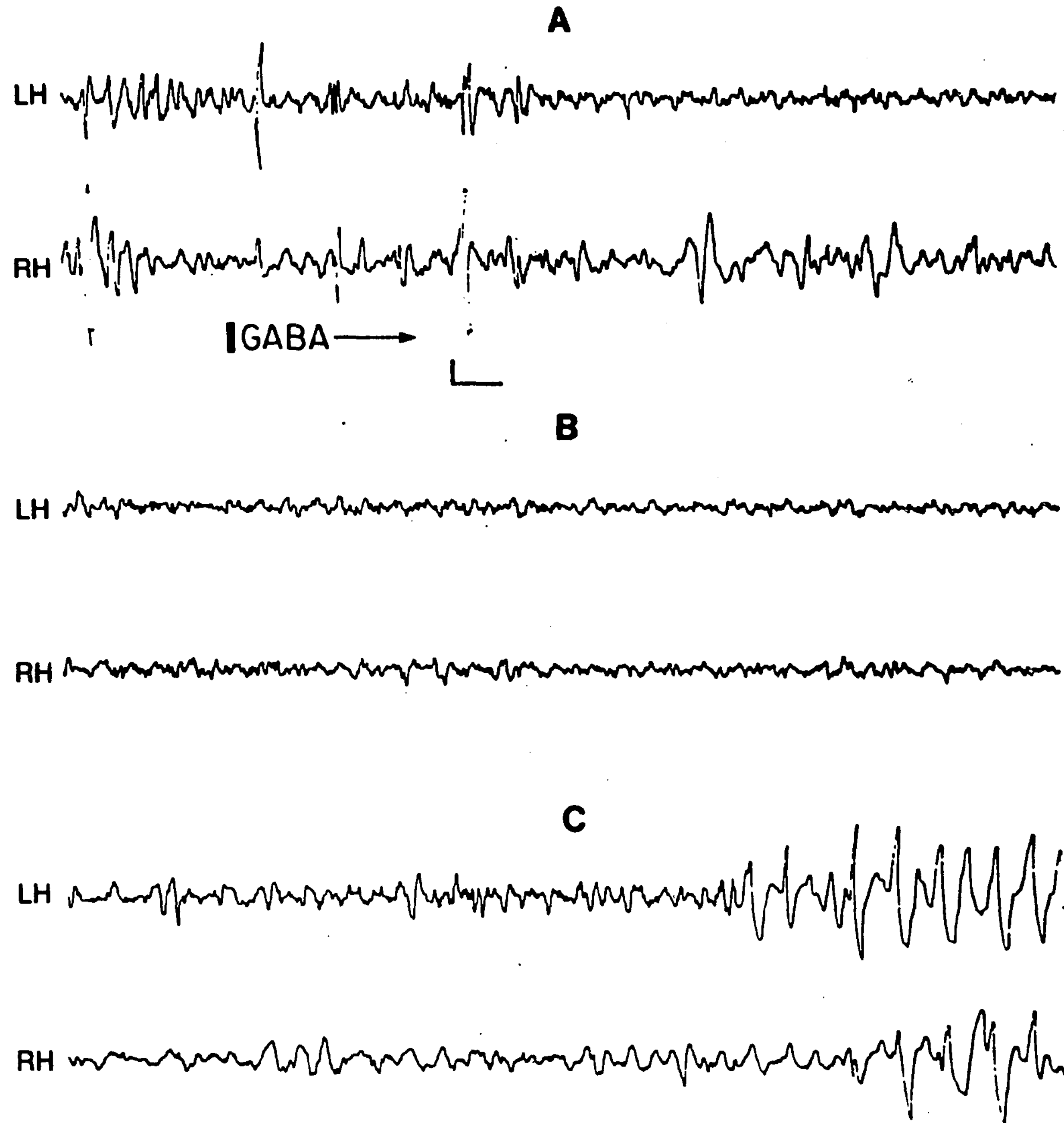


Figure 30

