GENERALIZED EPILEPSY EVOKED BY FREEZE LESIONS IN THE MESENCEPHALON OF THE CAT

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Abstract. Epilepsy was induced by making freeze lesions bilaterally in the mesencephalic reticular formation of cats. The convulsive activity was an equivalent of the generalized status epilepticus, developing 15–20 min after the freeze lesions had been made. The animals died in this state 16–20 h after it had developed. The generalized seizure activity may have spread to rostral structures from the lesioned mesencephalic reticular formation generalizing instantaneously, or have been triggered and maintained by a local irritative zone, surrounding the necrotized tissue caused by freezing. The cooling of the same cryoprobes, by which the freeze lesions had been made, had no effect on the status epilepticus, probably because only the necrotized tissue was cooled. The cooling of the inferior thalamic peduncles changed the pattern of epileptic discharges, indicating that thalamocortical structures played a role in sustaining it.

INTRODUCTION

Epileptic seizures can be induced in many ways in experimental animals. Generalized convulsions may best be elicited by the intravenous administration of pharmacological agents like Metrazol or by the application of electroconvulsive shock. A frequently used method is to give
excessive doses of Penicillin parenterally. It is also known that a focal seizure, starting from a — usually lesioned — part of the cerebral cortex, may become generalized.

The lesioned cortex, sooner or later, is very likely to become the focus of epileptic activity, the mechanism of which still remains to be determined. Among other types of lesions, a local freezing of the cortical tissue results in the development of focal or generalized seizures (29, 8, 11, 27). A characteristic feature of the abnormal EEG after freezing is its variability: both the pattern of the epileptic discharges, and the duration of the developed convulsive state is unpredictable (14, 25).

Few investigations are known of the effects of subcortical freeze lesions. Although it was shown that freeze lesions of various nuclei of the thalamus and basal ganglia may become epileptic foci, these seizures did not generalize spontaneously (12). The role of the mesencephalic reticular formation in the development and maintenance of generalized seizures is still a debated issue. According to Freedman and Moossy (5) and Freedman and Ferris (4), lesioning of the MRF significantly reduces the duration of generalized convulsions in the monkey. On the other hand, Walker and Serrano (29), after lesioning the midbrain reticular formation of monkeys, were not able to detect significant changes in the time course of focal or generalized convulsions elicited by Penicillin or electroshock. Fernandez-Guardiola and coworkers (3), and Quesney and coworkers (17) found that stimulation of the mesencephalic reticular formation strongly inhibits the generalized seizure activity induced by Metrazol or Penicillin. Generalized seizures were evoked by the application of Penicillin solution to the MRF according to Ralston and Langer (19). Gloor and coworkers could not confirm these data (6).

In this study we present evidence that bilateral freeze lesions made in the mesencephalic reticular formation — part of the "ascending reticular activating system" — bring about generalized convulsions in the cat. Furthermore, we studied the effect of the functional blockade — cooling — of the mesencephalic reticular formation and that of the inferior thalamic peduncle on this generalized epileptic activity, because these structures are known to exert profound influence on the synchronized or desynchronized state of the cerebral cortex (13, 23).

METHODS

Nine adult cats of both sexes were used in this study. Surgical procedures were performed in Nembutal anesthesia in aseptic conditions. Electrode implantation was carried out on two successive occasions using the stereotaxic coordinates of the atlas of Snider and Niemer (26). During the first procedure, cryoprobes were placed in the left mesencepha-
lic reticular formation (MRF coordinates: A 2.5, L 2.5, V -2.0) and in the left inferior thalamic peduncle (ITP, A 13.5, L 3.0, V -3.5). A bipolar stainless steel electrode was implanted in the central medial thalamic nucleus (CM, A 10.0, L 0.0, V 3.0). Stainless steel screws were driven into the skull bilaterally above the anterior suprasylvian (ASS), medial ectosylvian (MES), posterior suprasylvian (PSS) gyri. A stainless steel screw, placed in the bone covering the frontal sinus served as reference electrode. The screws, electrodes and probes were fixed with dental acrylic to the skull. The use of elevated ear-bars that formed holes in the dental acrylic made it possible to place the animals' head again in the stereotaxic zero position during the second implantation procedure, which took place at least a week after the first one. On this occasion cryoprobes were placed in the right MRF and ITP, and a bipolar stainless steel electrode was implanted in the MRF on the right side (A 4.0, L 2.0, V -2.0).

