

A Steroid Derivative (RU 5135) Exhibits Epileptogenicity in the Presence of Deficient Blood-Brain Barrier

M. S. MYSLOBODSKY,¹ M. MINTZ, R. TARRASCH AND J. BAR-ZIV*

*Psychobiology Research Unit, Department of Psychology, Tel Aviv University and *Department of Radiology, Hebrew University Medical School, Jerusalem, Israel*

Received 5 July 1990

MYSLOBODSKY, M S, M MINTZ, R TARRASCH AND J BAR-ZIV *A steroid derivative (RU 5135) exhibits epileptogenicity in the presence of deficient blood-brain barrier* PHARMACOL BIOCHEM BEHAV **38**(2) 327-331, 1991 — A hypothesis was tested that epileptic spiking, induced by systemic administration of a steroid derivative (RU 5135), may be associated with surgery-related cortical damage rather than epileptogenicity of the agent. Cumulative doses of the drug were given to rats and rabbits. The lasting quasi-periodic seizure discharges were always seen initially only under an epicortical electrode implant. Brain damage inflicted by such an electrode was confirmed anatomically (by assessing the area of lesion) and noninvasively (by showing contrast-induced enhancement of CT brain images in the hemisphere homolateral to epicortical electrodes). The threshold of epileptic spiking varied inversely with the area of cortical damage inflicted by the electrode. In a number of cases no spiking was obtained from the hemisphere with nonpenetrating (intraosteal) electrodes. We caution that a breach of the BBB caused by conventional surgery in rodents can make a benign drug appear as excessively toxic thereby discrediting potentially useful compounds.

Epileptic spiking Brain injury Blood-brain barrier Computed tomography GABA antagonist (RU 5135)

OUR previous study has demonstrated that a novel steroid derivative, RU 5135 (3 α -hydroxy-16-imino-5 β -17aza-androstan-11-one) (8), induces peculiar quasi-periodic cortical spiking, lasting for many hours with no or very few clinical manifestations of epilepsy (15). While a unique phenomenon in itself, this effect was particularly surprising as RU 5135 was the only known GABA antagonist that caused focal rather than generalized petit-mal type activity after systemic administration. Given that focal epileptogenesis requires a certain matrix of structural damage (13) we reasoned that RU 5135 may have appeared excessively toxic due to an inadequate even though generally accepted practice of electrode implantation. This hunch was confirmed by modifying the electrode implantation technique and assessing brain damage caused by surgery.

EXPERIMENT 1

METHOD

Subjects

The study used male and female Wistar rats as subjects. Rats were of about 300 g at the time of surgery.

Surgery.

Surgery was conducted under Equithesin (Salisbury Jensen,

Kansas City, MO). In 14 rats *epidural* silver ball electrodes were implanted in symmetrical points over the visual cortex as described elsewhere (15). In another 11 rats nonpenetrating (*extradural*) electrodes (stainless steel needles) were implanted bilaterally over the visual and somato-sensory cortex, using dental files (Reamer No. 25). All electrodes were soldered to amphenol microconnectors and were fixed to the skull with dental acrylic.

Procedure

The procedure of EEG examination was modeled after that described elsewhere (15). In brief, animals were taken to the experimental cage a week after surgery. Freely moving rats were tested inside boxes whose sides were mirrors for testing, when needed, visual evoked potentials. Baseline EEG was monitored using swivel assembly, and amplified and displayed on a Beckman Type R Dynograph (bandwidth 0.53–30 Hz). Thereafter, four successive doses of 0.5 mg/kg of RU 5135 (kindly donated by Dr. Hunt, Roussel-UCLAF) were injected IP every 10 min, followed by doses of 1 mg/kg, until the spiking appeared unilaterally. The administration of the drug was continued until either the spiking was obtained bilaterally or the dose was quadrupled over the threshold. EEG was recorded continuously throughout the session.

¹Requests for reprints should be addressed to M. S. Myslobodsky, Psychobiology Research Unit, Department of Psychology, Tel Aviv University, Ramat-Aviv, 69978 Tel Aviv, Israel.

