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NICOTINE-INDUCED EEG AND BEHAVIORAL AROUSAL*†

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Summary—(−) Nicotine, in doses of 0·005–0·01 mg/kg given intravenously over 1 min, produced transient behavioral arousal and EEG activation in cats with chronic indwelling brain electrodes. The effects of nicotine were evident when the animals were in natural slow wave (deep) sleep as observed behaviorally and by the EEG with recordings from various cortical and subcortical sites. After nicotine administration, the animals were aroused for a few minutes and later became sleepy behaviorally and showed EEG slow waves. This was frequently followed by activated or fast wave sleep. This phenomenon was not evident following infusion of equal volumes of warmed saline solution. In equal doses (+) nicotine, nicotine-N-oxide and cotinine did not affect EEG and behavior in slow wave sleeping cats. However, equipressor doses of (+) nicotine (0·05 mg/kg) and nicotine-N-oxide (1·5 mg/kg) produced slight behavioral and EEG arousal in cats with slow wave sleep. Massive doses of cotinine (25 mg/kg) given intravenously were less effective than (−) nicotine in doses of 0·01 mg/kg. Equipressor doses of epinephrine (E) (0·002 mg/kg), phenylalanyl lysine vasopressin (50 milliunits/kg) and DMPP (0·005 mg/kg) produced weaker EEG activation and behavioral arousal than (−) nicotine.

Pretreatment with trimethidinium (2 mg/kg) prevented the cardiovascular effects of nicotine but did not alter greatly its behavioral arousal or EEG activation effects. On the other hand, mecamylamine (0·6 mg/kg) completely blocked the cardiovascular actions of nicotine as well as its effects on EEG and behavior. Larger doses of mecamylamine interfered with the natural sleep cycle of the cat. By use of such pharmacologic techniques it is concluded that the behavioral arousal and EEG desynchronizing effects of nicotine are due primarily to an action on the central nervous system rather than peripheral afferent stimulation or release of various neurohormones. However, these latter effects contribute to the total phenomenon produced by nicotine in intact animals.

INTRODUCTION

WIDESPREAD peripheral autonomic effects make it difficult to determine the actions of nicotine directly on the central nervous system. Investigators have reported that nicotine in subconvulsive doses produces EEG desynchronization in rabbits (LONGO *et al.*, 1954; SILVESTRINI, 1958; STUMPF, 1959; DUNLOP *et al.*, 1960; SILVETTE *et al.*, 1962). FLORIS *et al.* (1962) reported that large doses of nicotine produce EEG desynchronization or seizure discharges in mesencephalic transected rabbits. These investigators suggested that the origin of seizure discharges was in the hippocampus. Most studies on the central actions of nicotine have been made using fairly large doses. In contrast, KNAPP and DOMINO (1961–1963) showed that nicotine in doses of 0·01 to 0·02 mg/kg given intravenously to intracollicular, high pontine brainstem transected animals caused EEG desynchronization. EEG desynchronization was noted in acute brainstem transected rabbits,

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cats, dogs, and monkeys. These investigators demonstrated that nicotine-induced EEG desynchronization was due to an action on the central nervous system and not to stimulation of peripheral nerves or release of various neurohumoral substances.

EEG studies in acute animals provide little information regarding the behavioral consequences of nicotine. In intact preparations, EEG desynchronization recorded from neocortical structures can occur in at least two different behavioral states. One is that associated with behavorial alertness or wakefulness and the other with activated, 'paradoxical' or fast wave phase of sleep (DEMENT and KLEITMAN, 1957; DEMENT, 1958; YAMAMOTO, 1959; YAMAMOTO and KIDO, 1962; JOUVET, 1961). In order to ascertain if the EEG desynchronizing actions of nicotine are related to behavioral arousal or to the activated phase of sleep, it was necessary to study the effects of nicotine in intact animals with chronically implanted brain electrodes. Various pharmacologic treatments were used in an attempt to demonstrate whether the actions of nicotine observed in such intact preparations were due primarily to peripheral or central components. This manuscript describes some of the results obtained.

METHODS

Experiments were performed in fifteen cats with chronically indwelling brain electrodes utilizing a Latin square design for drug administration at 2-week intervals. The cats were prepared for placement of indwelling electrodes using modifications of conventional techniques. Adult cats of both sexes were used. Surgical preparation of the animals was under pentobarbital sodium anesthesia. Stainless steel wires of 0·22 mm in diameter (insulated except for tips of 0.5 mm) were used as the depth electrodes. Bipolar depth electrodes were inserted into the amygdala and hippocampus with the aid of the stereotaxic atlases by JASPER and AJMONE-MARSAN (1954) and SNIDER and NIEMER (1961). Physiologic recordings of injury discharges by insertion of electrodes were used for location of the hippocampus and olfactory-induced waves for the amygdala. Bipolar silver ball electrodes of 0·5 mm in diameter were applied as epidural surface electrodes to the somatosensory cortex. Additional depth electrodes were occasionally placed in the posterior hypothalamus and mesencephalic reticular formation. Each electrode was soldered to a Cannon plug and fixed on the scalp by means of dental cement. Silastic tubing of 0·7 mm in diameter was inserted into the right jugular vein with the other end fixed to a connector on top of the skull. The animals were allowed to recover for a 2-week period before being used for drug studies. In the meantime, they were given antibiotics prophylactically to reduce infection. At the time of the experiment, EMG of the posterior neck muscles, EKG and respiratory movements were recorded along with the brain waves on a Grass polygraph. The animals were each placed in a sound proof box with a one-way viewing window. Behavioral changes were observed and correlated with EEG activity. Crucial sequences of behavior were recorded on 16 mm movie film. In order to promote naturally occurring sleep, the animals were made as warm and comfortable as possible. With care and patience it was possible to observe all stages of natural sleep.

