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Pharmacologic Dissociation of Behavior and EEG "Sleep Patterns" in Dogs: Morphine, N-Allylnormorphine, and Atropine. (19345)

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Clinical experience has demonstrated that alterations in the state of consciousness are usually associated with changes in the electroencephalogram(1). In particular, characteristic changes in the electroencephalogram occur during natural sleep or after the administration of certain hypnotic drugs, notably the barbiturates(2,3). However, there is evidence that the mechanisms which subserve sleep may be dissociated from those which subserve synchronization and desynchronization of the electroencephalogram. Thus, Magoun(4) has shown that after destruction of the posterior hypothalamus in the cat, afferent sensory impulses may produce "arousal" patterns in the electroencephalogram, while the animal remains quite somnolent. The reverse has also been noted in a few instances. Thus, Andrews(5) observed that in one human subject, a single "analgetic" dose of morphine produced "sleep waves" in the EEG while the patient was wide awake. Cahen and Wikler(6) noted that in the rat, morphine produced "burst" and slow activity in the EEG, although the animal had to be restrained to permit recording. In this species, the "burst and slow wave" pattern produced by morphine was indistinguishable from that produced by pentobarbital, although in the latter case, the animal was anesthetized.

The purpose of the present report is to demonstrate that such dissociations between "sleep patterns" in the EEG and behavior can be produced regularly by administration of a number of drugs under certain experimental conditions, and to discuss the significance of these phenomena in relation to the mechanisms of sleep and the regulation of spontaneous rhythmic cortical electrical activity.

Methods. Twenty-three experiments were made on 12 mongrel adult dogs, in which permanent "mercury-platinum cup" dural electrodes had been implanted in the anterior, middle and posterior portions of the skull (corresponding roughly to the "frontal," "parietal" and "occipital" regions of the cerebral cortex) on both sides of the head symmetrically, according to the method of Wikler and Altschul(7). For recording, the animals were trained to lie quietly on a table in an electrically shielded room, without anesthesia or curarization. "Unipolar" tracings were obtained by pairing leads from each mercury-platinum electrode with a needle electrode inserted in the ipsilateral ear. Various "bipolar" tracings were also made. An eight-channel Grass ink-writing electroencephalographic apparatus was employed throughout.

