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Effects of nicotine on electrocortical activity and acetylcholine release from the cat cerebral cortex

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1. The effects of small amounts of nicotine on electrocortical activity and central acetylcholine (ACh) release have been studied on anaesthetized cats.

2. The most common effect of nicotine given intravenously in a dose of 2 μg/kg every 30 sec for 20 min was to cause desynchronization of the electrocorticogram, indicating cortical activation, and an increase in the release of cortical ACh.

3. A larger dose given less frequently (4 μg/kg every min for 20 min) caused, in some experiments, an increase and in others a decrease in cortical activity. Such changes were accompanied respectively by an increase or decrease in cortical ACh output.

4. The amounts of nicotine that affected the electrocorticogram and ACh release are probably similar to those absorbed by the cigarette smoker who inhales.

5. The effects of nicotine on the electrocorticogram were transient, but the effects on ACh were prolonged. This suggests that at least two pathways are involved in the nicotine response.

Nicotine causes electroencephalogram (e.e.g.) activation and behavioural arousal in cats and other species when given as a single intravenous injection (Knapp & Domino, 1962) or as a short infusion (Yamamoto & Domino, 1965). It should, however, be possible to mimic more closely the nicotine intake of a smoker by the repeated intravenous administration of small amounts of nicotine (Armitage, 1965). The exact dose and its rate of injection may be critical because a small change in either parameter can alter the effect on behaviour (Armitage, Hall & Morrison, 1968). In the present experiments the effects on electrocortical activity of repeatedly injecting small amounts of nicotine (1–4 μg/kg) have been investigated. Concurrent effects on the spontaneous release of acetylcholine (ACh) from the cerebral cortex (MacIntosh & Oborin, 1953 ; Mitchell, 1963) have also been studied, because it is believed that nicotine may act at some central sites to release ACh (Armitage & Hall, 1967 ; Armitage, Milton & Morrison, 1966 ; Morrison, 1968).

Methods

Experiments were performed on forty male and five female cats, weighing 2.7–3.6 kg. Anaesthesia was obtained by a single intraperitoneal injection of diallyl barbituric acid and urethane ("Dial," Ciba Ltd. 0.7 ml./kg, or 0.8 ml./kg of a similar product prepared in our laboratories), which was sufficient to produce surgical anaesthesia and a steady electrocorticogram for more than 6 hr. Blood pressure was recorded from a femoral artery with a Bell and Howell pressure transducer coupled to a Devices polygraph. The femoral vein was cannulated to allow the intravenous injection of nicotine or 0.9% NaCl solution. Each nicotine injection was contained in a volume of 0.1 ml. and washed in with 0.5 ml. of a 0.9% NaCl solution. A tracheal cannula was inserted and, with the cat lying on its abdomen, the head was positioned in a stereotaxic instrument.

Acetylcholine was recovered from the left parietal cortex in the region of the suprasylvian gyrus using the cup collecting technique described by Mitchell (1963). The release of ACh was expressed in ng/20 min/cm^2 cortex. The cup was refilled every 20 min with Ringer–Locke solution (NaCl 9.0, KCl 0.42, $CaCl_2$ 0.24, $NaHCO_3$ 0.2, glucose 2.0 g/l.), containing physostigmine 20 μg/ml. Solutions placed in the cup were pre-warmed to 37° C. The samples removed from the cup were assayed for ACh on the blood pressure of the eviscerated cat treated with physostigmine (Brown & Feldberg, 1936) or on the dorsal muscle of the leech sensitized with physostigmine 20 μg/ml. The assay procedure for the leech was similar to that described by Collier & Mitchell (1966). To determine the accuracy of the assay procedure, unknown solutions of ACh were assayed by matching as closely as possible the contraction of leech muscle or fall in blood pressure with the response caused by known amounts or concentrations of ACh. The error rarely exceeded 15%. The biological activity of the samples was considered to be due to ACh for the following reasons: (1) the effect on blood pressure was abolished by hyoscine; (2) incubation of the samples and of ACh with one drop of N-NaOH at 37° C for 15 min destroyed the biological activity; (3) parallel assays on the dorsal muscle of the leech and on cat blood pressure preparations gave similar values in terms of ACh. Szerb (1963) has previously shown that this ACh-like activity cannot be distinguished from ACh by paper chromatography.