The following drugs were administered as an intravenous infusion in saline solution over a one-minute period: (−) nicotine base, (+) nicotine base*, (+) nicotine ditartrate†,

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cotinine base‡, cotinine fumarate¶, nicotine-N-oxide‡, epinephrine hydrochloride, phenylalanyl lysyl vasopressin**, DMPP††, trimethidinium bismethosulfate‡‡, mecamylamine hydrochloride¶¶, serotonin creatinine sulfate and histamine phosphate. All drugs were given in doses calculated as base. The actions of these drugs and various combinations on behavior were compared with each drug infused in a constant volume of 1·5 ml over a 1-min period. After completion of a series of experiments the positions of the electrodes were determined histologically by the iron deposition technique (see DOMINO, 1955 for details).

Blood pressures were measured in acute animals prepared under ether anesthesia and subsequently given a local anesthetic, 1 mg/kg of decamethonium and artificial respiration at 300 ml of air/kg/min.

RESULTS

A. *Lack of effects of saline solution infusion on the EEG and behavior of the sleeping cat*

The normal EEG of the cat was classified on the basis of six behavioral levels: excited, alert, resting, drowsy-light sleep, slow wave (deep) sleep and activated (fast wave) sleep. The EEG and physiologic counterparts of these behavioral states have been described previously (YAMAMOTO, 1959). Under the conditions of the present experiments utilizing a sound-proofed chamber in which the animals were made very comfortable, a naturally occurring deep sleeping state could be obtained easily. During this period the animals showed a stable sleep pattern both in their EEG and behavior. High voltage slow waves of 1–3 c/s and spindle bursts in the somatosensory cortex, high voltage slow waves and spike-like discharges in the amygdala, and high voltage fast waves in the hippocampus were routinely observed. In addition, a minimal discharge of the EMG of the neck muscles and rhythmic respiration were recorded in such a sleeping state. These EEG and behavioral manifestations of slow wave natural sleep were easily reversed by direct high frequency electrical stimulation of the mesencephalic reticular formation, posterior hypothalamus or by external stimuli such as a loud noise. All fifteen cats studied illustrated that the EEG and behavioral manifestations of slow wave sleep were not altered by an intravenous infusion of 1·5 ml of physiologic saline solution warmed to body temperature. If cold solutions and/or rapid injection of large volumes were given, the animals could be aroused from slow wave sleep. Therefore, it was particularly important that the solutions administered were warmed and given slowly.

B. *Effects of nicotine*

1. *Single doses.* The intravenous infusion of nicotine (0·005–0·01 mg/kg) over a 1-min period in slow wave sleeping cats produced three distinct electroencephalographic and behavioral responses. These were: (a) electroencephalographic and behavioral

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††Kindly provided by Dr. R. F. PARCELL, Parke Davis Research Laboratories, Ann Arbor, Michigan.

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¶¶Kindly provided by Dr. C. STONE, Merck Institute for Therapeutic Research, Merck and Company, Inc., West Point, Pennsylvania.

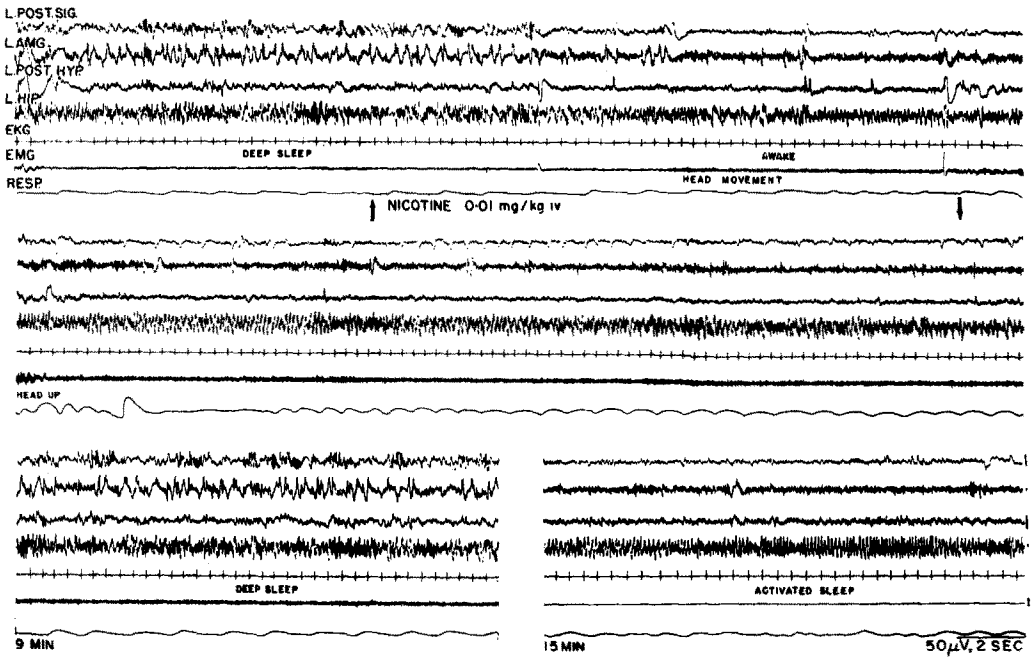

FIG. 1. EEG effects of nicotine in the cat with chronic indwelling brain electrodes. Nicotine was given between the two arrows during slow wave (deep) sleep. Note changes in EEG activity and behavior. The upper two records are continuous. The lower are 9 and 15 min later. All recordings were bipolar. Symbols: L. POST. SIG.—left posterior sigmoid gyrus; AMG.—amygdala; POST. HYP.—posterior hypothalamus; HIP.—hippocampus; EKG.—electrocardiogram; EMG—electromyograph of neck muscles; RESP.—thoracic respiration. Time bases and voltage calibrations are as indicated. These symbols when used apply to all subsequent figures.