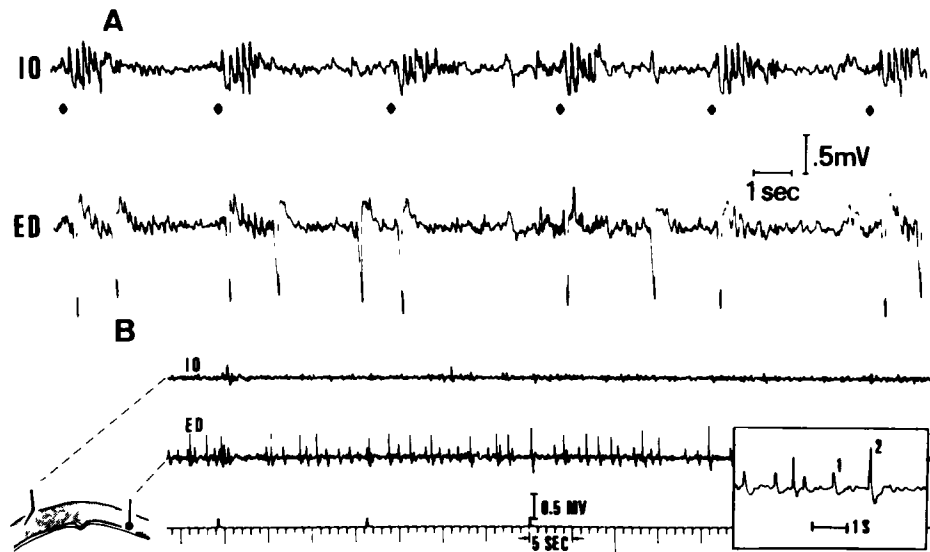


FIG 1 (A) A quasi-periodic cortical spiking recorded from the epidural (ED) electrode concurrently with normal EEG from the contralateral area with an intraosteal (IO) implant. Waking freely moving rat. Note that photic stimuli (stroboscopic flashes) marked with diamonds elicit sensory afterdischarges in the hemisphere with the intraosteal implant. (B) Asymmetric spiking activated by RU 5135 in a rabbit implanted with the epidural and intraosteal electrodes. Waking rabbit restrained in a perspex box. Note that the paper movement was kept slow to record spikes as vertical deflections. An inset obtained with a higher speed of paper shows that in this experiment there were two categories of spikes of similar configuration but of different amplitude (1,2). The smaller spiking focus was attributed to cortical damage inflicted by a jeweler screw positioned 8 mm away from the epicortical electrode. It was found to penetrate the inner table.

With four animals, semi-acute experiments were conducted. The latter animals were implanted intraosteally (three rats) or epidurally (one rat) and one hour thereafter they were taken to the experiment.

Brain Morphology

Evans blue (2% in 0.9% NaCl, 2 ml/kg) was infused into the femoral vein 5 min before perfusion. The distribution of the dye was scored after (19). The cortical tissue damage under electrodes was further examined using alternate 80 μ sections of the frozen brain stained with Nuclear Fast Red (Gurr).

RESULTS

Electrophysiological Findings

Increasing doses of RU 5135 lead, at a mean dose of 4.0 ± 1.8 mg/kg (range 1–7 mg/kg), to unilateral spiking in both chronically and acutely implanted animals with one or two epidural electrodes (Fig. 1A). An additional dose (1.9 ± 1.3 mg/kg) of the drug had to be administered in order to recruit the other side of the brain into seizure activity. It is of interest that photic stimuli (stroboscopic flashes) used in such preparations were capable of eliciting sensory afterdischarges only in the hemisphere with the intraosteal implant. In 5 rats, a cumulative dose of 11 ± 5.6 mg/kg was arrived at, without reaching the stage of bilateral spiking. In all of these, a nonpenetrating electrode was located homolaterally to the spike-resistant side.

Behavioral Correlates

Unilateral spiking was associated with no characteristic behav-

ior. Most often reduced motility, ataxic walk, sniffing and grooming were noted. With bilateral spiking, animals became increasingly adynamic with head resting on the floor and the forelimbs stretched out. Myoclonus was seen infrequently. When it appeared, it seldom evolved into generalized seizures even when intense spiking was seen under penetrating electrodes implanted over the somatosensory area. In three animals with nonpenetrating electrodes spiking was not obtained with a dose of 15 mg/kg. All three developed a progressive loss of postural tone with no seizure manifestations.

