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Effects of nicotine and tobacco smoke on the electrical activity of the cerebral cortex and olfactory bulb

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Summary

1. Effects of nicotine, cigarette smoke and carbon monoxide have been compared in the cat *encéphale isolé* preparation, exhibiting a synchronized electrocorticogram (ECoG) and behavioural sleep.

2. 2 ml samples of smoke, containing approximately 7 μg nicotine (approximately 2 μg nicotine/kg for a 3 kg cat), introduced into the lungs at 30 s intervals from a smoking simulator, caused desynchronization of the ECoG and behavioural arousal.

3. Effects of smoke were matched in the same experiments by intravenous injections of nicotine, 2 μg/kg every 30 s.

4. The use of specific nicotine antagonists, for example mecamylamine, and filters for removing nicotine, indicated the presence in smoke of other agents capable of exerting a pharmacological response.

5. Cigarette smoke contains approximately 5·0% carbon monoxide. Introduced into the lungs of cats pretreated with mecamylamine (2 mg/kg), 2 ml samples of 5% carbon monoxide caused changes in the ECoG similar to those caused by smoke.

6. Effects of nicotine or smoke were not modified by pretreatment with chlorpromazine (2·0–4·0 mg/kg). Atropine (0·3 mg/kg), however, prevented the cortical activation, but not the behavioural arousal.

7. 2 ml samples of smoke applied to the nostrils caused the occurrence in the olfactory bulb of a discharge or burst of " induced " waves. This discharge was sometimes accompanied by a transient period of cortical activation.

8. These studies demonstrate that in cats, nicotine is the principal pharmacological constituent of tobacco smoke as far as effects on the central nervous system are concerned, although other constituents of smoke may play a contributory role.

Introduction

Studies in man have provided conflicting evidence for changes in cortical activity during smoking. Hauser, Schwarz, Roth & Bickford (1958) and Wechsler (1958) were unable to differentiate changes in the electro-encephalogram (e.e.g.) occurring in subjects smoking normal, low nicotine, nicotine free, or glass cigarettes filled with cotton. Since changes in activity also occurred in non-inhalers, it was suggested

that the response may be due to abnormal attention perhaps resulting from the physical act of smoking. Lambiase & Serra (1957), however, concluded that smoking caused characteristic changes in cortical activity, of short duration, consisting of a flattening of potentials. Effects of smoking, as reflected in the e.e.g., were found by Murphree, Pfeiffer & Price (1967) to be stimulant rather than tranquillizing, although subjects exhibited individual differences. Brown (1968) has recently reported that the spontaneous e.e.g. patterns of heavy smokers contained significantly less alpha and more high frequency rhythmic activity than did those of non-smokers. Nevertheless, this author stated " the nicotine content of cigarettes would not seem to be responsible for the activated and high frequency rhythmic e.e.g. patterns of heavy smokers ".

Small amounts of nicotine (2 or 4 μg/kg) injected intravenously every 30 or 60 s for 20 min, caused changes in cortical activity and acetylcholine release from the cerebral cortex of the anaesthetized cat (Armitage, Hall & Sellers, 1969b). These small doses of nicotine are probably similar to the amounts absorbed by the smoker each time he inhales a puff of cigarette smoke (Armitage, Hall & Morrison, 1968). Nicotine also caused behavioural arousal in addition to e.e.g. activation, when given as a short intravenous infusion to unanaesthetized cats (Yamamoto & Domino, 1965).

The present studies were undertaken (*a*) to ascertain the actions of tobacco smoke on cortical activity, (*b*) to determine the contribution of nicotine to any changes observed, and (*c*) to investigate the possibility that constituents of cigarette smoke, other than nicotine, may also contribute to the central response.

Methods

Experiments were performed on cats of either sex weighing 2·3–3·4 kg. Sixty-eight experiments were made on *encéphale isolé* preparations. In two further experiments, effects of nicotine were studied on the cortical activity and behaviour of unrestrained cats, carrying chronically implanted cortical electrodes.