The percentage change in ACh output was obtained by comparison of the average output for three samples before, and three samples after, the injection of nicotine. The resting output of ACh was taken as the mean of the three samples preceding administration of saline or drug.

Electrocortical activity was recorded on a Kaiser TR60 electroencephalograph with bipolar silver ball electrodes placed on the suprasylvian gyrus within the cup and also from a similar position on the contralateral hemisphere. Body temperature was maintained at 37° with an Electrophysiological Instruments homeothermic blanket.

In some experiments the Ringer–Locke solution within the cup contained nicotine thus allowing its direct application to the cortical surface. The contents of the cup were later assayed for nicotine on the blood pressure of the same cat.

The following drugs were used: atropine sulphate, acetylcholine perchlorate, nicotine hydrogen tartrate and physostigmine sulphate. Doses are expressed in terms of the base.

Results

Spontaneous release of ACh

There was considerable variation in ACh output from the cerebral cortex of different animals which is in agreement with the findings of other authors (Collier & Mitchell, 1966). The mean spontaneous release of ACh from the cerebral cortex in forty-five experiments was 7.4 ± 1.3 ng/20 min/cm^2, the highest output in any one experiment being 36.1 ng/20 min/cm^2 and the lowest <1 ng/20 min/cm^2. The reason for such a wide range of ACh outputs can probably be attributed to the absence of atropine in the cortical cup. Other workers (Mitchell, 1963; Kanai & Szerb, 1965) have included atropine in the bathing solution in order to increase ACh output from the cortical surface. We therefore studied effects of atropine in two selected experiments in which the average value for the resting output of ACh exceeded 10 ng/20 min/cm^2 and in two experiments in which the resting output of ACh was less than 5 ng/20 min/cm^2. The results of these experiments are shown in Fig. 1. When the resting output was high, the addition of atropine to the bathing fluid in ascending concentrations of 100, 200 and 400 ng/ml. increased only slightly the spontaneous release of ACh from the cortical surface. When the resting output was low, however, atropine considerably increased the resting release of ACh to a level approaching the higher values sometimes obtained in the absence of atropine. Independent of whether the ACh output was high or low, atropine (200 and 400 ng/ml.) caused the appearance of high voltage slow waves in the electrocorticogram.

Effects of nicotine

Initially, two series of experiments were performed. In the first, the animals were given forty injections of 2 μg/kg at 30 sec intervals for 20 min, that is, a total of

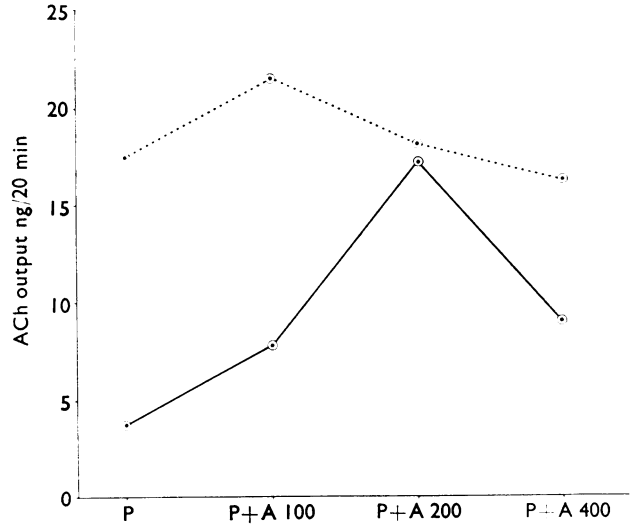

FIG. 1. Effect of atropine in the Ringer–Locke solution bathing the cortex on ACh output from left parietal cortex of anaesthetized cats. ⊙- - -⊙, Experiments in which resting output was greater than 10 ng/20 min/cm^2 cortex. ⊙——⊙, Experiments in which resting output was less than 5 ng/20 min/cm^2 cortex. Ordinate: ACh output in ng/20 min/cm^2 cortex. Abscissa: Ringer–Locke containing 20 μg/ml. physostigmine (P), or Ringer–Locke with physostigmine and atropine (P + A). Doses of atropine expressed in ng/ml.

80 μg/kg ; in the second, the same total amount was given but in twenty injections of 4 μg/kg at 1 min intervals. In six out of ten experiments in which nicotine (2 μg/kg) was injected intravenously every 30 sec for 20 min there occurred increases in the ACh output ranging from 29 to 100% (Fig. 2), and in these experiments changes in electrocortical activity occurred which were consistent with cortical activation. In the remaining four experiments there was no change in acetylcholine output and no change in the electrocorticogram. In two experiments, forty intravenous injections of saline (0.6 ml.) did not affect ACh output or electrocortical activity.