arousal immediately following the nicotine injections, (b) a few min later, a subsequent stage of slow wave sleep and, (c) the occurrence of activated sleep. The EEG manifestations of these three stages in one animal are illustrated in Fig. 1. Bipolar EEG activity was recorded from the posterior sigmoid gyrus, amygdala, posterior hypothalamus and hippocampus as well as the EKG, EMG of neck muscles and thoracic respiration. In the period immediately prior to nicotine injection, the cat was in a slow wave sleeping state. This was evidenced by the occurrence of EEG slow waves and spindle bursts in the posterior sigmoid gyrus, high voltage slow waves and spikes in the amygdala, and hippocampal fast activity. In addition, respiration was slow and regular. During this state nicotine (0·01 mg/kg) was given over a one-minute period. After approximately half of the injection, the cat showed evidence of head movement and behavioral arousal. This was preceded a few seconds earlier by low voltage, fast wave activity and typical *theta* rhythm in the hippocampus. The mean duration $\pm$ S.E. for the EEG and behavioral manifestations of arousal was 2·93 $\pm$ 0·52 min. Subsequently, the cat began to relax and returned to his previous slow wave sleeping state. It was noted that frequently this state was much deeper and more persistent than prior to nicotine administration. A portion of the EEG tracing taken 9 min after intravenous nicotine infusion is illustrated in Fig. 1. At this time the animal showed more evident EEG manifestations of the slow wave sleeping state. Approximately 15 min after the intravenous infusion of nicotine the cat lapsed into activated

sleep as evidenced by low voltage, fast wave activity in the posterior sigmoid gyrus, amygdala, and posterior hypothalamus. At the same time the hippocampus showed marked *theta* activity. The EMG of the neck muscles completely disappeared, respiration and heart rate were irregularly increased.

It is of considerable interest that the EEG manifestations of arousal following nicotine nfusion usually were first evident in the hippocampus. Neocortical and amygdaloid EEG activation occurred a few seconds after the initiation of hippocampal *theta* rhythm. Following nicotine injection high voltage, rhythmic *theta* waves were obtained. This is in contrast to similar activity occurring in this structure in the cat which arouses spontaneously. Under these circumstances the amplitude of the hippocampal *theta* rhythm as well as its sinusoidal form is not as marked. The marked hippocampal EEG activity following nicotine administration suggests a strong activation of this system. Neocortical and amygdaloid EEG activation usually lagged behind the induced hippocampal *theta* activity. The high voltage rhythmic bursts in the amygdala, which are frequently seen in excited cats, were observed following nicotine administration. It is of interest that behavioral arousal due to nicotine usually was evident a few sec after EEG activation. The behavioral manifestations of arousal following nicotine administration included opening of the eyelids, sniffing, picking up and turning the head and occasionally standing. At no time was there evidence of nausea and emesis. A marked increase in respiratory exchange, EMG activity as well as an increase in blood pressure and alteration in heart rate were also noted. The physiologic and EEG manifestations 20 min prior to as well as 20 min after nicotine injection for the same cat as in Fig. 1 are plotted graphically in Fig. 2.

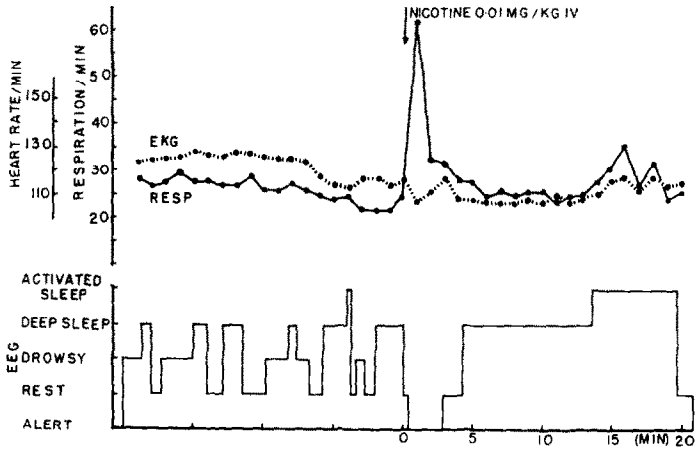

FIG. 2. Graph of the effects of nicotine.

It can be seen that prior to nicotine infusion the cat's EEG patterns fluctuated between the resting state, drowsiness, and slow wave (deep) sleep. Occasionally a brief period of activated sleep was observed during which EKG and respiration showed minor fluctuations. Following nicotine infusion intravenously in a dose of 0·01 mg/kg, marked respiratory stimulation, bradycardia and evidence of EEG activation and behavioral alertness was obtained. Within 5 min this was followed by a more consistent pattern of deep sleep and subsequently (15–30 min) by a stage of activated sleep.

In an attempt to quantify some of these findings, the percentage of time spent in different EEG stages of sleep were analyzed 5 min prior to as well as 5 min after nicotine injection. The data for a series of 9 animals given nicotine as well as other treatments are summarized in the bar graph illustrated in Fig. 3. Before any drugs were given the percentage of time

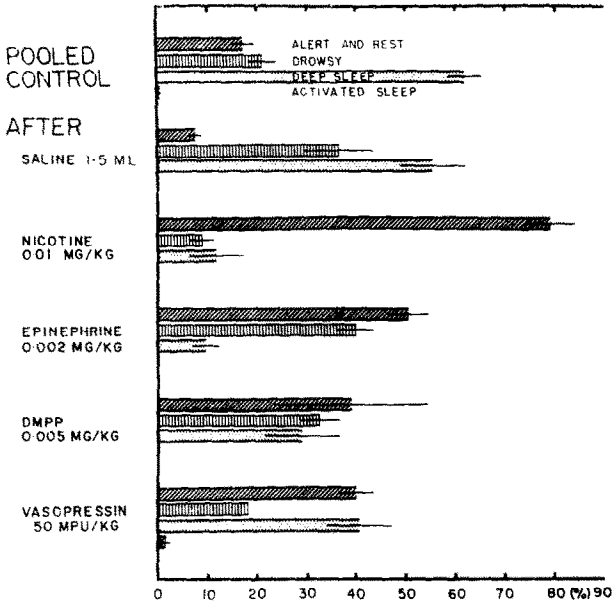

FIG. 3. Mean percent time ± S.E. in different EEG states 5 min before and after various treatments.

spent in deep sleep was much greater (62·1 per cent) than in the other states simply because the time of drug injection was determined by the presence of deep sleep. After saline solution injection the animals continued in the drowsy and/or deep sleeping state and thus showed a minimum of wakefulness. In contrast, the intravenous injection of nicotine caused a marked increase in EEG and behavioral arousal which was highly significant ($P < 0{\cdot}001$). This was associated with a decrease in drowsiness and deep sleep ($P < 0{\cdot}001$).