Anatomical Brain Lesion in the Area of Electrode Contact

In most cases the proof of anatomical damage was easy to obtain by just examining plain serial sections under low magnification. Evans blue infusion caused a detectable stain identifying locations of epicortical electrodes and/or jeweler screw implants (Fig. 2C,D). In the event of asymmetric discharges, higher magnification ($\times 200$ – $\times 400$) allowed us to confirm either a measurable cortical depression or a frank penetration involving layers I–III. In order to further ascertain the interrelation between spiking asymmetry and cortical damage, the damaged area was outlined and its short and long diameters were measured under low magnification ($\times 12$) using a 0.1 mm internal rule. The correlation coefficient computed between the areas values and threshold of spiking in the corresponding hemisphere yielded an r of -0.66 ($df=34$, $p<0.001$).

DISCUSSION

We were able to replicate our previous study (15), albeit only using epidural electrodes. On the side with an intraosteal implant

no reliable spiking can be obtained even after an excessive dose of the drug. Anatomical analysis and the distribution of Evans blue confirmed that 'epidural' implants could hardly be considered harmless in rats. The steel jeweler's screws that are widely used as electrodes and/or anchors [e.g., (20), for review] were especially damaging in the rat's skull with the average thickness of less than 1 mm, even when positioned 'part way.' In view of the above, an implantation of intraosteal electrodes might be considered an acceptable alternative when EEG/ERP analysis in waking, freely moving animals is entertained.

EXPERIMENT 2

The foregoing findings may be interpreted as suggesting that implantation-related focal blood-brain barrier (BBB) leakage made it possible to reveal a peculiar epileptogenic effect of RU 5135. We wondered whether implantation surgery in animals with larger calvaria (e.g., rabbits) would reproduce this effect. The additional advantage of working with rabbits is that a meager lesion of the cortical microvascular bed and consequent BBB lacunae may be examined noninvasively using iodinated contrast media in the course of computed tomography. It is known that normal brain tissue is protected by the BBB from direct penetration of the contrast medium when the latter is administered intravenously (21). In rabbits, it takes a dose of 25 ml/50 sec of 50% sodium diatrizoate to disrupt the BBB (7).

Therefore, using a protocol described above, EEG effects of RU 5135 were explored in the rabbit. Then a group of rabbits was submitted for computed tomography (CT) examination.

METHOD

Subjects

Fourteen Chinchilla rabbits weighing 2000 g at time of surgery were used.

Surgery

Surgery was conducted under Nembutal (Salisbury Jensen, Kansas City, MO). Stainless steel electrodes were implanted in symmetrical points over the visual cortex epidurally and intraosteally as described above. The reference electrode was implanted in the frontal sinus. All electrodes were soldered to amphenol micro-connectors and were fixed to the skull with dental acrylic.

Procedure

Unanesthetized rabbits were immobilized in a perspex box. RU 5135 (2 mg/ml/kg) was slowly administered into the marginal ear vein. EEG was continuously monitored for signs of epileptogenicity and the drug was readministered (1 mg/kg) if spiking was not obtained within 20–30 min of the observation period. The experiment was discontinued as soon as seizure potentials were fully developed.

CT Examination

The noninvasive assessment of the BBB was conducted with CT only in drug-naïve animals. Given that electrodes create artifacts obscuring the region of interest, a blunt perspex rod, 1.5 mm in diameter had to be implanted over the dura. Initially, brain scans were taken with aluminum jeweler screws in the burr holes. Although they highlighted the site of interest (14), the abrupt density changes that occur at the place where the screw interfaced with the brain reduced the enhancement effect due to the beam

hardening. This approach was thus abandoned. Instead, we controlled the place of the implant using the bone window to locate the burr hole or affixing 1 mm aluminum wire on the skull next to the burr hole.

The CT was performed on an Excel 2400 scanner (Elsint, Israel) with a matrix 512 × 512. A rabbit was scanned under Nembutal anesthesia given in a dose of 30 mg/kg (IP) 20 min prior to the scanning and 20 mg/kg (IV) immediately before it began. This dosage is sufficient since following IV administration of the contrast agent the depth of anesthesia is sustained due to contrast-induced displacement of the anesthetic from the albumin-binding sites (10).

A sequential set of high resolution direct coronal CT scans was taken with 1.2 and 2.5 collimation. Sixty percent meglumine iohalamate (IOT) was employed as a contrast medium (1–2 ml/kg infused IV over 2–3 min). This infusion rate is considered safe for rabbits (17). BBB disruption was confirmed by comparing tissue attenuation (in HU units) on both sides of the brain. Readings of HU absorption from a phantom during the period of the study proved stable.

