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Reversal of Morphine Tolerance after Medial Thalamic Lesions in the Rat

Abstract. *Tolerance, manifested by a diminished electroencephalographic response at cortical and subcortical recording sites, was found in rats subjected to repeated systemic injections of morphine sulfate. Reversal of tolerance to morphine resulted from destruction of the medial thalamus.*

After repeated administration of morphine, there comes a point when the same previously effective dose no longer has a soporific effect (*1*) on the organism. If the dosage is not increased, withdrawal symptoms frequently occur. In the rat, withdrawal is manifested by increased locomotor activity and "wet shakes." These symptoms are an indication of physical dependence on the drug.

Several hypotheses have been put forth to explain how drug tolerance develops. It has been generally assumed that morphine tolerance is manifested throughout the nervous system in a nonspecific manner. Wikler and Carter (*2*) studied the effect of repeated doses of morphine on spinal reflexes and demonstrated the development of tolerance in the caudal portion of the surgically sectioned spinal cord of the dog. Berkowitz and Spector (*3*) transferred tolerance from "addict" mice to previously untreated mice by injecting serum containing morphine immunogen. They suggested that these antibodies "reduced the concentration of morphine in critical sites in the brain." Pert and Snyder (*4*) showed that opiates do not bind equally to all cells in the central nervous system. For example, the caudate nucleus seems to have a greater affinity to opiates than does the cerebral cortex or cerebellum. Further evidence for a discrete locus of opiate action in the brain was provided by Wei *et al.* (*5*). In their experiments, withdrawal symptoms after morphine administration were precipitated by direct injections of minute amounts of naloxone (a potent antagonist) into a zone including the medial thalamus and midbrain. Naloxone injected at many other subcortical sites had no effect.

Despite biochemical evidence that morphine has a greater affinity for the basal ganglia of the brain than for other regions (*4*), there have been no reports of localized or selective bioelectrical changes in response to morphine injections. Changes in cortical electrical activity in response to morphine administration have been studied (*6*), but it is not known whether morphine response and morphine tolerance, manifested by electroencephalographic (EEG) responses to morphine, might show up in some regions of the brain earlier than in others. We compared the bioelectrical response of the caudate nucleus, medial thalamus, and cortex to repeated doses of morphine and studied the effect of medial thalamic lesions on naloxone withdrawal.

Ten male albino rats were anesthetized with sodium pentobarbital (50 mg per kilogram of body weight), and recording electrodes were implanted in the medial thalamus, caudate nucleus, and posterior cerebral cortex. After a 1-week recovery period, the animals were injected twice daily with morphine sulfate (30 mg/kg, intraperitoneally). At first, this regimen resulted in drastic alterations in brain bioelectrical activity and behavior. However, after repeated drug administration both the behavioral