The *encéphale isolé* was prepared under halothane anaesthesia using operative procedures similar to those described by Bradley & Key (1958). Anaesthesia was induced with halothane applied by mask, the trachea was cannulated and administration of anaesthetic continued via the tracheal cannula. In some experiments, the preparation was vagotomized before cannulation of the trachea, by section of both vagus nerves in the cervical region. The femoral vein was cannulated to allow intravenous injections, and blood pressure was monitored routinely from a femoral artery, using a pressure transducer coupled to a Devices polygraph. After positioning the head in a stereotaxic instrument, the skin, muscle and periosteum on top of the skull were reflected, and the muscles over the arch of atlas at the back of the neck removed by cautery. The arch was removed, the dura incised, and the spinal cord transected at the level of C1 with a blunt spatula. Any bleeding was arrested with absorbable gelatin sponge. Artificial respiration was applied and anaesthesia maintained. All wound margins were infiltrated with 2% lignocaine. Eight cortical silver ball electrodes (0·5–1·0 mm diameter), held and insulated in a nylon screw, were screwed into holes placed in the skull over both hemispheres. The electrodes were sited over lateral and suprasylvian gyri, the silver ball resting on dura.

In twelve *encéphale isolé* preparations, recording electrodes were implanted in the olfactory bulb. After opening the frontal sinus, a dental burr was used to expose the dorsal surface of the bulb at a point 1·5 mm from mid-line and immediately

anterior to the frontal pole of the brain. After piercing the overlying dura, a stainless-steel concentric bipolar electrode was inserted into the bulb to a depth of 4–6 mm. The electrode was fixed in position with dental cement placed in the frontal sinus.

Administration of halothane was terminated, the head removed from the stereotaxic instrument and placed on a soft cushion. Room lighting and sound were reduced to a minimum and at least 2 hr were allowed for recovery from anaesthesia before studies commenced. In some experiments, the nostrils were either sealed with adhesive tape or infiltrated with lignocaine, to prevent cigarette smoke acting on sensory receptors in the olfactory epithelium. Body temperature was maintained at 37° C with an Electrophysiological Instruments homeothermic blanket.

The *encéphale isolé* preparation exhibits patterns of electrical activity associated with the sleeping or waking state. To study changes from the sleeping to the waking state it was necessary to maintain the preparation, in so far as conditions allowed, in the sleeping state exhibiting a synchronized electrocorticogram. This was achieved by ensuring that the cat was not deprived of food before the experiment, and by adjusting the ventilatory volume for respiration as described by Abeles, Magness & Samueloff (1964). The ventilatory volume required to induce synchronization was predicted from body weight and the rate of respiration was maintained at 22/min.

Cigarette smoke from standardized cigarettes was either applied to the nostrils or introduced directly into the lungs. A smoking pump was devised which takes a standard 25 ml "man-sized" puff of cigarette smoke (Armitage, Hall & Heneage, 1969a). The facility is available for sampling from 1 to 5 ml of the 25 ml puff. Chemical analysis of the smoke samples showed that the 2 ml sample of tobacco smoke contained approximately 7 μg of nicotine (approximately 2 μg nicotine/kg for a 3 kg cat). 2 ml samples of cigarette smoke were injected at 30 s intervals directly into the airway from the respiratory pump, thus ensuring immediate passage of the smoke into the lungs. To study effects of smoke from which all particulate matter (including nicotine) had been removed, the 25 ml puff was first drawn through a glass paper Cambridge filter (Bentley & Burgan, 1961). Smoke could also be applied to the nostrils via a small plastic nozzle or via a filter funnel placed around the nose. In some experiments, the pump was also used to introduce 2 ml samples of 5% carbon monoxide into the lungs. A reservoir of 5% carbon monoxide was obtained by dilution with air.