RESULTS AND DISCUSSION

EEG Findings

Predictably, implantation surgery was more dependable in rabbits. Their thicker and more laminated cranial skull permitted the making of an accurate burr hole with minimal cortical lesions. Slowly administering the drug (2 mg/kg, IV) induced lasting homolateral spiking on the side of the epicortical implant (Fig. 1B) in only 3 of 7 rabbits. The pattern of spiking was similar to that observed in rats. Figure 1B demonstrates a case when two types of spikes, similar in shape but different in amplitude, appeared in one hemisphere 15 min after 3 mg/kg of the drug. This pattern was consistent with the possibility of two sites of neocortical damage. In fact, after the animal was sacrificed two sources of damage were established: one, caused by the epicortical electrode (apparently causing spike 2) and the other, produced by a neighboring jeweler screw (spike 1) that penetrated the inner table.

Unlike rats, rabbits were immobilized. Immobilization stress is known to increase BBB permeability (4), notably when associated with hypertension. The latter may have enhanced epileptogenicity of RU 5135. Yet hypertension could not account for the laterality of spiking and its association with signs of cortical damage.

CT Findings

The CT of the rabbit's brain is shown in Fig. 2A,B. Although the images suffered from rather low spatial resolution they clearly show major brain structures and hyperperfusion near the site of epicortical implants along with a more diffuse homolateral enhancement in the oral brainstem (Fig. 2B). It is of interest that the contrast enhancement was far outside the area of electrode pressure. The radiodensity varied widely from 10 to 60 HU.

The result of this experiment further documents that electrode implantation caused a disruption of the BBB, both locally and diffusely. One may argue that IOT is an agent of relatively high osmolarity which may in itself contribute to disruption of the BBB thereby facilitating its own entry into the CNS. This appears unlikely in view of the fact that sodium diatrizoate, a more neurotoxic agent than IOT (1), showed virtually no neurotoxicity in the absence of the concurrent BBB lesion even when given in larger doses (7).

CT was conducted while rabbits were under barbiturate anesthesia which is known to induce vasoconstriction. Constricted ce-