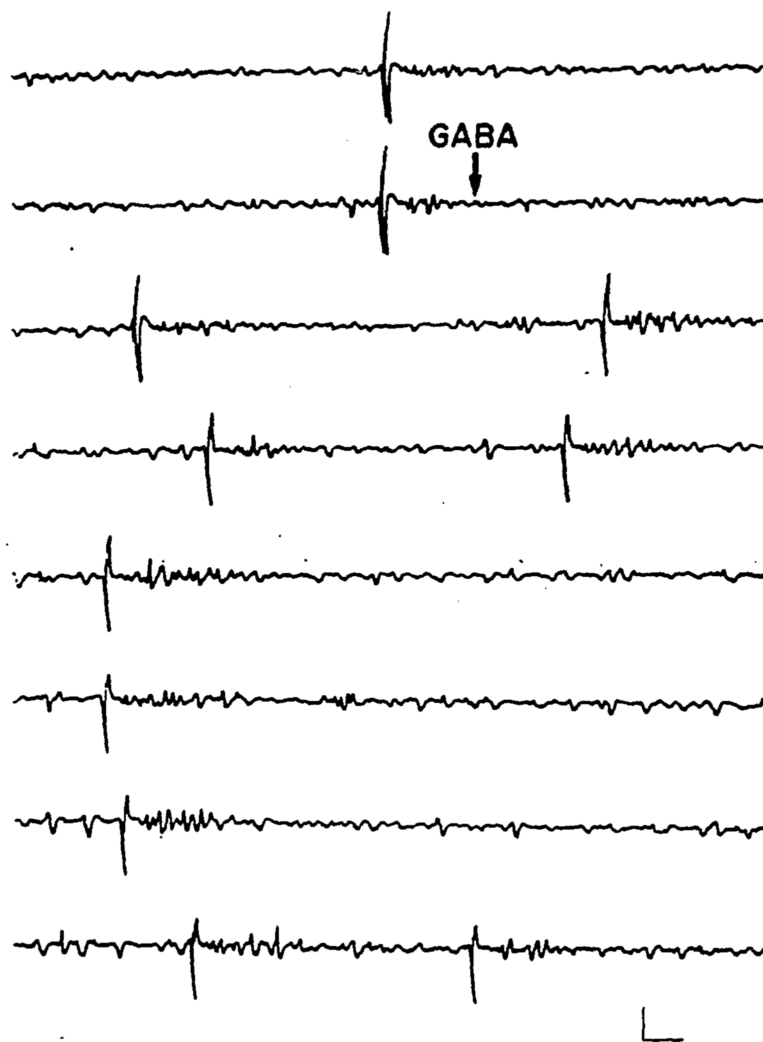
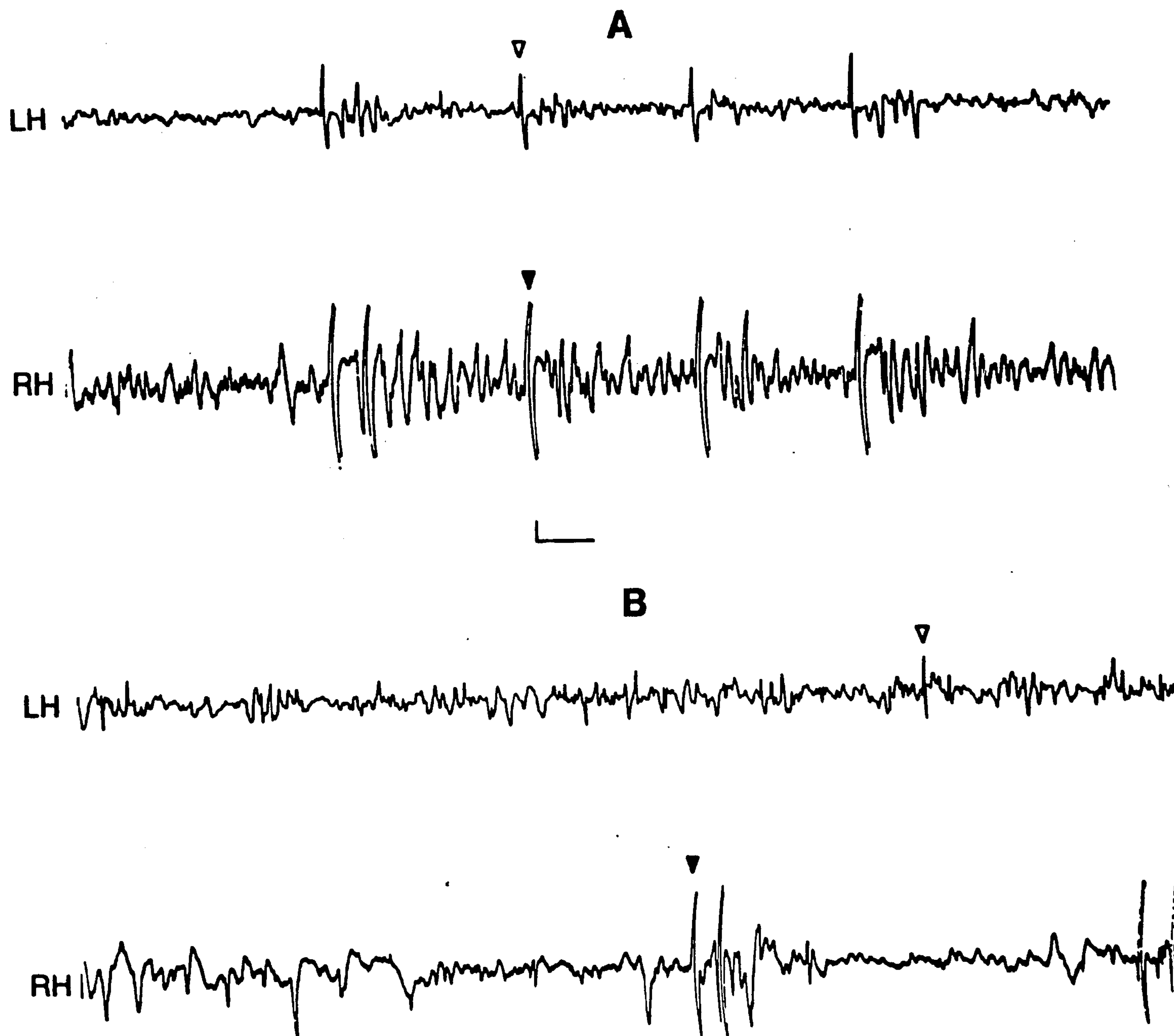