After the implantation of the various devices and electrodes the animals were allowed at least three weeks of post-operative recovery. During the experiments the animals were placed in a restraining box. Their heads — protruding from the box — were painlessly held by the elevated ear-bars. The animals adapted to this situation in about 4–5 days.

The EEG activity was fed into 8 channels of chopper-stabilized preamplifiers of a Beckman Type R polygraph.

The construction and operation of the system used for cooling and freezing had been previously described in detail by Skinner and Lindsley (24) and Skinner (21), and will be mentioned only briefly here.

The cryoprobes were constructed of untempered stainless steel tubing of 0.45 mm outer diameter. The tubes were bent in U-shape, the walls close to each other, but leaving the flow of cooled alcohol — used as coolant — free and sufficiently fast. The flow of alcohol was driven by nitrogen in a closed system at a pressure of 100–400 psi. The cooling of the pressurized alcohol was achieved by letting it flow through tanks in which dry ice was kept. A silver heater wire (0.025 mm diameter) was wrapped around the shaft of the probes. Thermocouples made of copper and constantan wires (0.05 mm diameter) were attached to the shaft and tip of the probes, which made the continuous monitoring of their temperature possible. The reference junctions of the thermocouples were placed in the dental acrylic that filled the frontal sinus. The heater wire and the thermocouples were fixed onto the probes with Epoxylite. The application of 3–4 amp. of D. C. current to the heater wire made it possible to keep the shaft at physiological temperature and to have the cooling effect localized to the probe's tip.

Functional blockade of the tissue, surrounding the tip of the cryoprobe
was produced by cooling the probes to 0-5°C. The effect is localized to
the very vicinity of the tip (24, 28). Freeze lesions were made by cool-
ing the probe’s tip to -25°C for 30 s while maintaining its shaft at
body temperature.

At the end of the experiments the animals were narcotized with
Nembutal and perfusion of the brain was carried out with 10% formalin
through the carotid arteries. The location of the probes and electrodes
was identified on frontal frozen sections of the brain.

RESULTS

Before making the freeze lesions, the effect of cooling of the implanted
probes was tested several times on each animal. The cooling of both
cryoprobes implanted in the MRF caused diffuse slowing of the EEG
both in the cortex and in the brain-stem. A typical example is shown
in Fig. 1. The effect took 12-20 s to develop and was accompanied by

Fig. 1. Effect of bilateral cooling of MRF cryoprobes on the EEG. l, left side; r,
right side. MES, medial ectosylvian gyrus; PSS, posterior suprasylvian gyrus; ASS,
 anterior suprasylvian gyrus; CM, centrum medianum; MRF, mesencephalic reticu-
lar formation. Generalized slowing of the EEG is recorded in all cortical leads
and in the brain-stem. After the termination of the cooling, the desynchronized
electrical activity suddenly reappears in all leads at the same time. Monopolar
recordings.
characteristic signs of natural sleep. After the cooling was stopped, the desynchronized EEG activity always returned quite suddenly as if it was a rebound phenomenon during the rewarming of the probes. Cooling of the ITP bilaterally caused widespread desynchronization of the cortical electrical activity.

The freeze lesions were made by cooling both MRF probes to \(-25^\circ\text{C}\) for 30 s. It was accompanied by bilateral slowing of the EEG and miosis, both lasting for 2–3 min. In some cases decerebrate rigidity was observed, which disappeared after 2–3 h. In some animals 1 or 2 min after the freeze lesions seizure activity developed in the MRF, which lasted for 20–30 s and consisted of sharp waves of 150–200 \(\mu\text{V}\) amplitude, as shown in Fig. 2. The characteristic feature of this phenomenon was that the epileptic activity did not generalize, but remained localized to the MRF. (Note that these are monopolar recordings. The low amplitude sharp waves that can be recognized in some other leads than the MRF are most probably the result of volume conducted signals.)

