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EEG After Intracerebroventricular Tetrodotoxin in Cats.* (29271)

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Tetrodotoxin (TT) is a potent poison extracted from the puffer fish. Systemic administration of TT causes death in mammals mainly as a result of neuromuscular paralysis(1). A direct depressant action of the toxin on vital centers in the brain stem has also been suggested(2). Borison *et al*(3) described in the cat a state of volitional paralysis following intracerebroventricular injection of TT in doses too small to cause peripheral effects. Hypothermia and respiratory depression also characterized the neurotoxic syndrome. On the other hand, TT did not appear to bring about anesthesia or analgesia since corneal, pinna, and nociceptive reflexes were not notably impaired.

This study was undertaken to examine the electroencephalographic correlates of the unusual behavioral response to TT administered intracerebroventricularly. Chronic experiments were performed in order to use unrestrained cats as their own controls and to observe concurrently the cortical and motor responses to the toxin. Acute experiments were performed on immobilized cats in order to maintain continuous artificial ventilation so as to obviate any indirect influence upon the EEG that might result from respiratory failure or other forms of motor disturbance.

Methods. Chronic preparations. Experiments were performed on 10 cats. The lateral cerebral ventricle was cannulated and epidural cortical electrodes were screwed into the skull under aseptic conditions in cats anesthetized with pentobarbital. In some animals a vinyl catheter was inserted into the carotid artery and secured to a plug fixed on a shoulder harness. This procedure permitted measurement of blood pressure in the unanesthetized animal. Intravenous injections were made through another catheter inserted into the external jugular vein. EEG tracings were recorded from sensorimotor and occipital cortical regions. Control recordings were taken for several days after surgery to provide representative tracings before drug treatment. The EKG was also monitored in selected animals. The crystalline toxin† was dissolved in isotonic saline and injected *via* the lateral ventricular cannula in volumes of 0.1 to 0.2 ml and total amounts of 0.1 to 0.2 μg. Effects were observed over the next 2 to 3 hours. Artificial ventilation with a Harvard pump was given as needed on emergency basis.

Acute preparations. Cats were prepared with cannulae, electrodes, and catheters as in the chronic preparations except that ether was employed as the anesthetic. When administration of ether was discontinued, lidocaine (1%) was infiltrated into the wound

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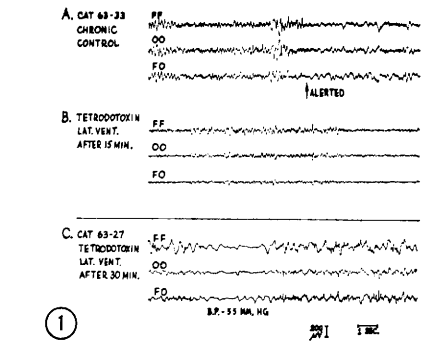

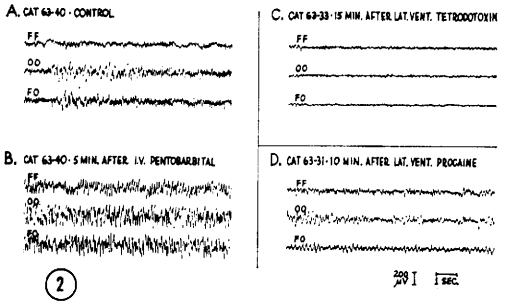

FIG. 1. Bipolar EEG recordings in chronic unrestrained cats. Effects of intraventricular tetrodotoxin. F, frontal; O, occipital. Cat "alerted" by handclap at arrow in panel A. Note fast activity at 15 min, during height of drug effect in Panel B. Panel C shows cortical depression due to low blood pressure.

FIG. 2. Comparison of effects on EEG of lateral ventricular tetrodotoxin (C) with i.v. pentobarbital (B) and lateral ventricular procaine (D).

edges, and the trachea was intubated through the mouth. The animal was placed on artificial ventilation and decamethonium bromide (500 μg/kg) was injected intravenously. Additional doses of decamethonium (250 μg/kg) were given thereafter as needed to maintain paralysis. EEG was recorded periodically for at least one hour and until cortical activity indicated recovery from anesthesia before TT was administered. In some preparations, body temperature, EKG, and arterial blood pressure were also monitored.