Two cats carrying chronically implanted cortical electrodes were prepared under pentobarbitone anaesthesia (40 mg/kg intraperitoneally) observing aseptic precautions. The skin, muscle tissue and periosteum on top of the skull were reflected, the external jugular vein cannulated and the cannula attached to a valve screwed into the skull (Hall, Gomersall & Heneage, 1968). Four silver-ball electrodes (0·5–1·0 mm diameter) held and insulated in a nylon screw, were screwed into the skull (two per hemisphere) over the suprasylvian gyri. The free ends of the silver wires were soldered to the terminals of a miniature socket which was attached to the skull with acrylic cement. The skin was sutured round the socket and valve, and penicillin G and streptomycin were injected intramuscularly. One week elapsed before the intravenous injection of nicotine or saline, during which the animal was acclimatized to the behaviour chamber. This consisted of an illuminated sound-attenuated constant environment chamber fitted with one-way glass for observation. For recording, a flexible array of fine wires was attached via a connector to the miniature socket on

the skull. Polyvinyl tubing filled with heparinized sterile 0·9% NaCl solution was attached to the valve via a connector, to allow the intravenous injection of nicotine or saline. Wires and tubing were bound together and attached respectively at the other extremity to a socket and stainless steel hypodermic tubing. From the roof of the chamber, leads were taken to the electroencephalograph for electrical recording, and to a syringe fitted with a three-way tap for injection.

Bipolar recordings of electrical activity were made with a Kaiser TR60 electroencephalograph. All drugs were injected intravenously in 0·9% NaCl solution.

The following drugs were used: D-amphetamine sulphate, atropine sulphate, chlorpromazine hydrochloride, mecamylamine hydrochloride, nicotine hydrogen tartrate, pentobarbitone sodium. Doses are expressed in terms of base.

Results

Electrocortical activity

Electrocortical activity was considered in four distinct stages as illustrated in Fig. 1. During stages A–C the animal showed behavioural signs of sleep, indicated by closure of the eyes and complete immobility of the head. In stage A, electrocortical

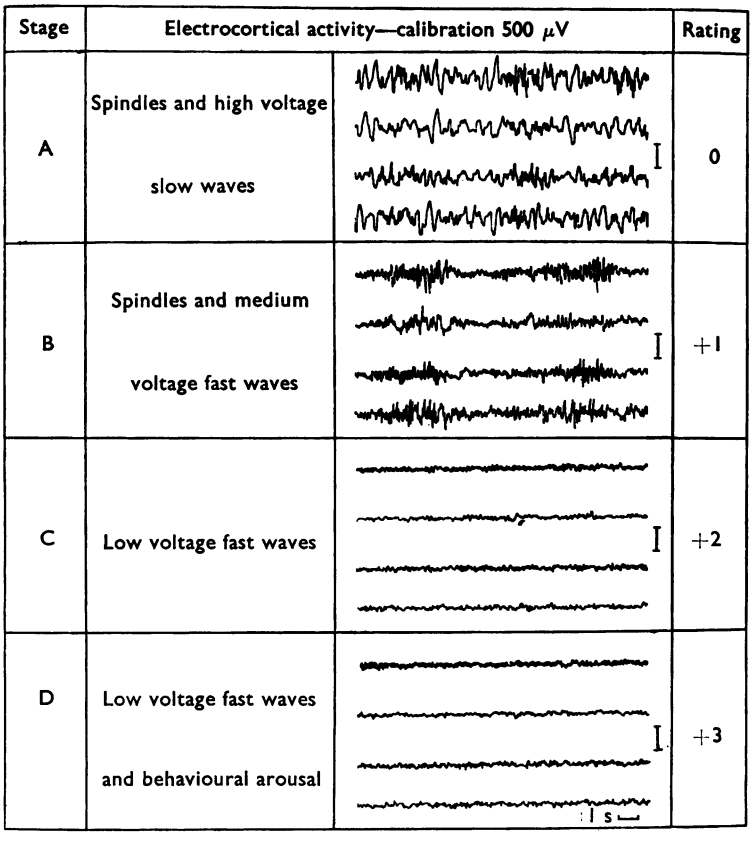

FIG. 1. Cat, *encéphale isolé*. Records of electrocortical activity relating to the various stages of sleep and arousal. Stage A, normal sleep; stage B, light sleep; stage C, pre-alert and stage D, behavioural arousal. Allotted to each stage is an activity rating.

activity was synchronized and consisted of spindles and high voltage slow waves characteristic of sleep. Spindles and medium voltage fast waves occurred in stage B, indicative of light sleep or drowsiness. Stage C represented a pre-alert stage, when the animal still exhibited behavioural signs of sleep, although cortical activity consisted of low voltage fast waves, corresponding to complete desynchronization or cortical activation. In stage D cortical activity resembled stage C, but in addition behavioural arousal was also present. The eyes opened and reacted to visual stimuli, often accompanied by movements of the ears, jaws and vibrissae.