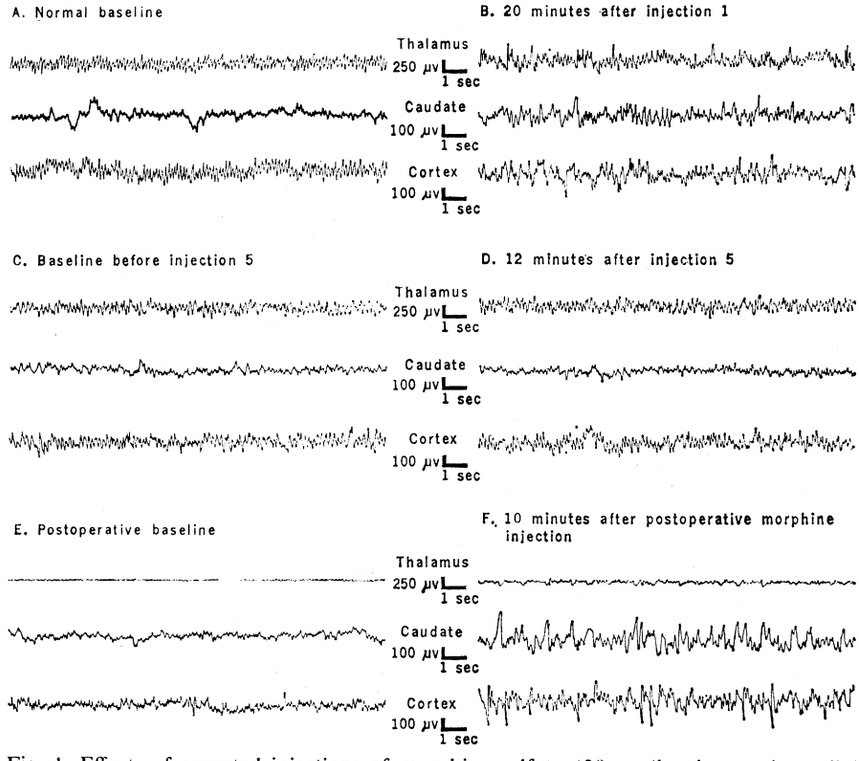

Fig. 1. Effects of repeated injections of morphine sulfate (30 mg/kg, intraperitoneally) on EEG tracings recorded from thalamic, caudate nucleus, and cortical sites in rat 256. Tolerance to morphine is demonstrated in (D). The medial thalamic lesion results in the reappearance of sensitivity to morphine (F).

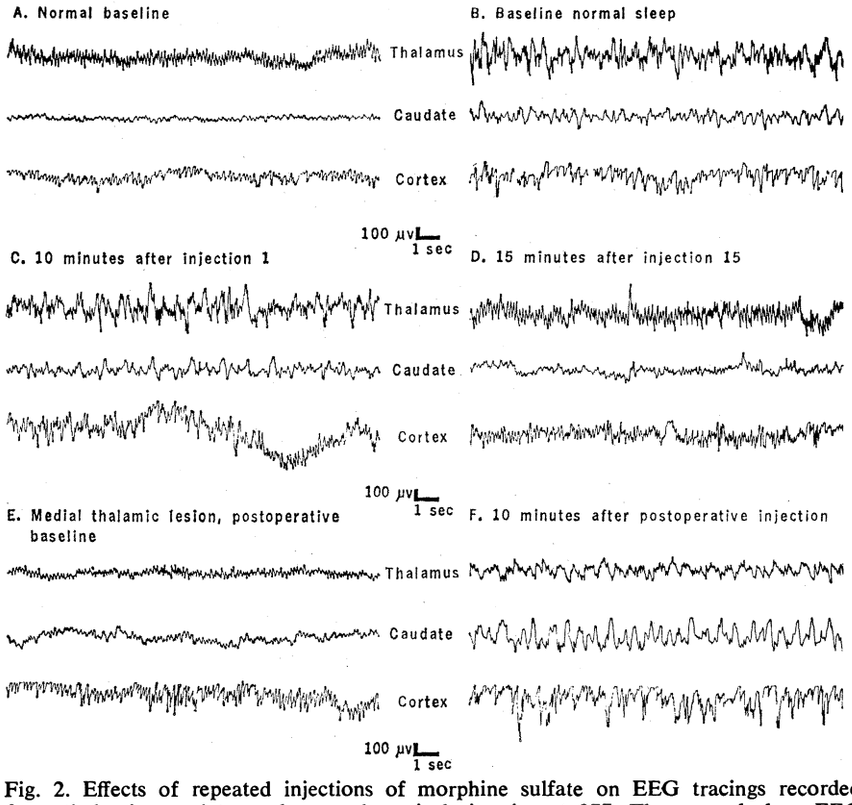

Fig. 2. Effects of repeated injections of morphine sulfate on EEG tracings recorded from thalamic, caudate nucleus, and cortical sites in rat 277. The normal sleep EEG (B) and that after morphine injection (C) are similar. Tolerance to morphine is demonstrated in (D). Reversal of morphine tolerance after medial thalamic lesion is seen in (F).

and EEG effects disappeared in all animals.

Figures 1A and 2A illustrate the characteristic wave patterns obtained from these recording sites in two rats. Subject 256 (Fig. 1) subsequently developed tolerance very rapidly; subject 277 (Fig. 2) required three times as many morphine injections before becoming tolerant to the drug. After the first morphine dose, EEG patterns were drastically altered (Figs. 1B and 2C).