![Fig. 2. Localized MRF seizures. Seizure activity appearing predominantly in the mesencephalic reticular formation 2 min after the MRF freeze lesion. Monopolar recordings. Abbreviations as in Fig. 1.](image)

The seizures developed explosively 15–20 min after the freeze lesions had been made without any alarming clinical or electrophysiological sign. The picture was the equivalent of status epilepticus, e.g., continuous seizure activity without normal background EEG. Typically, sharp waves with 2–3 cps frequency were recorded both in the cortex and in the brain-stem with maximal amplitude in the central and parietal areas.
Fig. 3. Status epilepticus developing after bilateral freeze lesions in the MRF. Fifteen minutes after the freeze lesion generalized monotonous seizure activity is recorded. The epileptic discharges are most prominent in the central and parietal areas. Still continuous convulsive activity, but a changed pattern can be recorded 15 h after the freeze lesion was made. Abbreviations as in Fig. 1. Bipolar recordings.

The seizure potentials in the brain-stem had a much lower amplitude than in the cortex. Irregular jerks of the muscles of the snout, neck and trunk accompanied this condition.

Fig. 4. Effect of bilateral cooling of the MRF (+2°C) in status epilepticus. The beginning and cessation of cooling is indicated by arrows. No recognizable change can be observed in the EEG. Abbreviations as in Fig. 1. Bipolar recordings.
In a few hours the EEG notably changed and polyspike-and-wave complexes were seen in the cortical leads with 0.8–0.9 cps frequency (Fig. 3). Jerks of the muscles of the snout, neck and trunk were observed synchronously with these complexes. The animals died in status epilepticus 16–20 h after it had developed.

The effect of cooling of the MRF probes in status epilepticus can be seen in Fig. 4. The characteristic slowing of the EEG, typically caused by the cooling of both cryoprobes implanted in the MRF before the freeze lesion, is absent in both the cortical and brain-stem leads. Apparently no change can be observed either in the frequency or in the amplitude of the seizure potentials.

Two hours later the ITP probes were cooled in the same animal. The effect is shown in Fig. 5. Although computerized waveform-analysis was not performed, it is clear that during the cooling the polyspike complexes became simpler in form owing to the decrease of the number of spikes within the complexes. However, the complexes themselves occurred with the same amplitude as before cooling. Unlike the effect of ITP cooling in the unlesioned animal, which ended abruptly several seconds after the termination of cooling, the above-described changes caused by ITP cooling in status epilepticus lasted for 2–3 min. A typical experiment is shown in Fig. 5 where 2 min after the cessation of the cooling its effect was still clearly present.
In Fig. 6 two unstained histological sections of the midbrain are shown. The most rostral (A) and most caudal (B) levels were selected from a typical animal where the freeze lesions destroyed bilaterally nearly the whole reticular formation. The extension of the lesions were 4–5 mm in the rostrocaudal direction.

![Image of two unstained sections of the midbrain](image)

**Fig. 6.** Two unstained sections of the midbrain of a typical animal, showing the most rostral (A) and most caudal (B) sections of the brain-stem where the freeze lesions destroyed nearly the whole reticular formation.

**DISCUSSION**

According to our data, the generalized seizure activity can be elicited by freeze lesions in the mesencephalic reticular formation of the unanesthetized cat. Data of the literature would suggest that it is quite unlikely that hypersynchronized seizure activity might originate from the MRF. Kreindler and coworkers (10) and Bergman and coworkers (1) elicited tonic, or tonic-clonic seizures in cats and rabbits by the electrical stimulation of large areas of the reticular formation extending from the medulla to the midbrain. It was noted that these seizures were characterized not by synchronized, but by desynchronized cortical EEG patterns.
Data of Gloor and Testa (7) and Testa and Gloor (28) seem to prove that blockade of the MRF by cooling or by pharmacological means markedly enhances the "corticoreticular" or "centrencephal" type convulsive activity induced by Penicillin. However, it can clearly be seen in their Fig. 5 and 6 (28) that the generalized seizure develops during the period when the brain-stem rewarms after the MRF cooling, which fact does not support their conclusion. This period corresponds to a rebound desynchronization in the normal animal, which we always observed 15–20 s after the cessation of the cooling of the MRF. It is obvious that Gloor and his coworkers identified the generating mechanism of the Penicillin induced generalized spike-and-wave activity with the one responsible for the spindle activity that could be elicited by the stimulation of various nuclei of the thalamus, but not by the stimulation of the MRF. The conclusion was drawn on the basis of their observation that the most effective stimulation sites for triggering generalized epileptiform discharges were those from which spindle activity could be elicited in the normal animal (17). They postulated the existence of multiple pacemakers distributed throughout the thalamus that are responsible for precipitating generalized epileptiform activity (15). The hypothesis that the MRF counteracts this mechanism is supported by several observations, one of them being that epileptic discharges are suppressed by arousal (16). Buser and Horvath (2) mapped those sites in the MRF, the cooling or lesioning of which markedly enhanced synchronized (spindle) activity in the cortex of the cat.