Nicotine

In each of twelve experiments, the effects of forty injections of nicotine 2 μg/kg at 30 s intervals for 20 min were compared with those of twenty injections of 4 μg/kg given at 1 min intervals. The dosage regime was alternated for each experiment. The preparation was allowed 5 min in stage A (spindles and high voltage slow waves) before the injections of nicotine commenced. A rating scale was used (Fig. 1) to determine changes in cortical activity and behaviour. The electrocorticograms were examined visually, and a rating allotted to the dominant activity for each minute during the 20 min injection period and for the succeeding 5 min. During this period the preparation was observed for the presence or absence of behavioural arousal. A change from A–B rated one, from A–C two and from A–D three. The ratings from all experiments, for each dosage regime and for a given time, were summed and averaged. Thus a representative change in cortical activity with time was obtained (Fig. 2). Nicotine 2 μg/kg per 30 s caused a steady change in cortical activity which was generally maintained between stages B and C. Nicotine 4 μg/kg per min initially caused a greater change in activity (between stages C and D), but the effect was not maintained. After completing the injections of nicotine (2 μg/kg per 30 s or 4 μg/kg per min), activity declined.

Considering each individual experiment, cortical desynchronization usually occurred during the injection period, sometimes accompanied by behavioural arousal. This is not apparent in Fig. 2, which shows an overall change in activity for

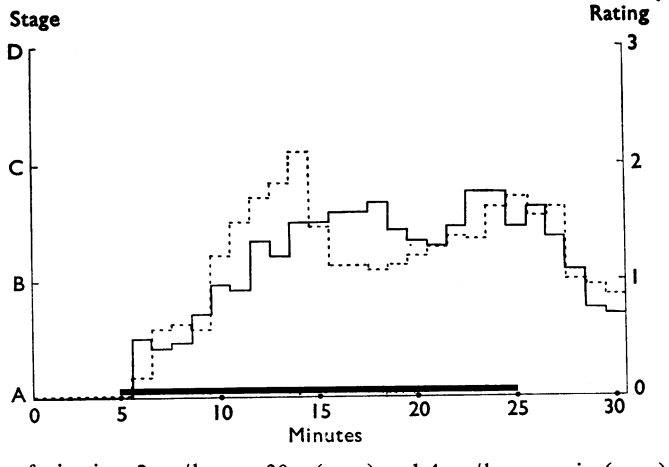

FIG. 2. Effects of nicotine, 2 μg/kg per 30 s (——) and 4 μg/kg per min (- - - -) injected intravenously, on cortical activity and behaviour as defined in Fig. 1. Duration of nicotine injections indicated by horizontal black bar. Activity ratings are the mean of twelve experiments.

all twelve experiments. In eight of these experiments, behavioural arousal was either absent or short lasting. In the other four experiments, however, behavioural arousal was more prominent, the time for which behavioural arousal occurred with nicotine 2 μg/kg per 30 s varying from 5 to 10 minutes. In three of these experiments in which nicotine 2 μg/kg per 30 s caused behavioural arousal, nicotine 4 μg/kg per min had little or no effect. In the remaining experiment, however, the behavioural response caused by nicotine 4 μg/kg per min was of longer duration than that following nicotine 2 μg/kg per 30 s.

In the two chronic cat preparations, intravenous injections of nicotine (2 μg/kg per 30 s or 4 μg/kg per min) caused cortical activation and a behavioural " wake-up " effect. The previously sleeping animal became aroused, and moved around the behaviour chamber whilst injections of nicotine continued. When injections ceased, the cat gradually reverted to the sleeping rate. 0·9% NaCl solution similarly injected had no effect.