2. *Effects of repeated doses.* Marked tachyphylaxis to the blood pressure increase, stimulation of respiration as well as behavioral and electroencephalographic arousal were obtained if nicotine was administered repeatedly at time intervals of less than 30 min. When nicotine was given in a dose of 0·005 mg/kg at a one-hr interval reproducible EEG and behavioral arousal were obtained. However, marked tachyphylaxis to the respiratory stimulant effects of nicotine were still evident (see Fig. 4). If nicotine was administered at a two-hr interval no evidence of tachyphylaxis was obtained to any of the physiologic parameters measured (Fig. 5).

C. Effects of various derivatives of nicotine on EEG and behavior

The effects of (+) nicotine, nicotine-N-oxide, and cotinine were compared with (−) nicotine on the basis of equal dosage (0.01 mg/kg) and also on the basis of doses producing

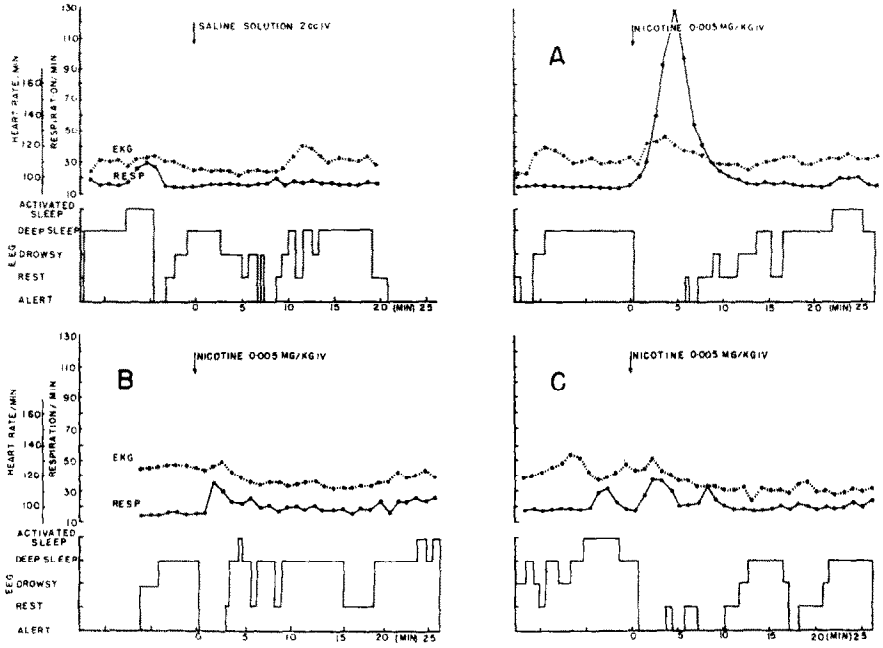

FIG. 4. Graph of the effects of nicotine given at 1-hr intervals. Note tachyphylaxis of respiratory stimulation but not EEG arousal to repeated hourly injections of nicotine.

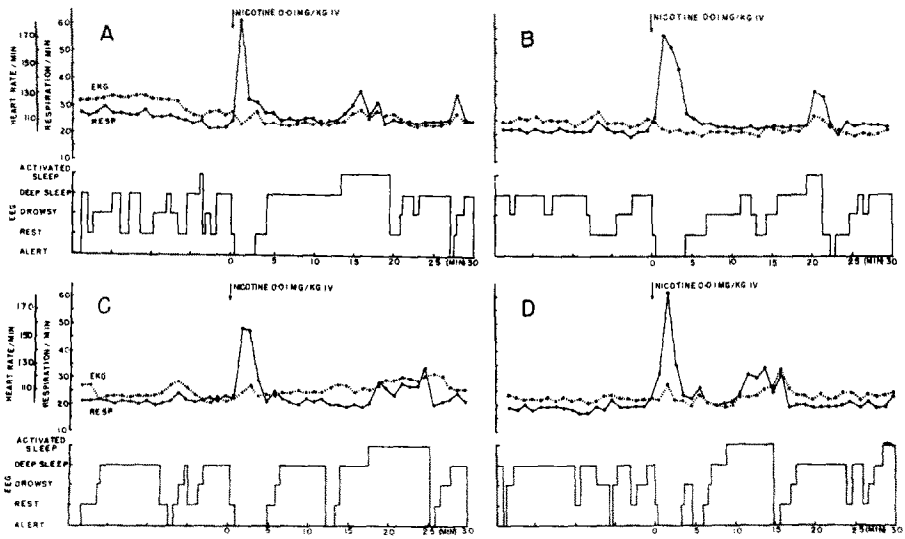

FIG. 5. Graph of the effects of nicotine given at 2-hr intervals. Note lack of tachyphylaxis.

an equipressor response. The increase in blood pressure usually obtained with (−) nicotine was in the order of 60–70 mm mean Hg pressure. In doses of 0·01 mg/kg given intravenously (+) nicotine did not produce any change in blood pressure nor was there

alteration of the EEG or gross behavioral pattern in deeply sleeping cats. On the other hand, equipressor doses of (+) nicotine (0·05 mg/kg) produced transient EEG activation and behavioral arousal. The mean duration ± S.E. of (+) nicotine EEG activation was 1·34 ± 0·25 min. Behavioral arousal induced by (+) nicotine was much weaker than that of (−) nicotine. In addition, in equipressor amounts, respiratory stimulation produced by the (+) isomer was not as marked. As was observed after (−) nicotine slow wave and activated sleep followed the initial arousal phase.

