manifested in infant rats suckling an immune dam: many of the ingested *T. spiralis* were bound in mucus, and those that penetrated the intestinal epithelium were relatively immobile.

The relative importance of immunity to *T. spiralis* which is acquired in utero versus that conferred in colostrum or milk was determined by exchanging pups between immune and nonimmune dams at intervals after whelping (Fig. 2). Pups born to immune dams and fostered by a normal dam beginning on the first or third day of life were vulnerable to infection when challenged at 14 to 16 days. Evidently any immunity transferred in utero or in colostrum dissipates within 2 weeks. When pups were shifted from immune to nonimmune dams 1 day before challenge the immunity transferred in milk persisted for at least 24 hours. Pups shifted from nonimmune to immune dams were protected after fostering for only 24 hours.

We next confirmed an observation reported by Culbertson (*4*), namely that normal rat pups can be protected against *T. spiralis* by an intraperitoneal injection of specific serum from immune animals. We then used $(NH_4)_2SO_4$ precipitation to prepare a fraction of serum from immune animals which was rich in immunoglobulins (mainly IgG). This serum protected pups when injected intraperitoneally or given orally (Table 1). The protection was equivalent to that provided by an infected dam. Absorption of serum Ig with antiserum to rat IgG resulted in a significant loss of protection, while absorption with antiserum to rat IgA or IgM had no obvious effect. Therefore it is significant that antibodies to *T. spiralis* can be passaged serially through a lactating dam to her suckling young. This was demonstrated by infusing Ig from immune donors into lactating but otherwise normal rats. Antibodies originally present in the transferred material appeared in the blood of suckling pups after 24 hours (Fig. 3).

Antibodies probably mediate the immunity to *T. spiralis* that is transferred passively from a specifically immunized rat dam to her suckling young. Immunoglobulins G and A are the major antibody classes in rat milk, and they are present in similar amounts (*5*). That IgG is protective was revealed in the finding that IgG-rich serum (rat serum contains relatively little IgA) from immune donors protected newborn rats when ingested or injected intraperitoneally. The protective action of such antibodies may be attributable to the ready transport of serum antibodies to *T. spiralis* from the dam's blood into her milk and thence into the blood of suckling pups. Whether milk antibodies of other isotypes also protect remains an open question.

Our failure to diminish the protective capacity of specific serum antibody by adsorbing it to antibody to IgA does not mean that IgA is not involved in the mediation of rapid expulsion. Milk IgA may be qualitatively as well as quantitatively different from that in serum. The idea that IgG and IgA both mediate protection is appealing in light of (i) the capacity of IgA to protect mucosal surfaces, (ii) the association of *T. spiralis* larvae with the intestinal mucus of immune pups, and (iii) the ability of IgG to be transported readily across the intestinal epithelium of suckling rats (*6*) into the niche where the parasite lodges and is immobilized in immune rats. Mucus-associated muscle-stage larvae have been reported by others (*7, 8*) studying rapid expulsion in adult rats. Our experiments show that immunity to *T. spiralis* in neonates is passively transferred by antibodies, does not require previous intestinal exposure to the parasite, and is remarkably similar to rapid expulsion in adult rats.

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Interruption of the Mammillothalamic Tract Prevents Seizures in Guinea Pigs

Abstract. *Interruption of the connection between the mammillary bodies and the anterior nucleus of the thalamus in guinea pigs, by discrete bilateral electrolytic lesions of the mammillothalamic tract, resulted in essentially complete protection from the behavioral and electroencephalographic convulsant action and lethal effect of pentylenetetrazol. This result demonstrates that the mammillary bodies and their rostral efferent connections are important for the propagation and perhaps initiation of generalized seizures.*

The concept that subcortical regions in the mammalian brain propagate and perhaps initiate generalized seizures has been part of the biomedical literature for more than four decades (*1*). Several subcortical structures including various thalamic nuclei (*2*), the reticular formation (*3*), and, most recently, the substantia nigra (*4, 5*) have been suggested to play important roles in processes responsible for mediating generalized seizures.

We recently observed that guinea pigs infused with a combination of the convulsant drug pentylenetetrazol (PTZ) and the anticonvulsant drug ethosuximide, so that a prolonged state of minimal seizure activity was produced, had selective metabolic activation of the mammillary bodies, the mammillothalamic tracts, the anterior nuclei of the thalamus, and the ventral tegmental nuclei (*6*). This observation suggested that the mammillary bodies and their connections may be involved in the convulsant actions of PTZ. We now present evidence that supports this hypothesis and demonstrates that the pathway between the mammillary bodies and the anterior thalamus (mammillothalamic tracts) is important for the expression of experimental generalized seizures.