Cigarette smoke

2 ml puffs of cigarette smoke (containing nicotine, approx. 2 μg/kg) introduced into the lungs at 30 s intervals caused cortical activation and behavioural arousal in twenty-four experiments. In two of these experiments, the effects of smoke were compared directly with the effects of nicotine 2 μg/kg per 30 s injected intravenously. One such experiment is illustrated in Fig. 3. Electrocortical activity before smoke (Fig. 3A) and nicotine (Fig. 3D) consisted of spindles and high voltage slow waves accompanied by behavioural sleep. After five puffs of smoke, the high voltage slow activity was replaced by low voltage fast waves (Fig. 3B) and behavioural arousal also occurred. A similar response was seen after seven injections of nicotine 2 μg/kg every 30 s (Fig. 3E). Two minutes after the tenth and last puff of smoke (Fig. 3C) or injection of nicotine (Fig. 3F), the preparation again showed high voltage slow waves and behavioural sleep. The longer the period of cortical activation, the greater the time taken for recovery of the sleeping state. If smoking was continued, cortical activation persisted. In contrast, the continued injection of nicotine did not always maintain the cortex in a state of activation. Cigarette smoke or nicotine would not again cause desynchronization if applied or injected immediately after cortical activity had become synchronized. To obtain reproducible changes with either nicotine or smoke, it was usually necessary to allow intervals of at least one hour. Vagotomy did not affect the cortical response to cigarette smoke.

Olfactory bulb

In three of twelve experiments in which recordings were made from the olfactory bulb, cigarette smoke was applied to the nostrils with a filter funnel. 2 ml puffs of smoke caused transient periods of desynchronization of 5–30 s duration, occurring whilst smoking continued. In one of these three experiments, the 2 ml puffs caused cortical desynchronization, whilst a larger volume of smoke (25 ml) failed to do so. To present smoke more efficiently to the olfactory epithelium which lies deep in the nasal cavity, 2 ml puffs of smoke were applied directly to the nostril with a small plastic nozzle containing a narrow orifice. In a further nine experiments, cigarette smoke applied in this way caused a discharge or burst of activity in the olfactory bulb. These large rhythmic oscillations appearing in the olfactory bulb during the

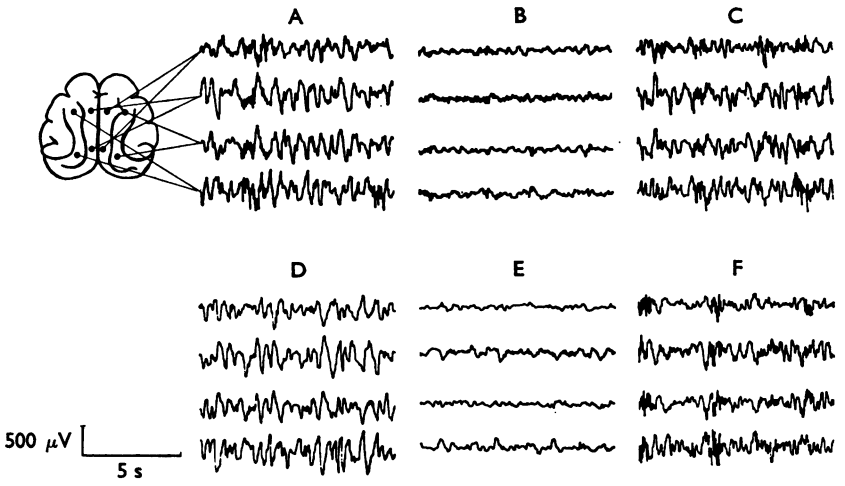

FIG. 3. Cat, 2·8 kg, *encéphale isolé*. Records of electrocortical activity. A and D, control activity (asleep); B, low voltage fast waves after five 2 ml puffs of cigarette smoke introduced into the lungs; E, a similar response after seven intravenous injections of nicotine, 2 μg/kg; C and F, 2 min after the tenth puff of smoke and tenth and last injection of nicotine.