Figure 31



BIBILIOGRAPHY

Ajmone Marsan, C. Acute effects of topical epileptogenic agents. In H.H. Jasper, A.A. Ward & A. Pope (Eds.), Basic Mechanisms of the Epilepsies. Boston: Little Brown, 1969.

Bloor, B., Wrenn, F.R. & Margolis, G. An experimental evaluation of certain contrast media used for cerebral angiography: Electroencephalographic and histopathological correlations. J Neurosurg., 8, 585-594, 1951.

Bradbury, M. The Concept of the Blood-Brain Barrier. New York: Wiley and Sons, 1979.

Broman, T. & Lindberg-Broman, A.M. An experimental study of disorders in the permeability of the cerebral vessels (the blood-brain barrier) produced by chemical and physico-chemical agents. Acta Physiol Scand [Supp], 10, 102-145, 1945.

Broman, T. & Olsson, O. Experimental study of contrast media for cerebral angiography with reference to possible injurous effects on the cerebral vessels. Acta Radiol., 31, 321-334, 1949.

Chang, L.W. & Hartmann, H.A. Blood-brain barrier dysfunction in experimental mercury intoxication. Acta Neuropath. (Berl.), 21, 179-184, 1972.

Collins, R.C., Kennedy, C., Sokoloff, L. & Plum, F. Metabolic anatomy of focal motor seizures. Arch. of Neurol., 33, 536-542, 1976.

Cutler, R.W.P. & Barlow, C.F. The effect of hypercapnia on brain permeability to protein. Arch. Neurol., 14, 54-63, 1966.