We examined the effects of interrupting the mammillothalamic tracts on the convulsant and lethal actions of PTZ. The mammillothalamic tracts were destroyed by stereotaxic electrocoagulation, and lesions were confirmed histologically. Clinical seizures following administration of various doses of PTZ were evaluated by a scoring system designed to rank the severity of clonic convulsant activity. The effect of PTZ on electroencephalographic (EEG) activity was also examined in paralyzed and ventilated animals. In these experiments, the duration of EEG seizure discharges in animals with lesions during a 1-hour experimental period was compared with that in control animals after injection of 150 mg of PTZ per kilogram of body weight; this supramaximal dose, in control animals, resulted in prolonged electrical seizures.

Guinea pigs (250 to 300 g), anesthe-

sized with a mixture of 4 percent halothane (Fluothane, Ayerst) in O_2 received lesions while being held in a rat stereotaxic frame (Kopf) equipped with guinea pig adaptor; the stainless steel electrode was 250 μm in diameter, with a 100-μm exposed tip, attached to a d-c constant-current lesion maker (Grass). Current ranged from 0.5 to 1.5 mA with a pulse time of 5 to 15 seconds. After surgery, the scalp was closed and animals were allowed to recover for 1 week before further testing. Mortality from the surgery was <5 percent, and no abnormal behavior was noted after the procedure. Experiments on mortality and behavior after administration of PTZ were performed using intraperitoneal injections of the convulsant (50 mg/ml) dissolved in saline. Clinical behavior following PTZ was observed and scored (5): 0, no seizure; 1, mild clonic; 2, severe clonic (explosive motor activity); 3, severe clonic within the first 10 minutes; 4, severe recurrent clonics; 5, steady clonic with the animal on its side; 6, same as 5 but within the first 10 minutes. The EEG effects of PTZ were examined with the animals paralyzed (Flaxedil, Davis, 15 mg) and ventilated (65 percent O_2, 35 percent, N_2O). The EEG was recorded 1 hour after the injection of PTZ. After being tested, the animals were killed and their brains fixed in 10 percent Formalin, imbedded in paraffin, sectioned, and stained for histological examination. Locating the lesions was done without knowledge of the response of the animal to PTZ.

Examination of control animals revealed that the threshold dose of PTZ for clonic seizures was 50 mg/kg, and this dose caused no fatalities. A dose of 75 mg/kg was lethal in 80 percent (LD_{80}) of the animals and 100 mg/kg was lethal in 100 percent (LD_{100}) (Fig. 1A). Doses of both 75 and 100 mg/kg produced severe clinical seizure activity beginning approximately 90 and 45 seconds, respectively, after injection. The mean for the control group approached the maximum possible seizure score of 6 at 75 mg/kg, and all controls had a maximum score at 100 mg/kg (Fig. 1C). Electroencephalograms from paralyzed and ventilated animals demonstrated that PTZ at a dose of 150 mg/kg produced frank electrical seizure discharges occupying slightly more than 50 percent of the recording time (Fig. 1D).

Comparison with control animals revealed that animals with lesions that spared the mammillothalamic tracts (missed MT) or those that had the tracts interrupted only unilaterally were indistinguishable from controls. As a group, the animals with bilateral destruction of more than 90 percent of the cross-sectional area of the tracts were significantly protected from the convulsant and lethal actions of PTZ (Fig. 1). However, there was considerable variability among these animals, and both size and location of the lesions influenced the degree of protection.

With regard to lesion size, animals with lesions larger than 1.5 mm in diameter (large MT) were indistinguishable from controls. These lesions not only destroyed the tracts (each 0.5 mm in diameter), but also injured portions of the thalamus, subthalamus, and hypothalamus (Fig. 2D). Lesions between 1.0 and 1.5 mm in diameter (medium MT) produced statistically significant protection; animals with such ablations exhibited less severe clinical seizure activity with both 75 and 100 mg/kg doses, and their mortality was reduced after injection of a 75 mg/kg dose. Animals with lesions less than 1.0 mm in diameter (small MT), which essentially limited the affected area to the two tracts and only very small portions of immediately adjacent regions (Fig. 2C), exhibited the greatest protection. They had only very mild clinical seizures after 75 and 100 mg/kg and very little seizure activity on EEG. None of these animals died after injection of 75 mg/kg PTZ, and a 100 mg/kg dose was only an LD_{40}. Animals with either medium or small lesions that died after receiving either 75- or 100-mg/kg injections survived longer than controls (Fig. 1B).