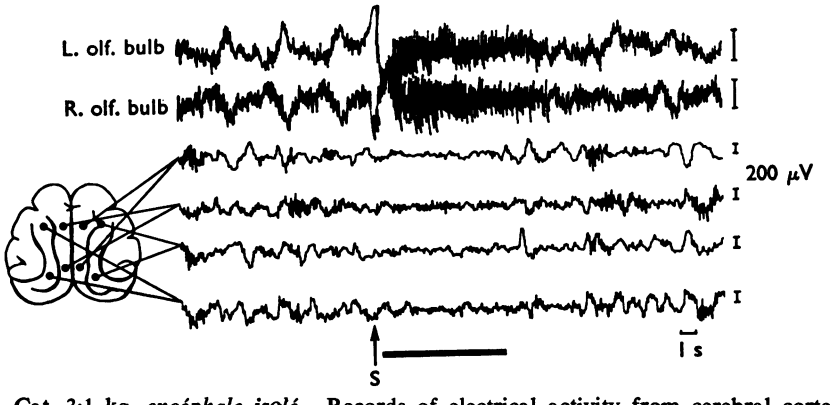

FIG. 4. Cat, 3·1 kg, *encéphale isolé*. Records of electrical activity from cerebral cortex and left and right olfactory bulb. At the arrow, a 2 ml puff of cigarette smoke (S) was applied simultaneously to both nostrils. The black bar indicates the duration of cortical desynchronization in relation to the "induced" waves occurring in the olfactory bulb.

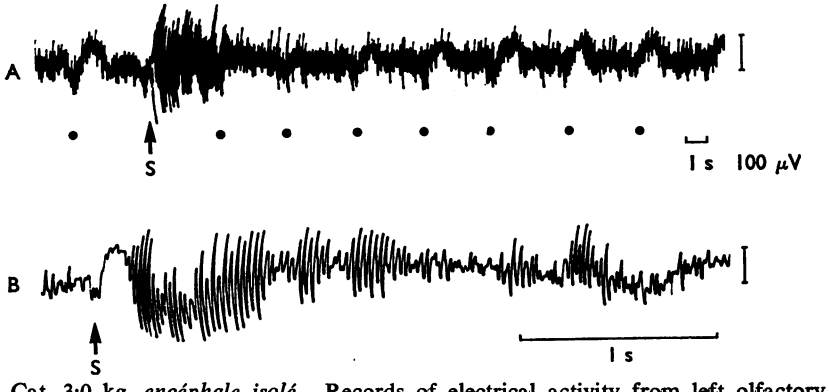

FIG. 5. Cat, 3·0 kg, *encéphale isolé*. Records of electrical activity from left olfactory bulb. A, "induced" waves elicited by a 2 ml puff of cigarette smoke (S) applied to left nostril, and for comparison effects of room air (●). B, "induced" waves recorded at a faster speed to indicate discharge frequency of approximately 40 Hz.

inspiration of an odour were referred to by Adrian (1950) as " induced waves ". In the absence of an olfactory stimulus, the spontaneous activity of the olfactory bulb was characterized by rhythmic oscillations of lower amplitude, the so-called " intrinsic waves ". Figure 4 illustrates a typical experiment in which a 2 ml puff of cigarette smoke was applied to both nostrils. Induced waves were recorded from both olfactory bulbs, together with a transient period of desynchronization of cortical activity. The period of cortical activation lasted for about 10 s, the approximate duration of the discharge recorded from the left and right bulb. In some experiments, smoke caused induced waves in the olfactory bulb without modifying cortical activity. Occasionally, room air puffed up the nostril also caused the occurrence of induced waves, without affecting activity recorded from the cortex. Tolerance