Fieschi, C., Lenzi, G.L., Zanette, E. & Passero, S. Effects on EEG of the osmotic opening of the blood-brain barrier in rats. Life Sciences, 27, 239-243, 1980.

Flodmark, S. & Steinwall, O. A method for study of the interrelation between EEG and blood-brain barrier phenomena.

Acta Physiol. Scand., *56*, 112-119, 1962.

Flodmark, S. & Steinwall, O. Differential effects on certain blood-brain barrier phenomena and on the EEG produced by means of intracarotidally applied mercuric chloride. *Acta Physiol. Scand.*, *57*, 446-453, 1963.

Flodmark, S. & Steinwall, O. Reversible blood-brain barrier alteration induced by certain organic acids and indicated by means of EEg and dye tests. *Acta physiol. Scand.*, *58*, 368-375, 1963.

Flodmark, S. Clinical detection of blood-brain barrier alteration by means of EEG. *Acta Neurol. Scand.*, *41* (Suppl. 13), 163-177, 1965a.

Flodmark, S. Blood-brain barrier alteration after experimental cold injury of the rabbit brain indicated by penicillin G in EEG and by dye tests. *Acta Physiol. Scand.*, *63*, 225-235, 1965b.

Foltz, E.L., Thomas, L.B. & Ward, A.A. Electroencephalographic effects of intra-carotid injection of Diordrast. *Trans. Amer. Neurol. Ass.*, *76*, 115-117, 1951.

Gloor, P. Epileptogenic action of penicillin. *Ann. N.Y. Acad. Sci.*, *166*, 350-360, 1969.

Hayashi, T. The inhibitory action of beta-hydroxy-gamma-amino butyric acid upon the seizure following stimulation of the motor cortex of the dog. *Jour. Physiol.*, *145*, 570-578, 1959.

Horrall, O.H. *Bile: Its Toxicity and Relation to Disease*. Chicago: University of Chicago Press 1938.

Johansson, B. & Nilsson, B. The pathophysiology of the blood-brain barrier dysfunction induced by severe hypercapnia and by epileptic brain activity. *Acta Neuropath*. (Berl.), *38*, 153-158, 1977.

Lee, J.C. & Olszewski, J. Increased cerebrovascular permeability after repeated electroshocks. *Neurology*, *11*, 515-519, 1961.

Levine, M.E., Spigelman, M.K., Zappulla, R.A., Povlishock, J.T., Malis, L.I. & Holland, J.F. Anatomic studies of blood brain barrier disruption following intracarotid bile salt infusion. In S. Ishii, H, Nagai & M. Brock (Eds.). Intracranial Pressure V. Berlin: Springer/Verlag 1983.

Lorenzo, A.V., Shirahige, I., Liang, M. & Barlow, C.F. Temporary alteration of cerebrovascular permeability to plasma protein during drug induced seizures. Amer. Jour. of Physiol., 223, 268-277, 1972.

Lorenzo, A.V., Hedley-White, E.T., Eisenberg, H.M. & Hsu, D.W. Increased penetration of horseradish peroxidase across the blood-brain barrier induced by Metrazol seizures. Brain Research, 88, 136-140, 1975.

Meldrum, B.S. & Horton, R.W. Neuronal inhibition mediated by GABA and patterns of convulsions in baboons with photosensitive epilepsy Papio papio. In P. Harris & C. Mawdsley (Eds.). Epilepsy. Edinburgh: Churchill Livingston, 1974.

Neuwelt, E.A., & Frankel, E.P. Is there a therapeutic role for blood-brain barrier alterations? Ann Intern. Med., 93, 137-139, 1980.

Oldendorf, W.H. Permeability of the blood-brain barrier. In D.B. Tower (Ed.) The Nervous System. New York: Raven, 1975.

Oldendorf, W.H., Brain uptake of radiolabeled amino acids, amines and hexoses after arterial injection. Amer. J. of Physiol., 221(6), 1629-1639, 1971.