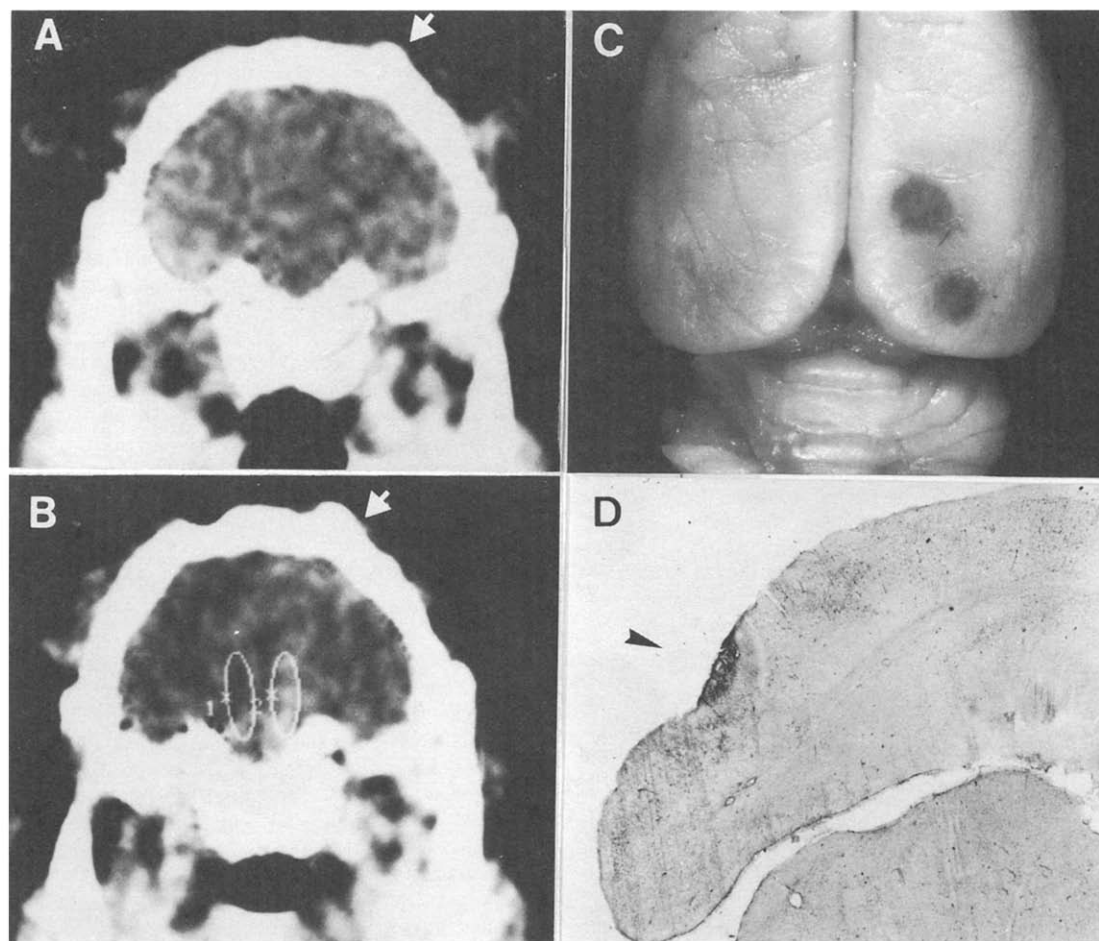


FIG 2 (A) Coronal CT of an anesthetized rabbit. Noncontrast cut shows no abnormality at a site of implantation (arrow head). (B) Contrast-enhanced image demonstrates diffusion of the contrast medium into the abnormal region and with a parallel enhancement in the homolateral mesencephalic area (Indicated by geometric areas of interest). (C) Top view of a rat's brain showing Evans blue staining under epidural jeweler screws. (D) Close view of an 80 μ coronal section showing the depth of damage under a screw (arrow head).

rebral vessels are less likely to develop BBB leakage in the presence of hypertension (9). Also, the contrast medium used in the present study was shown *not* to cause hypertensive response in the rabbit (18).

GENERAL DISCUSSION

The major result of the present study is that RU 5135, which was seen to produce lasting focal spiking (15), appeared rather ineffective in activating epileptiform activity when nonpenetrating electrodes were used. In three cases even its massive doses proved insufficient to activate electrographic and/or clinical signs of epileptogenicity in spite of a clear picture of general neurotoxicity.

It was indicated (3) that cerebral lesions akin to that caused by electrode implants can open the BBB thereby leading to an accumulation of various substances at the lesioned site at higher than physiological concentrations. Therefore, drugs with low toxicity may achieve high CNS concentrations and erroneously appear as more toxic than they actually are when administered intracisternally and/or intracerebrally. Feldberg (6) mentioned numerous studies, including his own, demonstrating that intracerebroven-

tricular administrations of minute amounts of drugs activate a variety of behavioral symptoms such as akinetic behavior, sleep-like conditions, catalepsy, catatonia, stupor, and seizures. The same drugs, administered systemically, produce different and often opposite behavioral responses. Marcus et al. (12) observed that with unimpaired BBB, only very high doses of estrogen could induce seizure discharges.

Although RU 5135-induced spikes were local, there is a possibility that surgery-related damage of the cortex may cause a more generalized BBB deficiency. Thus, the administration of IOT showed CT enhancement in the whole dorso-lateral aspect of the cortex with involvement of the rostral brainstem. The pathophysiology of this effect was not studied in any detail and only a tentative explanation could be provided. Given that IOT is a noxious agent (1), it may easily precipitate seizure discharges in a preparation with an area of preexisting breakdown of the BBB. These local seizures may conceivably elicit secondary and a more generalized disruption of the BBB. Such a scenario can be derived from anecdotal evidence that contrast media originate epileptiform EEG discharges in patients with local breakdown of the BBB due to brain pathology (2) and that seizure-induced increase of BBB permeability is proportional to the seizure duration (11).

While we have limited experience with EEG effects of contrast media, in informal trials we observed typical seizure afterdischarges in one of three waking rabbits after IV administration of 3 mg/kg of IOT. Yet Nembutal anesthesia that was employed during CT examination must have controlled seizure activity.

Alternatively, a rapidly acting central system controlling the BBB could be entertained to account for the more generalized extravasation of IOT. Yet if the latter mechanism is part of an intraaxial autonomic system controlling cerebral microvascular permeability and flow (16) one still needs to explain how it is triggered by the local BBB lesion.