In doses of 0·01 mg/kg nicotine-N-oxide did not produce any increase in blood pressure or affect the EEG and behavior of deeply sleeping cats. On the other hand, equipressor doses of nicotine-N-oxide (1·5 mg/kg) showed transient EEG activation and behavioral arousal from the deeply sleeping state. The mean duration ± S.E. of nicotine-N-oxide induced EEG activation was 0·95 ± 0·22 min when given in doses of 1·5 mg/kg intravenously. In contrast to the effects of (−) nicotine respiratory stimulation was not as evident with nicotine-N-oxide.

Doses of 0·01 mg/kg of cotinine did not affect the EEG or behavior of deeply sleeping cats. On the other hand, doses of 10 mg/kg given intravenously showed transient EEG activation and behavioral arousal although such doses did not produce any change in blood pressure. Larger doses of cotinine in the order of 25 mg/kg intravenously produced a slight fall in blood pressure. Such large doses of cotinine also produced brief episodes of EEG activation and behavioral arousal (see Fig. 6). The mean duration ± S.E. of cotinine-induced EEG activation was 1·53 ± 0·24 min.

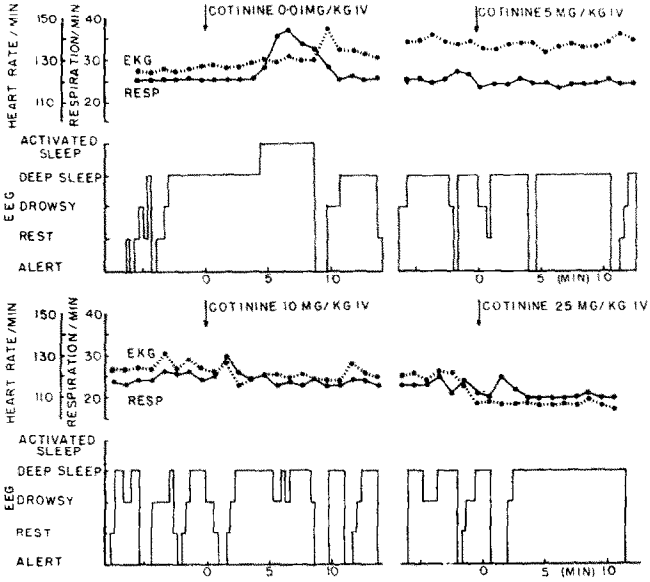

Fig. 6. Graph of the effects of increasing doses of cotinine.

The quaternary ganglionic stimulant, DMPP, was used to mimic the peripheral actions of nicotine. Doses of 0·005 mg/kg of DMPP caused pressor responses equivalent to those produced by 0·01 mg/kg of (−) nicotine given intravenously. In such equipressor doses

DMPP produced minimal EEG activation and behavioral arousal in deeply sleeping cats. Interestingly, respiratory stimulation was minimal. The mean duration ± S.E. of DMPP-induced EEG activation was 1·39 ± 0·48 min.

D. *Effects of some neurohumoral agents released by nicotine on EEG and behavior*

Nicotine is known to release various neurohumoral substances including E, vasopressin, serotonin, and histamine. The effects of these substances in maximal amounts which could be reasonably released by nicotine were tested on behavioral and EEG arousal in deeply sleeping cats.

1. *Epinephrine (E).* Doses of 0·002 mg/kg of E given intravenously produced equivalent pressor responses to 0·01 mg/kg of (−) nicotine. These amounts of E produced behavioral and EEG arousal in deeply sleeping cats. The mean duration ± S.E. of EEG activation following E injection was 1·95 ± 0·19 min. In contrast to the effects of equipressor doses of nicotine, behavioral arousal induced by E was limited to the animals opening their eyes or picking up their heads. Respiratory stimulation was not as marked as with nicotine itself. Thus, the overall effects of equipressor doses of E were far weaker than those obtained with nicotine.

2. *Vasopressin.* In a dose of 50 milliunits/kg synthetic phenylalanyl lysyl vasopressin produced a pressor response comparable to 0·01 mg/kg of (−) nicotine. Following the administration of vasopressin in the blood pressure continued to be elevated for approximately 6–8 min. During the pressor response a marked bradycardia was observed. EEG activation was obtained following intravenous injection of vasopressin, but spindle bursts reappeared while the blood pressure was still maintained. Vasopressin did not produce as dramatic a behavioral arousal compared to E and especially (−) nicotine. The mean duration ± S.E. of vasopressin-induced EEG activation was 1·63 ± 0·18 min. A particularly important finding following vasopressin administration was the marked percentage increase in the appearance of activated sleep.

3. *Serotonin.* Doses of 0·022 mg/kg of serotonin produced approximately a 39 mm Hg fall in blood pressure when given intravenously. The EKG showed an initial bradycardia and a secondary tachycardia. Respiratory stimulation was evident. During this time the cat showed EEG activation and behavioral arousal. The mean duration ± S.E. of serotonin-induced EEG activation in five cats was 4·20 ± 0·42 min. Subsequently, the cats fell into a light sleep behaviorally and electroencephalographically. Slow wave sleep was seen after the administration of serotonin but activated sleep was never seen within 25 min after injection.

4. *Histamine.* Doses of 0·001 mg/kg of histamine produced only a tachycardia in cats in slow wave sleep but did not alter the EEG and behavioral state. Within 5 min after injection the cats showed even slower wave patterns. Doses of 0·002 mg/kg of histamine produced a more marked tachycardia and hypotension of approximately 45 mm Hg. This was associated with a mean ± S.E. EEG and behavioral arousal of 2·44 ± 0·66 min in seven cats. Subsequently, the cats lapsed into slow wave sleep. Activated sleep was not seen within 20 min after histamine, in contrast to nicotine and vasopressin injections.