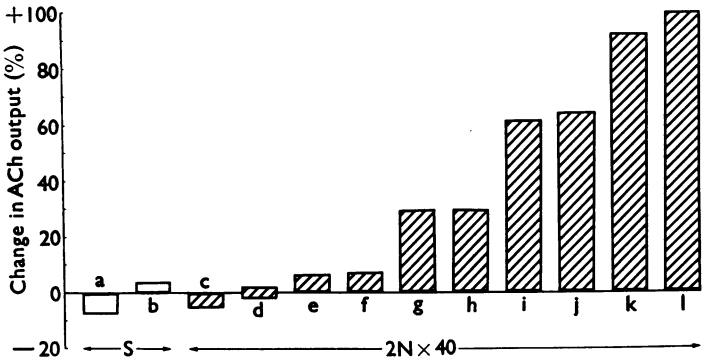

FIG. 2. Percentage change in acetylcholine output from the left parietal cortex of anaesthetized cats after intravenous saline (S) in two cats (a and b) and intravenous nicotine (N), 2 μg/kg every 30 sec for 20 min in ten cats (c–i). The percentage change in ACh was obtained by comparison of the average output for three samples before and three samples after the injection of nicotine.

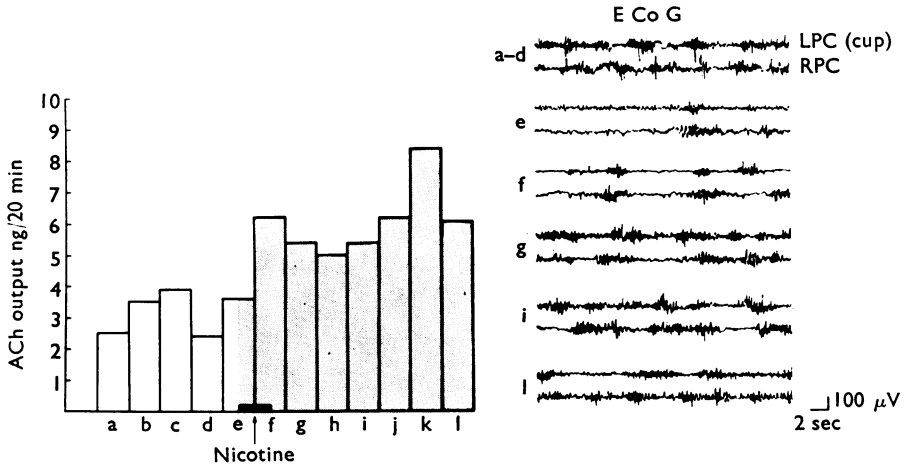

FIG. 3. Cat, 3.5 kg, " Dial " anaesthesia. Left: ACh output from left parietal cortex in ng/20 min/cm² cortex. Each column represents ACh release in successive 20 min samples. At the black bar nicotine was injected intravenously (2 μg/kg every 30 sec for 20 min), and the shading represents ACh release after intravenous nicotine. Right: electrocortical activity from left and right parietal cortex (LPC and RPC) at the period indicated during the experiment.

Figure 3 illustrates a typical effect of nicotine (2 μg/kg/30 sec) on ACh release from the left parietal cortex and on electrocortical activity. The resting output of ACh in this experiment was in the range 2.5 to 4 ng/20 min/cm² and typical electrocortical activity is shown in a–d. Electrocortical activity recorded from both hemispheres consisted of slow waves punctuated by bursts of spindle activity and resembled light sleep. Shortly after the start of the injection period, electrocortical activity became desynchronized indicating cortical activation. The spindle bursts became less frequent and slow wave activity was reduced in amplitude (e and f). The increased cortical activity was accompanied by an increased release of ACh which continued to the end of the experiment. The electrocorticogram assumed its original pattern (g) while the ACh levels were still elevated.

In six experiments in which nicotine was injected in a dose of 4 μg/kg every min, a reduction in ACh output (13–25%) occurred in three experiments and an increase in output (29–86%) in the remainder (Fig. 4). The changes in electrocortical activity that occurred were consistent with the changes in ACh output. The control experiments previously illustrated in Fig. 2 are again shown for comparison.