On the basis of these and other observations it would be tempting to hypothesize that it is the activity of the synchronizing mechanisms, released from the influence of the desynchronizing system after the MRF lesion that is responsible for triggering the epileptic activity seen in our experiments. However, it is well known that the region into which the MRF probes were implanted had frequently been lesioned in earlier studies. Still, no subsequent epilepsy had ever been noticed.

When the cortex is subjected to intense or prolonged freezing, hemorrhagic necrosis develops (20). Bleeding is probably caused by damage of the capillaries by ice crystals (9). These processes may act like local irritation to the periphery of the frozen tissue. It is possible that the local seizures observed in the MRF right after the freezing were direct consequences of these events that occur during the freezing and rewarming of the tissue. The status epilepticus may have developed from an instantaneously generalizing seizure, starting from the mesencephalon and spreading to thalamocortical structures, the latter being involved in the generation of synchronized cortical activity. It is also
possible that a constant triggering zone developed in the region immediately adjacent to the necrotized area and maintained the generalized seizure. Computerized analysis of the timing of the cortical and brain-stem seizure potentials was not performed, although it might have clarified the matter.

No pathological alterations were found in the cortical regions over which the recording electrodes were placed epidurally. Thus, cortical sites of "locus minoris resistentiae" could not be observed, which could have generated or even modified the epileptic discharges.

The comparison of the effects of the coolings of the MRF and ITP probes after the freeze lesion is hardly possible, because the MRF probes were in necrotic tissue, whereas the ITP probes were not. This may explain why the cooling of the lesioned MRF with the same probes with which the lesions were made had no effect in status epilepticus. In this respect the question of how far the effect of cooling extends in the necrotized tissue must be taken into consideration. According to Quinn and O'Brien (18), who performed their measurements on the same cooling system that we used in our experiment, a 20°C isotherm was found having a 4 mm diameter around the tip of the probe, when its temperature was maintained at 3°C. These conditions must be different in the necrotized tissue, although temperature gradients were not taken. For this reason and on the basis of the ineffective MRF cooling in status epilepticus we can speculate that if the focus of the epileptic activity was in the tissue adjacent to the necrotized area, it was not affected by cooling.

Bilateral cooling of the ITP in status epilepticus reduced the number of spikes in the polyspike-and-wave complexes while leaving their amplitude unchanged. The bidirectional pathway interconnecting the frontal granular cortex with the medial thalamus runs through the ITP and is considered to be a part of the nonspecific thalamocortical system. Its blockade is known to abolish spontaneous spindle bursts and several other forms of cortical synchronization (22, 23). According to Pellegrini and Gloor (15), bilateral cooling of the ITP reversibly abolished epileptic discharges. However, their conclusion was that the effect must be the consequence of an inadvertent cooling of the nearby preoptic area, releasing mesencephalic reticular mechanisms from inhibition, because bilateral complete lesions of the ITP were without effect.

In our experiment, where the MRF was bilaterally nearly completely destroyed, the cooling of the ITP still affected the generalized epileptic activity, although the time course of the effect was longer than that observed in the unlesioned animal, for which difference no clear explanation can be given at present. Thus, our data do not support the
theory of Pellegrini and Gloor about the mechanism of ITP cooling. However, it may indicate that the integrity of thalamocortical structures is necessary for the maintenance of this continuous seizure activity.

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REFERENCES


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