E. *Modification of nicotine-induced EEG and behavioral arousal by various ganglionic blocking agents*

In an attempt to dissociate the peripheral and central contributions of (−) nicotine in causing EEG and behavioral arousal two different ganglionic blocking agents were used as pretreatments. One was the quaternary ganglionic blocking agent, trimethidinium. Because of the permanent positive charges on this compound, it would not be expected to penetrate the blood-brain barrier easily. The other ganglionic blocking agent used was the secondary amine, mecamylamine. This compound has been shown previously to block the central as well as peripheral actions of nicotine (KNAPP and DOMINO, 1962). The intravenous administration of trimethidinium (2 mg/kg) did not alter the EEG and gross behavior of deeply sleeping cats. There was, however, some increase in heart rate as would be expected from a ganglionic blocking drug. Pretreatment with trimethidinium completely blocked the blood pressure elevating and bradycardic actions of nicotine. On the other hand, respiratory stimulation was still evident although somewhat reduced from control. Similarly, EEG activation and behavioral arousal produced by nicotine in the deeply sleeping cat still appeared as before trimethidinium pretreatment. The data obtained in one of the cats are plotted graphically in Fig. 7. Prior to trimethidinium

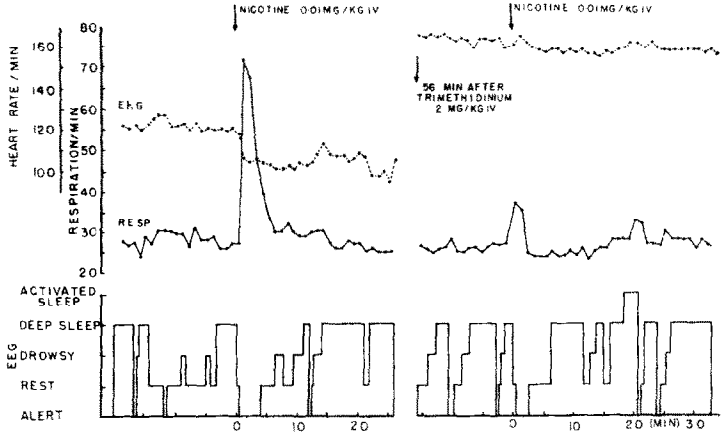

FIG. 7. Modification of the effects of nicotine by trimethidinium. Note that the respiratory stimulant and EEG arousal effects are only partially blocked.

administration, nicotine produced the characteristic physiologic alterations including EEG activation as illustrated. After trimethidinium administration heart rate was markedly increased. Nicotine infusion in a dose of 0·01 mg/kg produced no subsequent alteration in heart rate, although respiratory stimulation was reduced. It was still possible to obtain EEG and behavioral arousal but this was not as intense as in the control situation. The mean duration of EEG and behavioral arousal $\pm$ S.E. to nicotine after trimethidinium was 2·05 $\pm$ 0·29 min in comparison to nicotine alone which was 2·93 $\pm$ 0·52 min. In contrast to the lack of effects of trimethidinium, doses of mecamylamine in the order of 2 mg/kg given intravenously prevented naturally occurring sleep. After such doses of mecamylamine, the animals would lay on their sides but did not show characteristic deep

sleep. Instead the electroencephalographic pattern was one of desynchronization. Therefore the effects of low doses of mecamylamine in modifying nicotine actions were determined. It was found that doses of 0·6 mg/kg of mecamylamine did not affect the EEG or gross behavior of deeply sleeping cats. Therefore this was used for premedication. As might be expected, following mecamylamine administration all of the effects of 0·01 mg/kg of nicotine were blocked. These are graphically illustrated in one typical experiment in Fig. 8. Prior to mecamylamine administration, a dose of 0·01 mg/kg of nicotine produced the usual EEG and behavioral alterations as well as respiratory stimulation. On the other hand, after 0·6 mg/kg of mecamylamine, nicotine had no significant effect.

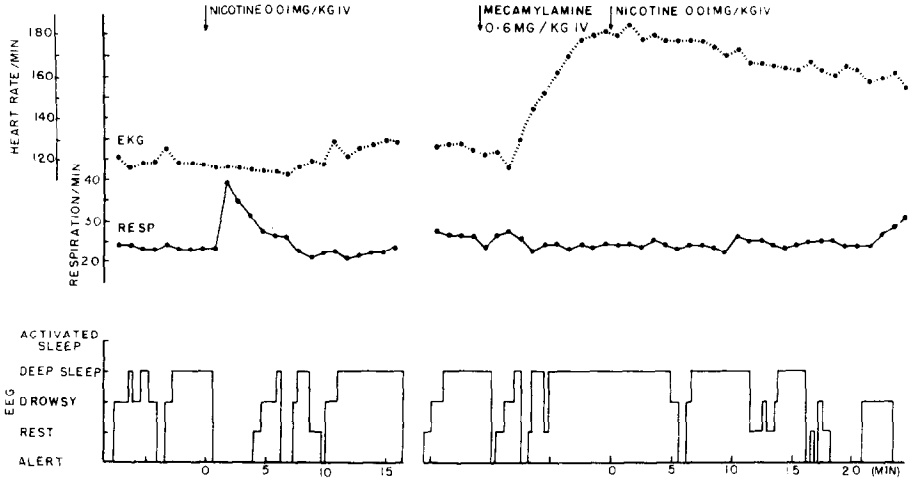

FIG. 8. Modification of the effects of nicotine by mecamylamine. Note that all of the effects of nicotine are blocked.