In some rats, the change occurred first at caudate recording sites; in others, the cortex showed high-amplitude slow waves first. There was no predictable pattern from one subject to another. Tracings from normally sleeping subjects looked almost identical to recordings from the same subjects after morphine administration (Fig. 2, B and C). The onset of drug effect varied from 2.5 to 15 minutes from subject to subject. The daily baseline recordings before injection of the drug were remarkably constant from day to day. However, the duration of the morphine effect (Figs. 1B and 2C) decreased gradually with each succeeding dose. By dose 5, subject 256 showed no behavioral response after morphine injection, but remained alert and often showed abstinence signs characterized by head bobbing and wet shakes. The EEG (Fig. 1D) remained the same as the baseline recordings (Fig. 1C). Tolerance to morphine developed much more slowly in subject 277. By dose 15, the tracing from the caudate leads was completely flat (Fig. 2D), the cortical EEG showed a slight amplitude effect, but the dominant frequency was much higher than after the initial dose of morphine. To obtain the pharmacological effect of the initial dose (Figs. 1B and 2C) it was necessary to repeat the morphine injection at least five times with about 20 minutes between injections (total dose, 150 mg/kg). This dose would have been lethal if given initially.

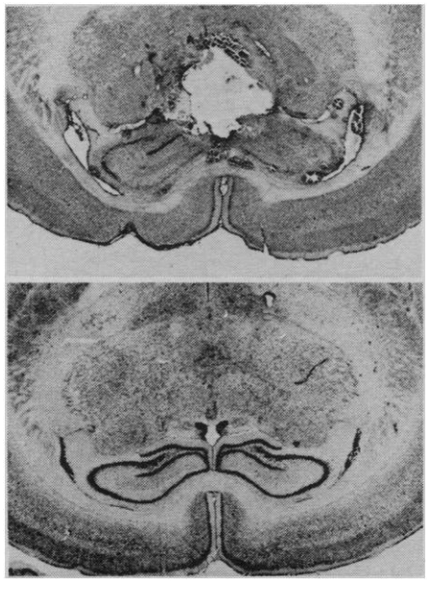

Fig. 3. Photomicrographs of thionin-stained frontal sections of the rat brain. Normal thalamus (top) and medial thalamic lesion (bottom) are shown. This lesion was effective in reversing morphine tolerance in the rat.

After the dose necessary to reinstitute the morphine effect in tolerant subjects was administered, naloxone hydrochloride (0.8 mg/kg) was injected intraperitoneally. This caused a nearly immediate reversal of the morphine effect. That is, the rats became alert, the high-amplitude slow waves were abolished, and the EEG from all three recording sites reverted to baseline. The medial thalamic tracings did not lead the others as might be expected from the results of Wei *et al.* (*5*). The naloxone effect lasted about 15 minutes, then the rats became somnolent again. To evaluate whether naloxone could reverse the effect of morphine on the EEG when the medial thalamus was ablated, lesions in this region were made through the implanted recording electrodes in four animals. Lesions of the caudate nucleus and hippocampus were made in six control subjects. Despite extensive damage to the medial and lateral habenular nuclei, the fasciculus retroflexus, and dentate gyrus and damage to the dorsomedial nucleus (Fig. 3), naloxone still reversed the effect of morphine. However, the action of the antagonist was of slightly shorter duration in subjects with medial thalamic damage. Lesions of the caudate nucleus and dorsal hippocampus had no effect.

The next day, EEG recordings from caudate and cortical sites were normal (Figs. 1E and 2E). The subjects with lesions were alert; they were sensitive to tactile stimuli and showed normal righting responses. The medial thalamic tracing from subject 256 was completely flat as a result of the lesion; that from subject 277 was drastically reduced in amplitude but not completely abolished. As soon as a single morphine dose was administered, the EEG from the two intact recording sites showed

an intense morphine response. All four subjects with medial thalamic lesions became somnolent (Figs. 1F and 2F), and the righting response disappeared. These effects were absent in animals with caudate and hippocampal lesions.

While medial thalamic lesions had little, if any, effect on precipitated withdrawal effects of the morphine antagonist naloxone, these lesions had a drastic effect on sensitivity to morphine in tolerant rats. It is difficult to reconcile these results with the view that tolerance to morphine is a generalized phenomenon occurring throughout the central nervous system. If this were the case, lesions in different parts of the brain should be equally ineffective in altering morphine sensitivity in tolerant subjects. Kerr and Pozuelo (*7*) demonstrated that lesions of the ventromedial nucleus of the hypothalamus and, to a lesser extent, lesions of the septal nucleus of tolerant rats resulted in increased sensitivity to morphine. Since behavioral criteria were used in their experiments to assess tolerance, whereas EEG criteria were used in the present study, it is not possible to compare the magnitude of the effects of lesions in the two studies.

