PICROTOXIN-BARBITURATES ANTAGONISM IN DECORTICATED ANIMALS*

H. R. MILLER AND E. A. SPIEGEL

The problem of the sleep-waking mechanism may be approached by a study of the toxins known to induce the states of sleep or awakening, and by investigation, particularly, of the antagonism of sleep- and wake-inducing drugs.

A primary consideration is the localization of the effects of these agents. The studies of Maloney, Fitch, and Tatum have shown that picrotoxin is very effective in awakening an animal from barbiturate sleep. This antagonistic action between picrotoxin and barbiturates proved to be of clinical value also.

The series of experiments here presented was designed to ascertain whether the presence of a functioning cerebral cortex is essential for the awakening action of picrotoxin. Koppanyi suspected that the cortex participates in this mechanism. Together with his co-workers, he observed that the excitability of the motor cortex in dogs, abolished after intravenous administration of pentobarbital, was restored upon intravenous injection of picrotoxin. Whereas the convulsive effect of this toxin may still be observed after destruction of the hemispheres (Grünwald, Morita) or section below the optic thalami (Pollock and Holmes), direct application to the cerebral motor cortex, according to Sollman, produces some stimulation.

For the experiments reported in this communication rats were chosen because in them, as is well known, the cortex is readily extirpated by suction. Accordingly, the cerebral hemispheres were exposed under ether anesthesia and, by means of glass tubes connected to a suction pump, the cortex was sucked out. First, tubes with a diameter of 3 mm. were employed and then tubes ending in a capillary for the removal of remaining particles of the cortex. Careful attention to the control of hemorrhage is important. Within

* From the Department of Experimental Neurology, D. J. McCarthy Foundation, Temple University School of Medicine, Philadelphia, Pa. Aided by a grant from Mrs. Woodford T. Stewart to H. R. M.

Presented at the Eighth Annual Meeting of the Harvey Cushing Society, April 6-8, 1939, at New Haven, Connecticut.

3 to 4 hours post-operatively the animals were able to sit up, turn from a lateral position into a normal position, perform spontaneous movements of the head, tail, and extremities, and move about more or less actively.

At this stage, and also one to two days later, the anesthesia and awakening experiments were carried out. Sleep was induced by intraperitoneal injection of from 2 to 4 mg. of nembutal per 100 gm. body weight. After from 3 to 7 minutes the rats were deeply anesthetized and remained motionless, lying on one side. They failed to react or, at most, responded only slightly to painful stimulation, i.e., squeezing or pinching the tail or limbs. With the animal in this state, picrotoxin was administered and its awakening influence observed. This drug (1 to 2 mg.) was injected intraperitoneally at 3 to 5 minute intervals.

One to ten minutes after intraperitoneal injection of from 1 to 5 mg. of picrotoxin per 100 gm. body weight, the animal began to turn from its motionless lateral position to a normal position and to perform normal spontaneous movements of parts of the body (head, tail, and extremities). Painful stimuli increased these movements and often brought on running movements. This period of awakening was followed by a convulsive stage. During the early part of this stage, the convulsive seizures (opisthotonus and general clonic movements) appeared only occasionally and the normal spontaneous movements were to be observed in the intervals between attacks of convulsions. Later the convulsions became more and more frequent and severe. Some animals survived these convulsions and in them the anesthesia-awakening experiments could be repeated in one or two days. Others, the majority, eventually succumbed from exhaustion or failure of the respiratory center.

The following protocol illustrates the type of experiment and the results obtained.

White Rat Picro X. 260 Gms.
Feb. 28, 1939

12:00 Finished decortication by suction under ether anesthesia.
2:20 Walks, manège movements to left.
3:26 Intraperitoneal injection of nembutal, 1 mg.
3:31 Injection repeated.
3:36 Injection repeated.
3:45 Injection repeated (total amount of nembutal 4 mg.).

3:50 Animal is deeply asleep in lateral position; fails to react when tail is pinched.
3:51 Picrotoxin 1.5 mg. intraperitoneally.
3:55 Picrotoxin 1.5 mg. intraperitoneally.
3:56 Animal still sleeps but reacts by twitching when tail is pinched.
4:01 Picrotoxin, 0.6 mg. intraperitoneally (total amount of picrotoxin 3.6 mg.).
4:05 Animal reacts by a few running movements when tail is pinched.
4:07 Sits in normal position and walks spontaneously.
4:10 Has a few general clonic convulsions.
4:15 Convulsions are more frequent.

March 1, 1939

10:00 Animal is awake.

March 2, 1939

10:50 Animal sits quietly, makes occasional spontaneous movements. Pinching of the tail induces crying, struggling; animal walks for a few minutes following pinching of the tail. Manège movements to the left.
11:00 Nembutal, 2.5 mg. intraperitoneally.
11:13 Nembutal, 1.3 mg. intraperitoneally.
11:22 Animal sleeps in lateral position, but still cries out and twitches when tail is pinched.
Nembutal, 1.2 mg. injected (total amount of nembutal 5 mg.).
11:26 Deep sleep. Only slight movements of limbs when tail is pinched.
11:27 Picrotoxin, 3.0 mg. intraperitoneally.
11:37 Animal sits up, runs around and waves tail.
11:38 The spontaneous movements are interrupted by attacks of opisthotonus.
11:39 Attacks of running and rolling movements, also jumping movements. Extreme motor excitation.
11:41 Animal exhausted, lies in lateral position.
11:42 Running movements in lateral position.
12:30 Exitus.

Autopsy: Cerebral cortex is completely removed, except for a small strip of the right pyriform lobe. The caudal thalamus is covered by remnants of the cornu ammonis.

Reviewing this series of 15 animals in which autopsy showed in 9 cases an incomplete, and in 6 cases a complete, decortication, except for a small medial strip of the pyriform lobe, it may be stated that *picrotoxin is able to awaken animals (rats) from barbiturate sleep after removal of the cerebral cortex.* Further experiments are in progress in order to ascertain upon which subcortical centers the awakening action of this drug depends.

References

1. Grünwald, H. F.: Arch. f. exper. Path. u. Pharmakol., 1909, *60*, 249.
2. Koppanyi, T., Linegar, C. R., and Dille, M. J.: J. Pharmacol. & Exper. Therap., 1936, *57*, 130; *58*, 199.
3. Maloney, A. H., Fitch, R. H., and Tatum, A. L.: J. Pharmacol. & Exper. Therap., 1931, *41*, 465.
4. Morita, S.: Arch. f. exper. Path. u. Pharmakol., 1915, *78*, 188-208.
5. Pollock, L. J., and Holmes, W. H.: Arch. Int. Med., 1915, *16*, 213.
6. Sollman, T.: *Manual of Pharmacology*. 4th Ed., W. B. Saunders, Philadelphia, 1932.