DISCUSSION

The purpose of this research was to test two alternate hypotheses: (a) that the EEG desynchronizing effects of nicotine were accompanied by behavioral arousal or (b) that such EEG desynchronization represented a stage of activated sleep. The use of cats with chronic indwelling brain electrodes provided a means of testing these hypotheses. Such animals have a relatively normal sleep cycle and it was possible to record easily naturally occurring deep or slow wave sleep. In such a deeply sleeping state, the administration of nicotine in low doses intravenously caused prompt EEG activation and behavioral arousal. From these experiments it can be concluded that the initial effects of nicotine are to produce a behavioral wake up effect. The results of these experiments are in agreement with the data obtained in acute animals by KNAPP and DOMINO (1961–1963). The latter investigators showed that nicotine produced EEG desynchronization in intracollicular midpontine brainstem transected preparations. Inasmuch as nicotine injection altered the electrical activity of the isolated reticular slab, a pontomesencephalic site of action for the EEG activating effect of nicotine was proposed. In contrast FLORIS *et al.* (1962) showed that large doses of nicotine continued to produce activation or seizures following mesencephalic transection in rabbits. They pointed out that EEG convulsive activity was more prolonged and intense in the hippocampus rather than neocortex and suggested that such discharges originated in the hippocampus. Their findings also

are reminiscent of earlier work by DUNLOP *et al.* (1960) who reported that of three regions (hippocampus, neocortex and reticular formation) examined in rabbits the hippocampus had the lowest threshold for nicotine-induced seizure discharges. Furthermore, a few seconds after nicotine injection there was often observed an increased firing of neuronal units that were synchronous with the hippocampal EEG *theta* activity. These phenomena were obtained with relatively large doses of nicotine so that there is some question as to whether they are applicable to low doses of nicotine that produce EEG activation alone. However, in the present study it was found that EEG desynchronization in the neocortex usually followed by several sec the appearance of hippocampal *theta* activity. At the same time 35–45 c/s burst discharges were recorded in the amygdala. This was accompanied by behavioral sniffing. The appearance of hippocampal *theta* rhythm before characteristic neocortical EEG desynchronization suggests that the hippocampus is exquisitely sensitive to the actions of even small doses of nicotine.

The possibility that nicotine-induced EEG desynchronization is secondary to stimulation of respiratory medullary areas or to blood pressure elevation was ruled out by several different experiments. Repeated administration of nicotine at one-hour intervals produced tachyphylaxis to the respiratory stimulant effects although EEG activation still occurred. This clearly dissociates the site of action of EEG activation of nicotine from its site for respiratory stimulation. EEG activation and behavioral arousal induced by nicotine were obtained after complete blockade of its cardiovascular effects following tremethidinium pretreatment. Similar results were obtained in midpontine transected animals by KNAPP and DOMINO (1962). The peripherally acting ganglionic stimulant DMPP elevated blood pressure to the same extent as nicotine. However, in equipressor doses DMPP produced much weaker EEG and behavioral arousal than nicotine itself.

It might be argued that the EEG and behavioral arousal effects of nicotine were due primarily to the peripheral release of E or vasopressin. However, equipressor doses of E (0·002 mg/kg) or synthetic vasopressin (50 milliunits/kg) produced much weaker EEG and behavioral arousal than nicotine (0·01 mg/kg). Of course a study of the effects of naturally occurring arginine vasopressin are indicated. Nevertheless, these results strongly suggest a primary direct action of nicotine on the central nervous system. These results are also consistent with the findings of KNAPP and DOMINO (1962) who showed that in brainstem transected animals, EEG activation effects of nicotine were not mediated by epinephrine or vasopressin release. Inasmuch as nicotine is thought to release serotonin as well as histamine, it was possible that these substances could produce the initial EEG and behavioral arousal effects observed. However, relatively large doses of these substances were necessary to produce an arousal pattern which still was relatively weak so it seems unlikely that release of these substances accounts for the nicotine arousal phenomenon.

Could the EEG activating effects of nicotine be due to its metabolic conversion to another substance? The best known metabolite of nicotine is (—) cotinine. In these experiments equal amounts of cotinine had no effect whatsoever. Relatively massive doses of cotinine were necessary to produce EEG and behavioral arousal. These were most consistently obtained with a total dose of 25 mg/kg given intravenously. Under these circumstances a very transient arousal was evident accompanied by slight fall in blood pressure. No significant respiratory stimulation was obtained. These cardiovascular findings agree with the results of BORZELLECA *et al.* (1962) who showed that 50 mg/kg of (—) cotinine caused a transient hypotension without respiratory changes in the pentobarbital anesthetized dog. It is of interest that cotinine does not penetrate into the central

nervous system as easily as nicotine (BOWMAN *et al.*, 1964; HANSSON and SCHMITERLOW, 1962). In contrast to the widespread distribution of C^{14}-labeled nicotine in the mouse brain, tritium-labeled cotinine does not concentrate in the brain nearly as well. Other derivatives of nicotine such as nicotine-N-oxide and (+) nicotine were shown to be much less effective than nicotine in producing EEG desynchronization and behavioral arousal.

One of the most intriguing effects of nicotine administration to the deeply sleeping cat was that following a brief period of behavioral arousal and EEG desynchronization, the animals lapsed into a deeper state of naturally occurring sleep and a subsequent phase of activated sleep. JOUVET (1961) suggested that the induction of activated sleep depends upon neurotransmitter substances of a cholinergic nature. Furthermore, PEON (1964) used cats with chronic indwelling brain cannulae to show that slow wave as well as activated sleep could be obtained by the injection of cholinergic substances directly into the brain in the area of the limbic midbrain circuit of NAUTA (1958). SEKUL and HOLLAND (1961) have pointed out that nicotine has an obvious structural chemical resemblance to acetylcholine in that the spacial distribution of charges in the molecule are somewhat similar. Inasmuch as historically nicotine has been used to demonstrate the 'nicotinic' sites of action of acetylcholine, it seems reasonable to suggest that the actions of low doses of nicotine are probably related to stimulation of cholinergic receptors. This suggestion is further strengthened by the use of agents which produce selective nicotinic blockade. For example, ganglionic blocking agents which block the nicotinic actions of acetylcholine are also extremely effective in blocking the actions of nicotine. It has been shown in these series of experiments that a ganglionic blocking agent (mecamylamine) which can penetrate the bloodbrain barrier blocks both the central as well as peripheral actions of nicotine. This is in contrast to the effects of trimethidinium which only blocked the peripheral actions of nicotine but not its central effects, presumably because trimethidinium cannot penetrate into the brain in sufficient dosage to block nicotinic receptors.