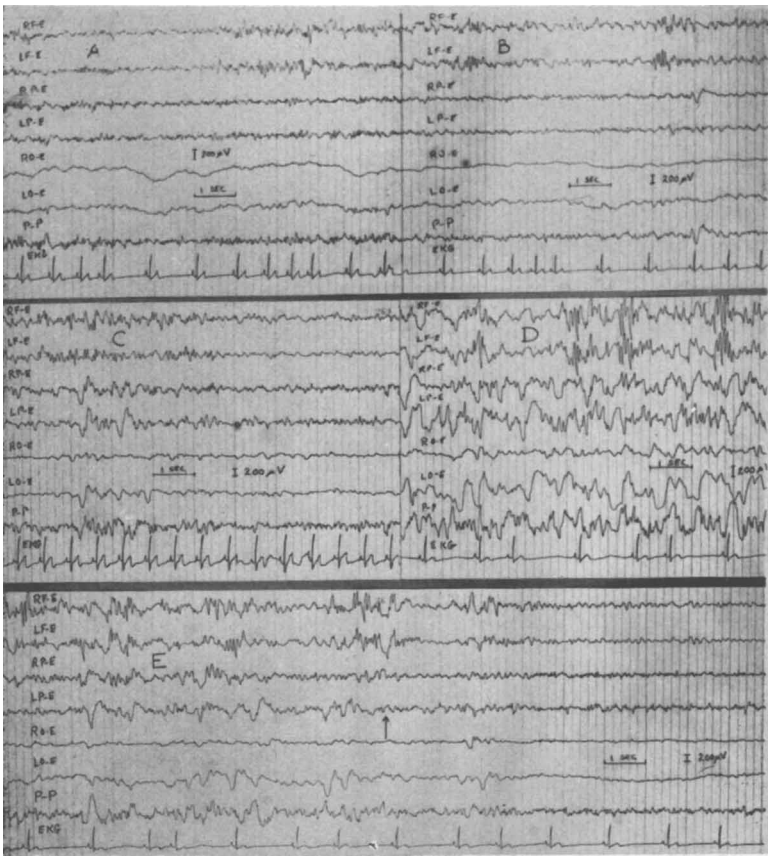

FIG. 1. Dog RCP-IC-1. Effects of "sedative" dose of morphine on the electroencephalogram. A, control. Note periodic "bursts" in frontal tracings. B, control at lower gain. C, 11 min after subcutaneous injection of morphine (approx. 5 mg/kg). Note admixture of random moderate to high voltage slow waves. Dog restless, vomited shortly before sample. D, 23 min after morphine. Note irregular high voltage slow activity in all tracings, and bursts of high voltage 12 cps. rhythms in frontal tracings. Dog apparently awake, eyes open, though dozing occasionally. E, 27 min after morphine, behavior same as in D. At arrow, dog alerted when called softly. Note sudden transformation of EEG from "sleep" to "waking" pattern.

All drugs were prepared in aqueous solutions and were administered subcutaneously in the following dose ranges: morphine 5-150 mg/kg, N-allylnormorphine 2.5-30 mg/kg, and atropine 2-8 mg/kg. An observer was always present in the electroencephalographic chamber and observations on behavior and the EEG were made simultaneously at frequent intervals. For present purposes, only the events which occurred early (*i.e.*, within 15-30 minutes) after injection of the drug will be described, since it was during this period that "sleep patterns" in the EEG were consistently recorded in each case.

Results. In most experiments, the control electroencephalographic patterns were characterized by a predominance of "low voltage fast" activity in all tracings, with an admixture of slower random or rhythmic activity in the anterior tracings. The latter were more prominent when the animal appeared to be "relaxed." Occasional "bursts" of high voltage rhythmic 8-14 cps. spike-like discharges also appeared in the anterior tracings when the dogs appeared to be dozing naturally, or in other experiments, after sleep was induced by intravenous injection of pentobarbital (approximately 15 mg/kg).

Within 15-30 minutes after injection of morphine, N-allylnormorphine, or atropine, the EEG exhibited marked changes. Regardless of the nature of the drug administered,

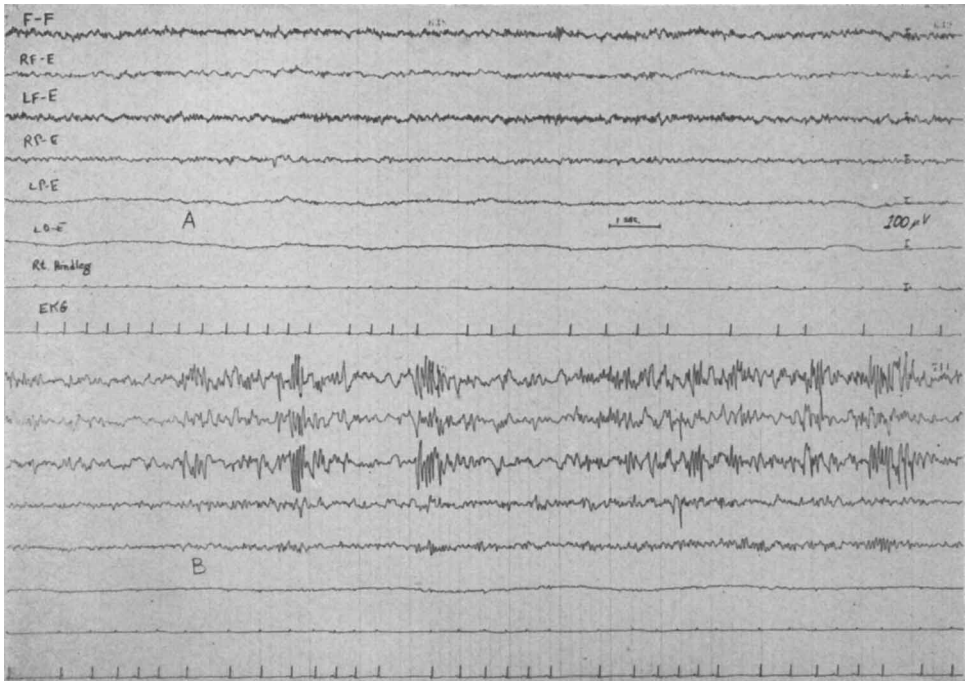

FIG. 2. Dog IC-16. Effects of N-allylnormorphine on the electroencephalogram. A, control. Dog awake. Note predominantly non-rhythmic fast activity in EEG. B, 12 min after subcutaneous injection of 2.5 mg/kg of N-allylnormorphine. Dog alert, moves eyes spontaneously and in following objects. Note typical bursts and slow wave "sleep" pattern in frontal EEG tracings and moderate diffuse slowing in parietal tracings.