In summary, these findings warrant calling attention to the

fact that a widely accepted technique of epidural positioning of electrodes can unwittingly produce BBB deficiency that is sufficient to distort EEG responses to drug. As a result, a number of benign and potentially useful chemical substances assayed electroencephalographically could be misclassified as being excessively neurotoxic

ACKNOWLEDGEMENTS

Partially supported by the Fund of the Research Authority Office of Tel Aviv University R Zuzowsky suggested the use of dental files (Reamer No 25) D Levin greatly assisted in running the study in rats

REFERENCES

- Adams, M D , Hopkins, R M , Ferrendelli, J A A rat EEG model for evaluating contrast media neurotoxicity *Invest Radiol* 23 S217-S219, 1988
- Avrahami, E , Weiss-Peretz, J , Cohn, D F. Focal epileptic activity following intravenous contrast material injection in patients with metastatic brain disease *J Neurol Neurosurg Psychiatry* 50 221-223, 1987
- Bakay, L. Blood-brain barrier and its alterations in pathological states. In Vinken, P J , Bruyn, G W , eds *Handbook of neurology*, vol 28, part II Amsterdam Elsevier, 1976 365-399
- Belova, T I , Jonsson, G Blood-brain barrier permeability and immobilization stress *Acta Psychol Scand* 116 21-29, 1982
- Clarke, H B , Gabrielsen, T O Seizure induced disruption of blood-brain barrier demonstrated by CT *J Comp Assist Tomogr* 13 889-892, 1989
- Feldberg, W A pharmacological approach to the brain from its inner and outer surface Baltimore Williams & Wilkins, 1963
- Hayman, L A , Hinck, V C Central nervous system angiography Contrast media In Taveras, J M , Ferruci, J T , eds *Radiology: Diagnosis, imaging, intervention* Philadelphia. Lippincott, 1988 1-7
- Hunt, P , Clements-Jewery, S A steroid derivative, RU 5135, antagonizes the GABA/benzodiazepine receptor interaction *Neuropharmacology* 20 351-361, 1981
- Johansson, B B. The blood brain barrier in acute and chronic hypertension In Eisenberg, H M , Sudith, R L , eds *The central vasculature Investigations of the blood-brain barrier* New York Plenum Press, 1980 211-226
- Lasser, E G , Elinzondo-Martel, G , Granke, R. C Potentiation of pentobarbital anesthesia by competitive protein binding. *Anesthesiology* 24 665-671, 1965
- Lorenzo, A V , Shirhige, J , Liang, M , Barlow, C R Temporary alteration of cerebrovascular permeability to plasma protein during drug-induced seizures *Am J Physiol* 223 268-277, 1972
- Marcus, E M , Watson, C W , Goldman, P L Effects of steroids on cerebral electrical activity *Arch Neurol* 15 521-532, 1966
- Mathieson, G Pathological aspects of epilepsy with special reference to the surgical pathology of focal cerebral seizures In Purpura, D P , Penry, J. K., Walter, R D , eds *Neurosurgical management of the epilepsies Advances in neurology*, vol 8 New York Raven Press, 1975 107-138
- Myslobodsky, M S., Bar-Ziv, J Locations of occipital EEG electrodes verified by computed tomography. *Electroencephalogr Clin Neurophysiol* 72 362-366, 1989
- Myslobodsky, M S , Kofman, O Regular and long lasting neocortical spiking produced by systemic administration of a steroid derivative in the rat *Neuropharmacology* 22 157-164, 1983
- Preskorn, S. H , Hartman, B K , Raichle, M E , Swanson, L W , Clark, H B Central adrenergic regulation of cerebral microvascular permeability and blood flow pharmacologic evidence. In: Eisenberg, H M , Sudith, R L , eds *The central vasculature Investigations of the blood-brain barrier*. New York Plenum Press, 1980 127-137
- Rapoport, S I Blood-brain barrier opening by isotonic saline infusion in normotensive and hypertensive animals *Acta Radiol Diagn* 19 921-932, 1978
- Rapoport, S. I , Thompson, H. K , Bidinger, J M Equi-osmolar opening of the blood-brain barrier in the rabbit by different contrast media *Acta Radiol Diagn* 15 221-234, 1974
- Sage, M R., Wilcox, J., Evill, C. A , Berness, G T Comparison and evaluation of osmotic blood-brain barrier disruption following intracarotid mannitol and methylglucamine iohalamate *Invest Radiol* 17 276-281, 1982
- Sheatz, G C Electrode holders in chronic preparation A multilead technique for large and small animals In Sheer, D E , ed *Electrical stimulation of the brain* Austin Texas University Press, 1961 45-54
- Wilcox, J , Sage, M R , Evill, C. A. Effects of intravenous contrast material on the integrity of the blood-brain barrier *AJNR* 5 41-43, 1984