It has been shown that following the brief period of nicotine-induced behavioral arousal a subsequent period of enhanced slow wave sleep and activated sleep are evident. Some of these effects, particularly the facilitation of activated sleep, was produced by synthetic phenylalanyl lysyl vasopressin. Inasmuch as nicotine is known to release large quantities of natural occurring argininine vasopressin, it appears that this neurohormone may be involved in the enhancement of activated sleep following nicotine. Further research along these lines is now being carried out.

Résumé—Chez le chat, porteur d'électrodes cérébrales à demeure, la nicotine (−) à des doses de 0·005–0·01 mg/kg en administration intraveineuse pendant 1 min, entraîne un éveil comportemental et une activation EEG transitoires. Les effets de la nicotine sont particulièrement évidents chez le chat en état de sommeil natural, profond (avec ondes lentes) ainsi qu'il a été observé tant du point de vue du comportement que de l'EEG dérivé de différentes aires corticales et sous-corticales. Après administration de nicotine, l'animal se réveille quelques minutes puis devient comportementalement endormi avec présence d'ondes lentes. Cet état se poursuit fréquemment par un sommeil "activé" avec des ondes rapides. Ce phénomène n'est pas évident à la suite d'une infusion de volumes égaux de solution saline chauffée. A dose égale, la nicotine (+), oxyde de nicotine-N, et la coltinine sont sans effet EEG ou comportemental sur le chat en sommeil à ondes lentes. Cependant, des doses équipressives de nicotine (+) 0·05 mg/kg et d'oxyde de nicotine-N, 1·5 mg/kg, déterminent un léger éveil comportemental et EEG chez l'animal en état de sommeil avec ondes lentes.

Une posologie massive de cotinine (25 mg/kg), par voie i.v., se révèle moins efficace qu'une dose de 0·01 mg/kg de nicotine (−). Des doses équipressives d'épinéphrine (E) 0.002 mg/kg, de vasopressine (50 mU/kg) et de DMPP (0·0005 mg/kg) engendrent une activation EEG et un éveil comportemental moins accentué que la nicotine (−).

Un prétraitement par le trimethidinium (2 mg/kg) prévient les effets cardiovasculaires de la nicotine sans altérer significativement ses effets d'éveil comportemental ou d'activation EEG. D'autre part, la mécamylamine (0·6 mg/kg) entraîne un blocage complet des effets cardiovasculaires de la nicotine de même que son action sur l'EEG et le comportement.

Des doses supérieures de mécamylamine interfèrent avec le cycle naturel de sommeil du chat. L'utilisation de ces techniques pharmacologiques permettent de conclure à des effets d'éveil comportemental et de désynchronisation EEG de la nicotine qui seraient dus essentiellement à une action sur le système nerveux central plutôt qu'à une stimulation périphérique afférente ou à une libération de différentes neurohormones. Ces derniers effets contribuent cependant au phénomène global induit par la nicotine chez l'animal intact.

Zusammenfassung—(−) Nikotin verursachte in Dosen von 0·005–0·01 mg/kg, die ueber eine Minute intravenoes gegeben wurden, voruebergehendes Aufwachen und EEG Aktivierung in Katzen mit Dauer-Hirnelektroden. Der Nikotineffekt war erkennbar, wenn sich die Tiere in natuerlichem, durch langsame Hirnwellentaetigkeit gekennzeichnetem Schlaf befanden. Das Aufwachen wurde auf Grund der Verhaltensweise des Tieres und mit Hilfe von corticalen und subcorticalen Elektroden registriert. Nach Verabfolgung de Nikotin erwachten die Tiere fuer einige Minuten, wurden dann wieder schlaefrig mit im EEG erkennbarer langsamer Hirnwellen-Taetigkeit. Dies war oft gefolgt von aktiviertem, durch schnelle Hirnwellentaetigkeit gekennzeichnetem Schlaf. Dieses Phenomen war nicht erkennbar, wenn gleiche Mengen warmer Kochsalzloesung verabfolgt wurden. Gleiche Dosen von (+) Nikotin, Nikotin-N-Oxyd und Cotinine hatten keinen Effekt auf das EEG und das Verhalten von Katzen, die sich im von langsamer Hirnwellen-Taetigkeit gekennzeichneten Schlaf befanden. Dosen von (+) Nikotin (0·05 mg/kg) und Nikotin-N-Oxyd (1·5 mg/kg) mit gleichem Pressoreffekt bewirkten jedoch leichtes Aufwachen, gekennzeichnet durch das Verhalten des Tieres und durch das langsame Hirnwellentaetigkeit zeigende EEG. Hohe Dosen von Cotinine (25 mg/kg), intravenoes verabfolgt, waren weniger wirkungsvoll as (−) Nikotin in Dosen von 0·01 mg/kg. Dosen von Epinephrine (0·002 mg/kg), Vasopressin (50 Millieinheiten/kg) und DMPP (0·005 mg/kg) mit gleichem Pressoreffekt verursachten schwaechere EEG Aktivierung und geringeren Aufwacheffekt als (−) Nikotin.

Vorbehandlung mit Trimethidinium (2 mg/kg) verhinderte das Auftreten von dendurch cardiovaskulaeren Effekten Nikotin erzeugten, aenderte jedoch wenig den Aufwacheffekt oder die Wirkung auf die EEG Aktivierung. Dagegen fuehrte Mecamylamine (0·6 mg/kg) zu einem kompletten Block der durch Nikotin verursachten cardiovaskulaeren Effekte und des Aufwacheffekts. Hoehere Dosen von Mecamylamine stoerten den natuerlichen Schlafzyklus der Katze. Die Anwendung einer solchen pharmakologischen Methode laesst den Schluss zu, dass die im Aufwachverhalten und im EEG registrierte desynchronisierende Wirkung des Nikotin in erster Linie der Erfolg eines Effektes auf das Zentralnervensystem ist und weniger mit einer peripheren afferenten Reizung oder mit der Wirkung verschiedener Nervenhormone im Zusammenhang steht. Letztere tragen jedoch zu dem durch Nikotin im intakten Tier verursachten Phenomen bei.

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