Fig. 5 shows an experiment in which changes occurred in the electrocorticogram indicative of cortical activation. The ACh output in this experiment was not steady but even after it had fallen to a low level, nicotine (twenty injections of 4 μg/kg) caused a 61% increase in output. During (e) and (f) spindle burst activity decreased, although after 60–80 min (i) there was some recovery in the electrocorticogram. An opposite effect is illustrated in Fig. 6, and in this experiment nicotine caused a fall in the acetylcholine output from about 5 ng/20 min/cm² to about 3 ng/20 min/cm², a decrease of 35%. The ACh output remained at this low level for more than 2 hr. The electrocorticogram changed from the typical pattern of spindle bursts and slow waves before nicotine (a–d) to one in which spindle burst activity was reduced and the amplitude of the accompanying slow waves was increased (e and f). The electrocorticogram had assumed its original pattern within 30–40 min (g) although at this time ACh levels were still low.

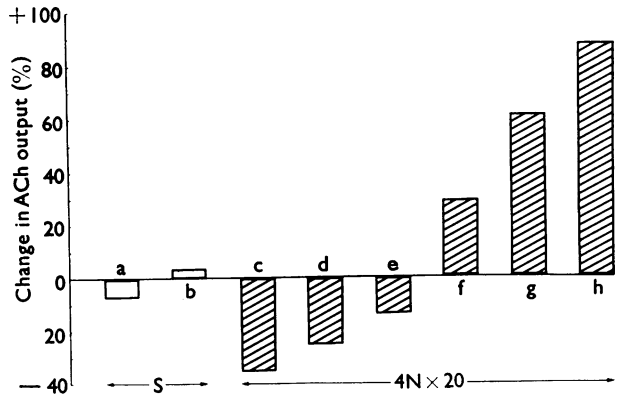

FIG. 4. Percentage change in acetylcholine output from the left parietal cortex of anaesthetized cats after intravenous saline (S) in two cats (a and b) and intravenous nicotine (N), 4 μg/kg every min for 20 min in six cats (c–h). The percentage change in ACh was obtained by comparison of the average output for three samples before and three samples after the injection of nicotine.

In the series of experiments in which the larger dose of nicotine (4 μg/kg) was given less frequently (once a minute), a decrease in ACh output occurred in half the experiments. Such a decrease was never observed when nicotine was injected at a dose of 2 μg/kg every 30 sec for 20 min. It was therefore of interest to observe the effects of nicotine 80 μg/kg given as a single injection, as a slow continuous

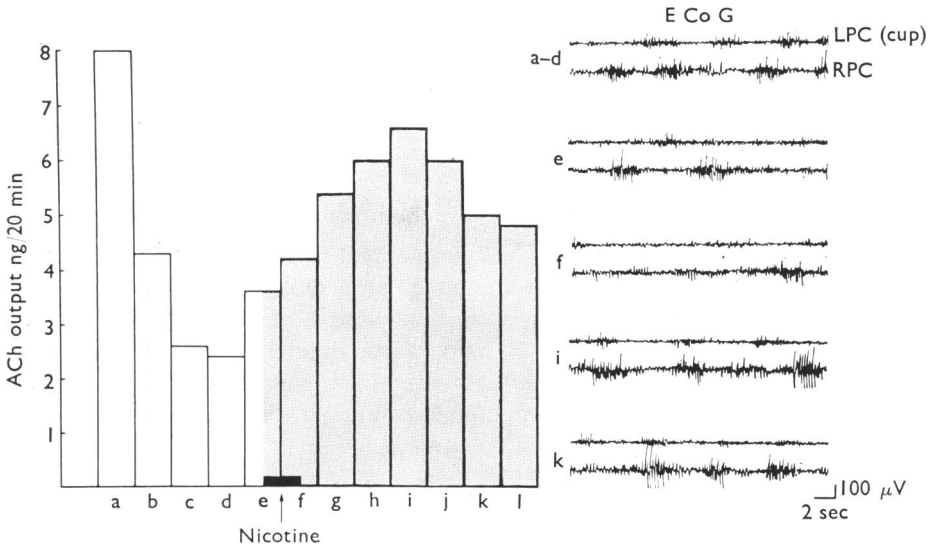

FIG. 5. Cat, 3.5 kg, "Dial" anaesthesia. Left: ACh output from left parietal cortex in ng/20 min/cm^2 cortex. Each column represents ACh release in successive 20 min samples. At the black bar nicotine was injected intravenously (4 μg/kg every min for 20 min) and the shading represents release after intravenous nicotine. Right: electrocortical activity from left and right parietal cortex (LPC and RPC) at the period indicated during the experiment.