A Grass model III electroencephalograph was used to record EEG and EKG. Arterial blood pressure was monitored with an anaeroid manometer and body temperature was measured with a rectal thermometer.

Results. Chronic studies. A typical resting EEG recording from an untreated animal is shown in panel A of Fig. 1. The record has a predominance of 8 to 15 cycle-per-second waves of intermediate voltage. This pattern was readily converted into one of lower voltage and higher frequency "activation" when a sudden noise (*e.g.*, handclap) was made near the animal, as indicated by the arrow in the Figure. Panel B of Fig. 1 shows the EEG 15 minutes after injection of 0.15 μg of TT into the lateral ventricle. At 3-5 minutes after injection the cat became hyperexcitable and ataxic. Within the next few minutes it lost its righting ability and showed nystagmus, tachypnea, and hyperextension of neck and forelimbs; it also vocalized loudly. Starting about 10 minutes after injection, skeletal muscle tone decreased, the eyes became fixed and mydriatic, and the animal lay quietly as though in a state of light anesthesia. Nevertheless, as seen in the Figure, the EEG pattern was characteristic of that observed in an alert animal. During this period the pupillary light reflex was obtunded. In contrast, mild pinching of a foot pad resulted in brisk withdrawal of the limb. In some animals lower EEG frequencies developed 30 minutes or so after injection of the toxin, but these reverted to the alerted pattern on presentation of a sensory stimulus. This EEG activation was often accompanied by appropriate behavioral signs, such as lashing of the tail, blinking, and vocalization. Recovery usually occurred in one to two hours after drug administration without permanent sequelae, although 3 animals died of fulminating pulmonary edema within 2 hours.

In those experiments in which blood pressure and EKG were recorded, there occurred a rise in arterial blood pressure (even exceeding 200 mm Hg) and a tachycardia in the initial 10 to 15 minutes of drug effect. Subsequently, the blood pressure fluctuated widely for a short period and then stabilized at a hypotensive level. Some animals showed a slowing of respiration at this time and a decrease in body temperature, as previously reported by Borison *et al*(3). An EEG pattern of high voltage slow waves progressing to isoelectric silence was associated with severe hypotension or respiratory embarrassment which resulted in depression of the nervous system. For example, as seen in panel C of Fig. 1,

the EEG exhibited an essentially isoelectric tracing during a transient fall of blood pressure to 55 mm Hg. Thus, EEG findings fail to demonstrate an influence of the toxin upon the cerebral cortex except through indirect means in those instances where vital functions were compromised.

Acute studies. The EEG pattern of acutely prepared cats was similar in most respects to the chronic pattern but the acute pattern contained somewhat fewer high-amplitude, low-frequency components. Intraventricular TT produced alterations in the EEG similar to those described above for the chronic studies. No EEG changes were discernible after administering *intravenous* TT in total doses of 2.5 to 5.0 μg/kg, that is, 10 to 20 times the amount of toxin that produced marked behavioral effects when injected into the lateral ventricle. Thus, impairment of ventilation or altered skeletal muscle activity did not complicate the EEG pattern observed in chronic cats treated with TT.

Comparison with central depressants. EEG effects of intravenous pentobarbital, and intraventricular procaine are compared with those of intraventricular TT in Fig. 2. All 3 treatments produced a similar form of gross motor deficit. After 10 mg/kg of pentobarbital, the animal exhibited the characteristic high-voltage, low-frequency EEG pattern of light anesthesia (panel B). Even strong sensory stimuli failed to alter this EEG pattern, although the nociceptive withdrawal response was only slightly depressed. Injection of procaine hydrochloride (1.5 to 3 mg in 0.1 to 0.2 ml isotonic saline) into the lateral ventricle caused loss of righting, nystagmus, and tachypnea starting 2 to 4 minutes after injection, a syndrome similar to that produced by TT. The pupillary light reflex remained intact, however, and the overall effects of procaine lasted only 10 to 20 minutes. During this time the EEG was not remarkably altered from the normal resting pattern (Fig. 2, panel D) but, unlike the state produced by TT, EEG activation was not readily evoked by presentation of sensory stimuli.