Because of the many complicated ways brain lesions and drugs interact, it would be imprudent to provide neuroanatomical speculation as to why lesions of the septum, hypothalamus, and medial thalamus should alter morphine sensitivity in tolerant rats. For example, localized lesions of one region can lead to a concomitant alteration in levels of neurotransmitters in distant regions of the brain (*8*). This effect is accompanied by an alteration of response to barbiturate anesthesia (*8*). Since morphine induces changes in central cholinergic metabolism (*9*), it would be useful to determine how medial thalamic, septal, and hypothalamic lesions affect this cholinergic response to morphine.

HERMAN TEITELBAUM
GEORGE N. CATRAVAS
WILLARD L. MCFARLAND
Armed Forces Radiobiology Research Institute, Defense Nuclear Agency, Bethesda, Maryland 20014

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Amphetamine Effects in Man: Paradoxical Drowsiness and Lowered Electrical Brain Activity (CNV)

Abstract. *Thirteen of 20 normal adults given 10 milligrams of dextroamphetamine exhibited paradoxical drowsiness accompanied by lowered electrical brain activity (contingent negative variation, or CNV) in the first hour post-drug. During this period, seven subjects showed behavioral alertness and increased CNV amplitude. Both groups of subjects showed heightened alertness 2 and 3 hours post-drug. Amphetamine is not a simple stimulant of the central nervous system but can also act as a depressant.*

Amphetamine is a stimulant of the central nervous system which has been widely used both clinically and socially. As therapeutic medication, it has been employed with some success in the treatment of obesity, hyperactivity in children, and narcolepsy (*1*). On the other hand, prolonged use has resulted in amphetamine psychoses, which are similar to paranoid schizophrenia and which have been associated with homicide (*2*). One hypothesis is that chronic amphetamine use stimulates arousal processes of the central and peripheral nervous systems and that overstimulation leads to overarousal, a disruption in attention, and psychotic behavior (*3*). This view is supported by findings that amphetamine increases behavioral arousal, exerts alerting effects on electroencephalographic activity, and increases brain norepinephrine levels (*4*). Therefore, we attempted to demonstrate in normal humans that amphetamine increases the amplitude of an event-related electrical brain wave called "contingent negative variation" (CNV) (*5*), which is an indicator of alertness (*6*). Unexpectedly, the drug produced a transient state of drowsiness and reduced CNV amplitude.

Subjects were 20 paid volunteers (*7*). The first experimental session was to acclimatize subjects to testing procedures. Drug or placebo was given in the second and third sessions, which were separated by 5 to 11 days. Subjects were randomly assigned to two groups of ten subjects each. One group received amphetamine first and placebo second. The second group received placebo first and amphetamine second. Neither subject nor experimenter knew the substance of the tablets (double blind procedure).

The experimental task consisted of a constant-foreperiod, simple reaction-time paradigm. The preparatory stimulus (first stimulus or S_1) was a brief (10-μsec) flash of dim light presented 1 m from the subject's eyes through a 2.5 cm aperture. The second stimulus (S_2) was a 1000-hz tone at a sound pressure level of 70 db (relative to 0.0002 dyne/cm^2) presented 1.5 seconds after the light flash and terminated by a telegraph key press. Intertrial intervals varied randomly from 13.5 to 21.5 seconds.

Experimental sessions began at approximately 9 a.m. A control (pretreatment) run consisted of 16 trials of S_1–S_2–motor response (light–tone–key press) and lasted 5 minutes. Either 10 mg of dextroamphetamine (two 5-mg tablets) or identical placebo tablets were administered orally. Eighteen runs with intervening rest periods (5 minutes) were given for 3 hours post-treatment.

Electroencephalographic (EEG) recordings were made from silver–silver chloride electrodes attached to vertex (Cz) and joined mastoid processes. Vertical eye movements were monitored by an electrooculogram (EOG) recorded from above and below the right eye (*8*).

The evaluation of amphetamine effects on CNV amplitude was complicated by an unexpected finding of drowsy behavior among the majority