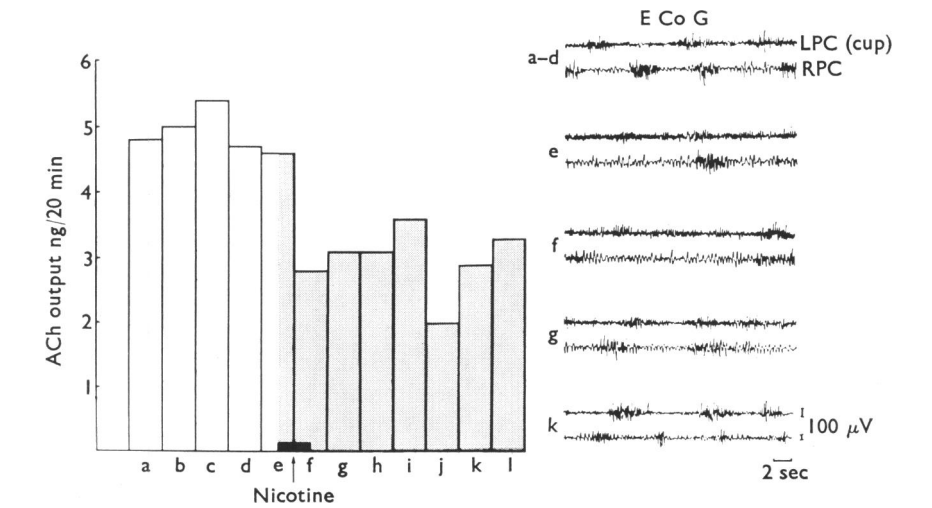

FIG. 6. Cat, 3.5 kg, "Dial" anaesthesia. Left: ACh output from left parietal cortex in ng/20 min/cm^2 cortex. Each column represents ACh release in successive 20 min samples. At the black bar nicotine was injected intravenously (4 μg/kg every min for 20 min), and the shading represents release after intravenous nicotine. Right: electrocortical activity from left and right parietal cortex (LPC and RPC) at the period indicated during the experiment.

infusion for 20 min, and as eighty divided doses of 1 μg/kg given every 15 sec. The injection of a single dose of 80 μg/kg in two experiments caused a decrease (24 and 41%) in ACh output. The electrocorticogram exhibited high voltage spiking from both hemispheres at a frequency of about 5/sec suggesting pre-seizure activity.

In a further two experiments, nicotine (1 μg/kg/15 sec) caused a decrease in ACh release (11 and 27%) and reduction in cortical activity similar to that illustrated in Fig. 6. Finally, a continuous infusion of nicotine 80 μg/kg for 20 min caused an increase of 10% in ACh output in one experiment and a decrease of 18% in another. In the experiment where ACh output increased, high voltage spikes occurred which preceded the desynchronization of electrocortical activity. In all these experiments, changes in ACh output persisted long after the electrocorticogram had reverted to spindle bursts and slow waves. Except when nicotine was given as a single injection of 80 μg/kg, there was no effect on blood pressure.

When nicotine (50, 100 or 200 μg/ml.) was added to the cup and left in contact with the cortical surface for 60 min, no change occurred in the electrocorticogram. The contents of the cup were removed and later assayed for nicotine on the blood pressure of the same cat. After 60 min, approximately 85% of the nicotine remained in the Ringer–Locke solution, suggesting that about 15% had been absorbed by the cerebral tissue at each concentration (7.5, 15 and 30 μg).