Discussion. The results indicate that tetrodotoxin (TT), injected into the lateral ventricle in cats, produces a state of volitional motor paralysis without concomitant loss of consciousness as in general anesthesia. Indeed, the initial period of drug effect appears to be one of alerting or excitation, as judged by the animal's behavior and the EEG activity. This interpretation necessarily depends upon the validity of our criterion for assessing the level of consciousness, *i.e.*, the EEG pattern. The classical interpretive relationship between EEG pattern and level of consciousness has been obscured somewhat by recent studies on the phenomenon known as "paradoxical sleep"(4,5). Episodes of apparent deep sleep are characterized by low-voltage, fast-frequency EEG waves very similar to the alerted state, at least as observed in neocortical leads. On the other hand, the hippocampus and associated regions exhibit a pronounced theta rhythm during paradoxical sleep.

That TT induced paradoxical sleep seems unlikely for several reasons. 1. No periods of spindling EEG (usually seen preceding and following episodes of paradoxical sleep) were encountered after administering TT. 2. Paradoxical sleep is associated with minimal skeletal muscle tone, and movements are limited to slight jerking of the eyes, eyelids and muscles of mastication. During the loss of postural control after injection of TT, there was often seen a marked nystagmus and hyperextension of neck and forelimbs. 3. EEG activation after TT was often associated with behavioral signs of arousal. In contrast, presentation of sensory stimuli to an animal in paradoxical sleep usually converts the EEG to a spindle-sleep pattern.

Behavioral effects brought about by intravenous pentobarbital were similar but certainly not identical to those produced by intraventricular toxin. EEG responses were in no way comparable; the pentobarbital-treated cat exhibited a slow-wave, high-voltage EEG which could not be activated even by strong stimuli.

Lateral ventricular procaine produced behavioral effects very similar to those of TT but of shorter duration. The EEG after procaine, at time of peak effect, was indistinguishable from a normal recording. Other workers(1) have shown that TT has local

anesthetic activity on peripheral nerves. Perhaps intraventricular TT acts as a local anesthetic on periventricular structures concerned with volitional control of skeletal muscle activity. Differences between effects of procaine and the toxin may be related to the extent of penetration into the periventricular substance and to the rate of inactivation of the drugs at their site of action. Nevertheless, the central neurotoxic effects of TT cannot easily be explained by a simple local anesthetic action since intracisternal and intrathecal injections of TT were ineffective in mimicking the action of procaine.

Summary. Tetrodotoxin injected into the lateral cerebral ventricle of cats produced a reversible loss of volitional control. This syndrome differs from general anesthesia in that the EEG showed a waking pattern which could be "activated" by sensory stimulation throughout the course of motor dysfunction. The behavioral effects of lateral ventricular procaine mimicked those of tetrodotoxin, but procaine interfered with EEG activation due to sensory stimulation.

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Effect of Dietary Calcium Levels on Production and Reversal of Experimental Osteoporosis in Cats.* (29272)

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It has been established from animal experiments that a diet deficient in calcium will cause loss of mineral from the skeleton(1-3), producing the radiologic appearance of osteoporosis(4,5). In young growing animals, in animals deficient in vitamin D, or in animals in which there is an excessive loss of mineral from the skeleton(6), this change is accompanied by rickets and the presence of osteoid tissue. The present study describes the osteoporotic condition produced in adult cats by a diet deficient only in calcium and determines whether the porosis so produced can be reversed by a high-calcium diet.

Methods. Experimental animals. Ten adult female cats were kept for 2 months on an adequate diet to standardize the animals. During this period, or during the first 2 months of the experimental period, each cat had a litter of 3 kittens which were suckled for 6 weeks. Throughout the experiment the animals were kept in a large pen which permitted them exercise. Six of the cats were then fed a diet deficient in calcium,‡ consisting of raw beef heart and distilled water supplemented with vit. D and A and trace amounts of iodine(7); this diet was fed *ad libitum*. Four of the cats were kept as controls and fed on the same diet as the experimental animals, except that they had milk instead of water and tricalcium phosphate was added to the beef heart to supply each cat approximately 40 mg of Ca per kg per day (this was the high-calcium diet).

At the end of 10 weeks the 6 experimental animals showed radiologic evidence of decreased mineral density; 3 of these 6 cats were then fed the high-calcium diet for 10 weeks, while the remaining 3 were maintained

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‡ Analysis, wet weight basis: nitrogen, 2.3%; phosphate 0.18%; and calcium, 0.007%.