the electroencephalographic pattern resembled strikingly that which was observed when the animal appeared to be asleep prior to medication, and consisted of alternate bursts of high voltage 8-12 cps. rhythmic "spike-like" discharges and random or rhythmic high voltage slow (2-6 cps.) waves. These were most prominent in the anterior tracings and were generally bilaterally symmetrical and synchronous. A variable amount of slow activity was also observed in the middle and posterior tracings.

Concomitantly, however, marked differences were observed in the animals' behavior. After the smaller doses of morphine (5-10 mg/kg), the dogs appeared to be dozing, but responded instantly to mild stimuli, such as whistling or calling by name. In these cases, alterations in behavior and EEG pattern appeared simultaneously (Fig. 1). After administration of larger doses of morphine, "arousal" reactions, both with respect to behavior and the EEG pattern, were more difficult to achieve, although the EEG pattern was indistinguishable from that which was observed after the smaller doses.

After administration of small doses of N-allylnormorphine (2.5 mg/kg), the dogs appeared to be at least as alert as before medication and followed objects with their eyes, although concomitantly "sleep patterns" were recorded in the EEG (Fig. 2). With larger doses, mild sedation occurred, but the animal continued to follow objects and respond to mild stimuli.

Following injection of atropine, the dogs were definitely "excited" and had to be restrained to permit recording, at a time when "sleep patterns" were evident in the EEG tracings (Fig. 3). When released, these atropinized animals jumped off the table and spontaneously returned to the animal quarters in the laboratory. Later, periods of excitement alternated with periods of sedation or apparent sleep, yet the "burst-slow wave" pattern in the EEG continued unchanged throughout.