Discussion

Yamamoto & Domino (1965) showed that nicotine in doses of 5–10 μg/kg infused intravenously over a period of 1 min caused e.e.g. activation in conscious cats. In the present experiments nicotine was injected over a period of 20 min in order to obtain a closer relationship to the nicotine intake of the inhaling tobacco smoker. Although the experiments were performed under anaesthesia, nicotine caused changes in electrocortical activity which could be related to changes in ACh output from the cerebral cortex. Baust *et al.* (1962) have previously described e.e.g. arousal induced by changes in blood pressure. The doses of nicotine injected in these experiments did not affect the resting blood pressure, so this factor did not contribute to the change in the electrocorticogram observed in the present experiments. Nicotine (2 μg/kg given every 30 sec) usually increased ACh output and desynchronized electrocortical activity; in no experiment did it cause the opposite effect. By increasing the dose of nicotine and reducing the rate of injection, synchronization of cortical activity and a reduction in ACh output sometimes occurred. The amounts of nicotine used in the present experiments are thought to be similar to those taken by the cigarette smoker who inhales (Armitage, Hall & Morrison, 1968). This suggests that blood nicotine levels, in the human smoker, which almost certainly depend on the rate of puffing and the puff volume, may be critical for producing either an increase or decrease in cortical activity. In unanaesthetized encéphale isolé preparations, these same small amounts of nicotine (2 μg/kg given every 30 sec for 20 min) or small amounts of tobacco smoke (Armitage, Hall & Morrison, 1968; Armitage & Hall, 1968) in addition to causing cortical desynchronization also caused behavioural arousal.

Celesia & Jasper (1966) concluded that changes in cortical activity could not be directly related to changes in the spontaneous release of ACh from the cortical

surface. Szerb (1967) has also suggested that the pathways involved in producing cortical e.e.g. activation and an increase in ACh release are distinct because parallel changes did not occur either when the reticular formation was stimulated at different frequencies or when other subcortical areas were stimulated. The fact that changes in ACh output following intravenous nicotine were maintained, sometimes for the duration of the experiment, while changes in electrocortical activity were generally transient, supports the suggestions of Celesia & Jasper (1966) and Szerb (1967). It is possible that nicotine acts on the ascending cholinergic nonspecific reticulocortical system described by Collier & Mitchell (1966, 1967), causing arousal or alerting of the preparation. This effect may be reflected in the anaesthetized animal by transient desynchronization of electrocortical activity and an increased release of cortical ACh. This possibility is consistent with the conclusion of Domino (1967) that intravenous nicotine causes e.e.g. desynchronization by an action on neurones of the pontine reticular formation. An additional contribution to the changes in ACh output may arise by way of a second cholinergic system, the thalamocortical after-discharge pathway (Collier & Mitchell, 1966, 1967) from actions of nicotine on brain structures particularly involved with changes in behaviour. This could account for our observations that effects of nicotine on ACh output and electrocortical activity are distinct. The experiments in which nicotine (4 μg/kg given every min for 20 min) caused opposite effects could be explained on the basis of depression of these systems or as a result of stimulation of inhibitory mechanism.

Previous studies (Armitage & Hall, 1967 ; Armitage, Milton & Morrison, 1966 ; Morrison, 1968) have indicated that nicotine acts at some central sites by releasing ACh. Nicotine applied topically to the parietal cortex in concentrations greater than those likely to reach the same site following repeated intravenous injections had no effect on electrocortical activity. This is in agreement with the findings of Krnjević & Phillis (1963) that there are no true nicotinic receptors in the feline cerebral cortex. Neither the increased ACh release from the cortex nor the increased electrocortical activity produced by intravenous nicotine is therefore evidence for a direct cortical action. It has been shown by Hernández-Péon *et al.* (1963) that the application of ACh to sites extending from the septal region into the mesencephalic reticular formation induces alertness, whereas the application of ACh to the limbic forebrain-limbic midbrain circuit of Nauta (1958) induced sleep. It has also been reported that brain structures associated with arousal are extremely sensitive to ACh, intracarotid injections of as little as 5 ng causing immediate e.e.g. activation (Bradley, 1960). Nicotine, by releasing ACh at various sites within the central nervous system, could therefore induce changes consistent with either an increase or decrease in cortical activity. These changes may depend on the dose of nicotine and its preferential action for a particular brain structure. In man, where the balance between the various systems may be slightly disturbed, nicotine, by interacting on any particular structure or structures may affect the balance.

The appearance of high voltage spikes on the electrocorticogram following a large single intravenous dose of nicotine 80 μg/kg is probably associated with convulsant activity. Convulsant drugs such as leptazol have been shown to increase ACh output in cats (Mitchell, 1963 ; Beleslin *et al.*, 1965 ; Celesia & Jasper, 1966), and rats (Hemsworth & Neal, 1968). That nicotine caused the opposite effect may result from differences in site of action because nicotine induces seizure discharge

by an action on the hippocampus (Floris *et al.*, 1964) whereas leptazol exerts its effects on other cortical and subcortical structures.

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