Pappius, H.M., Savaki, H.E., Fieschi, C., Rapoport, S.I. & Sokoloff, L. Osmotic opening of the blood-brain barrier and local cerebral glucose utilization. Ann. Neurol., 5, 211-219, 1979.

Purpura, D.P., Girado, M., Smith, T.G. & Gomez, J.A. Synaptic effects of gamma-amino butyric acid in cortical regions of increased permeability. Proc. Soc. Exp. Biol. & Med., 97, 348-353, 1958.

Quesney, L.F., Gloor, P., Kratzenberg, E. & Zumstein, H. Pathophysiology of generalized penicillin epilepsy in the cat: The R of cortical and subcortical structures. 1. Systemic application of penicillin. _Electroencephalogr. Clin. Neurophysiol._, _42_, 640-655, 1977.

Rapoport, S.I., Hori, M. & Klatzo, I. Testing of a hypothesis for osmotic opening of the blood-brain barrier. _Am. Jour. Physiol._, _223_(2), 323-331, 1972.

Rapoport, S.I. & Thompson, H.K. Osmotic opening of the blood-brain barrier without associated neurological deficits. _Science_, _180_, 971, 1973.

Rapoport, S.I., Matthews, K. & Thompson, H.K. Absence of brain edema after reversible osmotic opening of the blood-brain barrier. In H.M. Pappius & J.W. Feindel. _Dynamics of Brain Edema_. Springer/ Verlag: Berlin, 1976.

Rapoport, S.I., Matthews, K., Thompson, H.K. & Pettigrew, K.D. Osmotic opening of the blood-brain barrier in the rhesus monkey without measurable brain edema. _Brain Research_, _136_, 23-29, 1977.

Rapoport, S.I. Osmotic opening of the blood-brain barrier. In Purves, M. _Ciba Foundation Symposium-Cerebral Vascular Smooth Muscle and Its Control_. Amsterdam: Elsevier, 237-255, 1978.

Rapoport, S.I., Fredericks, W.R., Ohno, K. & Pettigrew, K.D. Quantitative aspects of reversible osmotic opening of the blood-brain barrier. _Am. J. Physiol._, _238_ (Regulative Intergrative Comp. Physiol. 7), R421-R431, 1980.

Rapoport, S.I., London, E.D., Fredericks, W.R., Dow-Edwards, D.L. & Mahone, P.R. Altered cerebral glucose utilization following blood-brain barrier opening by hypertonicity or hypertension. _Exper. Neurol._, _74_, 519-529, 1981.

Reese, T.S. & Karnovsky, M.J. Fine structural localization of a blood-brain barrier to exogenous peroxidase. _J. Cell Biol._, _34_, 207-217, 1967.

Remler, M. Focal stimulation of the brain by entirely extra-cranial means: An example of radiation controlled focal pharmacology. Conf. Neurol., 35, 50-62, 1973.

Remler, M. & Marcussen, W. EEG monitoring of focal lesions of the blood-brain barrier. Acta Neurol. Scand., 65, 51-58, 1982.

Reichenthal, E. & Hocherman, S. The critical cortical area for development of penicillin-induced epilepsy. EEG Clin. Neurophysiol., 42, 248-251, 1977.

Spigelman, M.K., Zappulla, R.A., Malis, L.I., Holland, J.F., Goldsmith, S.J. & Goldberg, J.D. Intracarotid dehydrocholate infusion: A new method for prolonged reversible blood-brain barrier disruption. Neurosurgery, 12(6), 606-612, 1983.

Torres, F. & Jacome, D. Secondary epileptogenisis in the human. In A. Mayersdorf & R.P. Schmidt (Eds.), Secondary Epileptogenesis. New York: Raven Press, 1982.

van Gelder, N.M. & Elliott, K.A.C. Disposition of gamma-amino butyric acid administered to mammals. Jour. of Neurochem., 3, 139-145, 1958.

Westmoreland, B.F., Hanna, G.R. & Bass, N.H. Cortical alterations in zones of secondary epileptogenesis: A neurophysiologic, morphologic and microchemical correlation study in the albino rat. Brain Res., 43, 485-499, 1972.