Discussion. These findings have added sig-

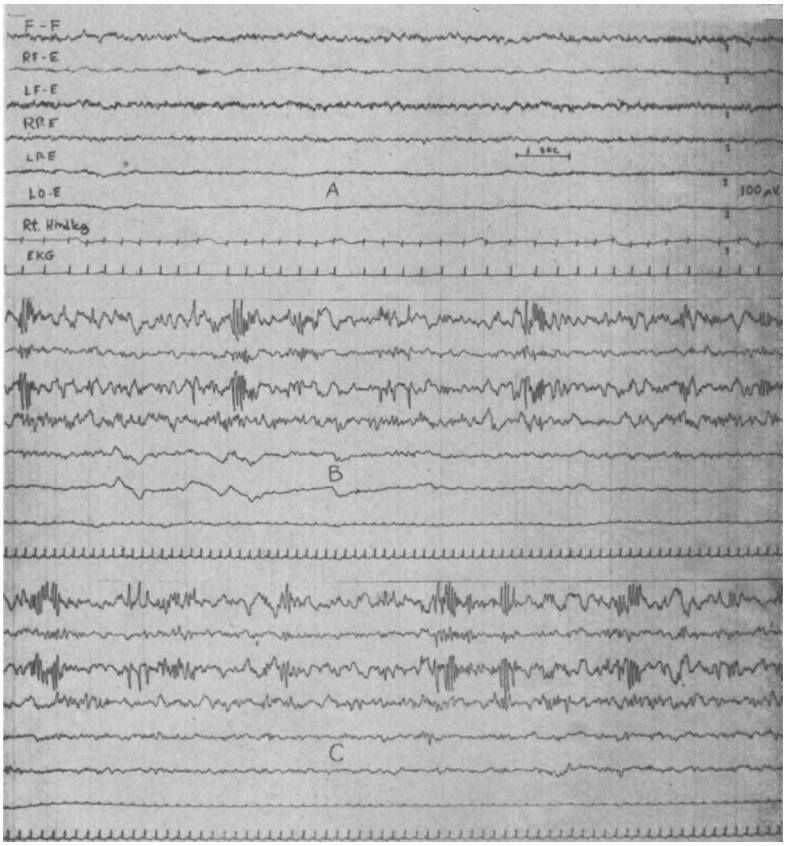

FIG. 3. Dog IC-20. Effects of atropine on the electroencephalogram. A, control. Dog awake. Note predominantly non-rhythmic fast activity in EEG. B, 23 min after subcutaneous injection of atropine 7.2 mg/kg. Dog excited, trying to right self, whining. Note concomitant burst-slow wave "sleep" pattern in EEG. C, 31 min after atropine. Dog quiet, eyes closed, apparently asleep. Note EEG pattern same as in B. This pattern continued unchanged for several hours after atropine while periods of excitement alternated with periods of sedation.

nificance when they are compared with the effects of pentobarbital. In amounts sufficient to produce "burst-slow wave" patterns in the EEG, this barbiturate produces a marked degree of unresponsiveness in both the rat(6) and the dog(8). It is evident, therefore, that the mechanisms which subserve "sleep" and those which subserve the "burst-slow wave" EEG patterns are distinct from each other, although they are very often closely interlocked. Furthermore, one cannot state that "sleep" causes the "burst-slow wave" patterns, or vice-versa. Rather, it appears that they are often concomitant phenomena and that it is necessary to investigate further the precise conditions under which they can be expected to occur simultaneously.

Direct neurophysiological evidence exists which indicates that the mechanisms which regulate synchronization and desynchronization of the spontaneous electrical activity of the cerebral cortex are subserved by neuronal systems which are independent of those which subserve the transmission of sensory impulses to cortex via relay nuclei in the thalamus(9). The pharmacological data here presented suggest that such synchronizing and desynchronizing mechanisms are also independent of those mechanisms which subserve consciousness. Furthermore, the fact that pentobarbital, morphine, N-allylnormorphine, and atropine can produce very similar EEG changes but quite diverse changes in many aspects of behavior(10), suggests that the spontaneous

electrical activity of the cerebral cortex reflects the activity of neuronal systems which, in part at least, are independent of those neuronal systems that subserve behavior in general. The present studies cast no light on what the functions of such hypothesized independent neuronal systems might be, but other data indicate that they may be related to cerebral (not necessarily organismal) homeostasis(11).

Summary. 1. In unanesthetized, uncurarized dogs certain dose of morphine, N-allylnormorphine or atropine produce similar changes in the EEG, which are identical with, or resemble closely, the "burst-slow wave" patterns which occur in natural sleep or during pentobarbital anesthesia. 2. The synchronizing mechanisms which regulate the electroencephalogram are distinct from those which regulate the state of consciousness, although frequently, they are functionally interlocked. 3. The significance of these findings is discussed with reference to the possible functions of the spontaneous electrical activity of the cerebral cortex.

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Chemically Induced Degeneration of Chick Mesonephros.* (19346)

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Considerable attention has been given to the study of the effect of chemical agents on the development of chick embryos. Various workers have treated chick embryos with substances such as insulin(1-4), nicotinic acid (5), lead(6-9), and sucrose(10) and observed a variety of abnormal morphological types.

During recent experiments on the toxicity of various chemicals on chick embryos, it was observed that injection of tantalum hydroxide induced hemorrhage in the capillaries of the chorioallantois membranes and in the kidney (11). The kidney was enlarged and congested with blood and lymph. This observation prompted a histological study of kidneys of chick embryos of various ages and of day-old chicks following injection of various substances.

Materials and methods. The eggs for this investigation were from Rhode Island Red hens at the Agricultural Research Center, Beltsville, Md. The eggs were placed in forced-draft incubators on the day laid and incubated from 10 to 18 days before injection. The following substances were injected: DL-alanine, anthranilic acid, 3-hydroxyanthranilic acid, and salts of tantalum, thorium, and lanthanum (Table I). Distilled water was injected into the eggs employed as controls. The procedure was to inject distilled water or chemical in aqueous solution, not exceeding 0.5 ml in volume, into the allantoic cavity. The opening was then sealed with melted paraffin. Approximately 30 eggs were injected with various chemicals or distilled water at

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