The large lesions destroyed a sizeable portion of the diencephalon as well as the MT, and animals differed little in the location of the damaged area. In the animals with medium lesions, the more ventrally placed lesions seemed to be less protective than those more dorsal. This impression was supported by the findings in animals with small MT lesions; those animals with dorsal lesions—that is, those that did not injure portions of the hypothalamus—were the most protected. None died after injections of 75 or 100 mg/kg, and they exhib-

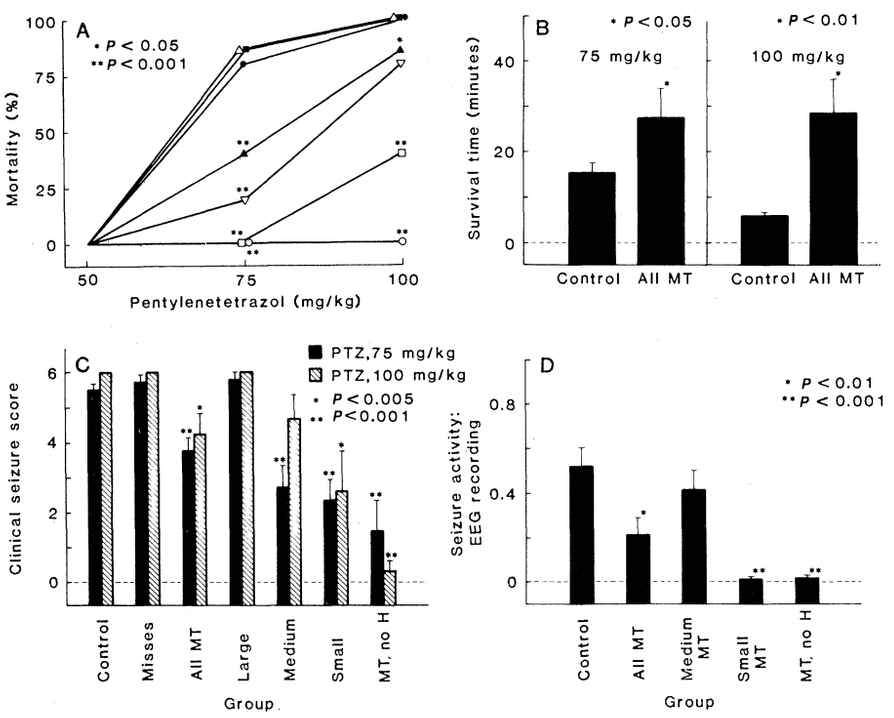

Fig. 1. (A) Effects of intraperitoneal injection of PTZ on mortality. Symbols: ●, control animals (n = 96 at 75 mg/kg; n = 52 at 100 mg/kg); ■, animals with lesions but without destruction of any portion of the mammillothalamic tracts (missed MT) (n = 15 at 75 mg/kg; n = 4 at 100 mg/kg); ▲, all animals with lesions >90 percent cross-sectional area of the MT destroyed bilaterally (designated MT-lesion animals) (n = 39 at 75 mg/kg; n = 19 at 100 mg/kg); △, MT-lesion animals with large lesions (>1.5 mm diameter) (n = 15 at 75 mg/kg; n = 2 at 100 mg/kg); ▽, MT-lesion animals with medium lesions (1.0 to 1.5 mm diameter) (n = 16 at 75 mg/kg; n = 13 at 100 mg/kg); □, MT-lesion animals with small lesions (<1.0 mm diameter) (n = 8 at 75 mg/kg; n = 5 at 100 mg/kg); ○, MT-lesion animals with small lesions not involving the hypothalamus (n = 4). Statistical evaluation was performed with the chi-square test with Yates correction. (B–D) Means (±standard error of the mean) analyzed with two-tailed t-tests comparing the control group with experimental groups. (B) Mean survival time of control (n = 76 at 75 mg/kg; n = 52 at 100 mg/kg) and MT-lesion guinea pigs (n = 16 at 75 mg/kg; n = 12 at 100 mg/kg) that died after intraperitoneal injections of PTZ. (C) Effects of PTZ on behavior in control (n = 96 at 75 mg/kg; n = 52 at 100 mg/kg) and treated (n = 4 to 16 in each group) guinea pigs. (D) Electroencephalographic (EEG) effects of 150 mg/kg injections of PTZ in paralyzed and ventilated control (n = 13) and treated (n = 4 to 12 per group) guinea pigs.

ited little or no clinical or EEG seizure activity.

None of the animals with lesions exhibited any apparent abnormalities of behavior or any change in feeding or growth.

The results demonstrate that small lesions destroying the dorsal tracts and little or none of the surrounding area offer essentially complete protection from the convulsant action of PTZ. The lesions not only block clinical convulsive activity and the lethal effects of the drug but also prevent the seizure discharges measured with cortical EEG. We believe that we have obtained lesions of such discrete size as to essentially restrict the ablated area to the two tracts and that the protective effects of these lesions are due primarily, if not solely, to the interruption of the tracts. Of course, one cannot exclude the possibility that other adjacent structures may still play a role either by influencing the expression of the seizure directly or perhaps by acting on the tracts to mediate the effects through the mammillary system.

The failure of large lesions to attenuate the PTZ seizures suggests that destruction of other neuronal structures near the mammillothalamic tracts has an opposite, facilitatory effect on the expression of generalized seizures. Other lesion and recording studies of the diencephalon (*2, 7*) have characterized numerous influences, both facilitatory and inhibitory, on seizure activity in this region of the brain. In light of the accumulated evidence demonstrating opposing influences in the diencephalon on the expression of seizures, it is clear that the effects of large lesions become difficult to interpret.

Most neurons in the medial mammillary body have bipolar axons that send processes rostrally to the anterior nucleus of the thalamus via the MT and caudally to the ventral tegmental nucleus (as well as to other structures) via the mammillotegmental tract. This connection between the upper brainstem and the anterior thalamus was first referred to as the "thalamic-midbrain circuit" (*8*). Our previous observation of selective metabolic activation of this circuit by a threshold convulsant stimulus was the first indication, to our knowledge, that it is involved in the expression of generalized seizures. Our finding that interruption of the MT prevent PTZ-induced seizures suggests that this system indeed mediates the convulsive action of PTZ between the thalamus and brainstem.

Numerous studies suggest that generalized seizures may originate in the subcortex and then propagate rostrally to affect the entire brain (*9, 10*). Supporting this possibility is the observation that the earliest detectable convulsive effects of PTZ on brain activity are found in the mesencephalic and pontine reticular formations, preceding subsequent involvement in other brain regions (*10*). By means of the mammillothalamic and mammillotegmental tracts, the mammillary bodies could influence the synaptic activity in the brainsteam and mediate the transmission to the thalamus. Interruption of this pathway may inhibit the rostral progression of seizure activity and result in diminished thalamic and cortical activation. Studies by Iadarola, Garant, McNamara, and their colleagues (*4, 5*) have shown that the efferents from the substantia nigra may serve, in part, as a gating mechanism for the expression of seizure activity. Whether our system is but an integral part of a larger circuit structure that includes the basal ganglia in controlling the expression of seizures or whether several independent networks exist, perhaps for different types of seizures, cannot yet be defined. Further characterization of the mammillary system in its relation to seizure activity and interaction with other neuronal systems may lead to a better understanding of the pathophysiological mechanisms underlying the expression of generalized seizures.

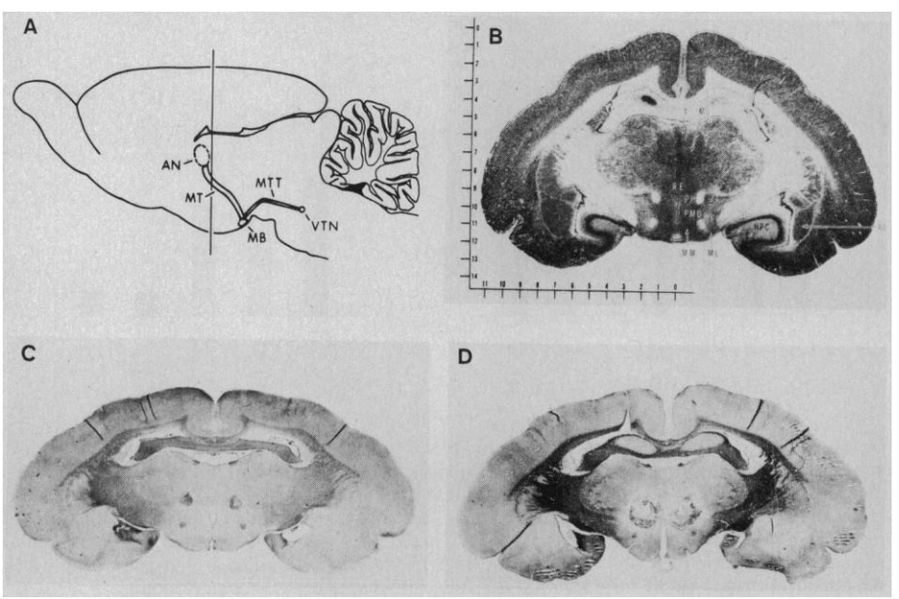

Fig. 2. (A) Parasagittal outline of guinea pig brain illustrating the midbrain-thalamic pathway consisting of the ventral tegmental nuclei (VTN), mammillotegmental tracts (MTT), mammillary bodies (MB), mammillothalamic tracts (MT), and anterior nuclei (AN) of the thalamus. The line perpendicular to the section indicates the approximate coronal level at which lesions were placed. (B) Coronal section from an atlas of guinea pig brain (*11*) at the anterior-posterior level of the mammillothalamic tracts. (C and D) Paraffin sections (20 μm) of guinea pig brains stained with Weil–neutral red demonstrating a small (C) and a large (D) lesion destroying the mammillothalamic tract bilaterally. [Courtesy of Karger]

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