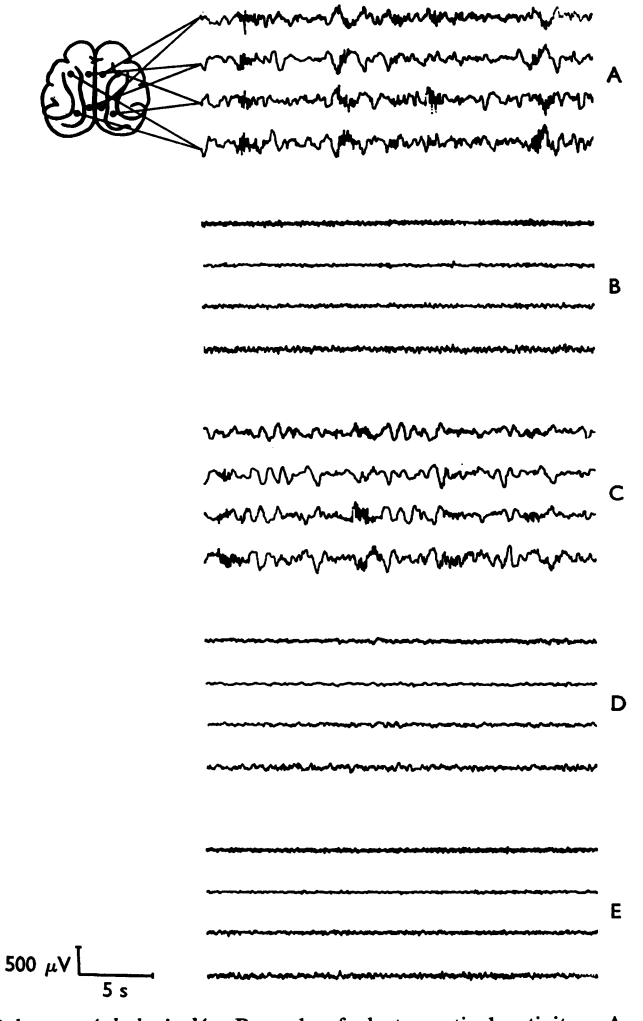

FIG. 6. Cat, 2·9 kg, *encéphale isolé*. Records of electrocortical activity. A, control activity (asleep); B, low voltage fast waves after seven 2 ml puffs of unfiltered cigarette smoke introduced into the lungs; C, high voltage slow waves interspersed with bursts of spindle activity after ten 2 ml puffs of filtered cigarette smoke; D, low voltage fast waves after twenty puffs of filtered cigarette smoke; E, low voltage fast waves after seven 2 ml puffs of unfiltered cigarette smoke.

developed rapidly when room air was used as the olfactory stimulus, whereas the effects of smoke were usually reproducible. The frequency of smoke-induced waves varied between 25 and 70 Hz in the nine experiments. A comparison of the discharge frequency for room air and smoke was made in one experiment which is illustrated in Fig. 5. The frequency was 15–20 Hz for room air as compared with 35–70 Hz for smoke. Smoke elicited a discharge with an amplitude of about 200 μV, whereas room air modified only slightly the intrinsic activity. In Fig. 5B the record was taken at a faster speed to illustrate a discharge frequency of about 40 Hz. Bulbar activity was unaffected by the injection of amphetamine (0·5 mg/kg) or nicotine (20 μg/kg), and was not modified by sensory stimulation induced by clapping or tapping. Cigarette smoke applied repeatedly to the nostrils never caused behavioural arousal.

Effects of Cambridge filters

The passage of smoke through such a filter reduced or abolished the cortical response. In two experiments, up to forty 2 ml puffs of filtered smoke failed to affect cortical activity. Approximately 60 min earlier, however, and also 60 min later, the electrocorticogram was desynchronized by less than ten 2 ml puffs of smoke which had not passed through a filter. In three further experiments, cortical activity was modified by filtered smoke, although when compared with the effects obtained in the absence of a filter, additional puffs of smoke were required to produce the same response. One such experiment is illustrated in Fig. 6. The electrocorticogram was desynchronized by seven 2 ml puffs of unfiltered smoke (B). Sixty-four minutes later the electrocorticogram remained synchronized after ten filtered puffs (C). Twenty filtered puffs, however, caused cortical activation (D). Chemical analysis showed that the total expected amount of nicotine (3·32 mg) had been retained on the filter. After a further 35 min, only five puffs of unfiltered smoke were required to cause cortical desynchronization (E).

Mecamylamine

Mecamylamine (0·5–2·0 mg/kg) always prevented the cortical activation and behavioural arousal caused by nicotine injected either as a single dose of 20 μg/kg, or repeatedly in a dose of 2 μg/kg per 30 s. In contrast, the similar changes in cortical activity caused by cigarette smoke were abolished by mecamylamine in only three out of ten experiments. Figure 7 illustrates an experiment in which the effects of nicotine, but not smoke, were blocked by mecamylamine. The nostrils were taped and both vagi sectioned. Before the administration of nicotine, or smoke, electrocortical activity consisted of spindles and high voltage slow waves (Fig. 7A). Nicotine injected as a single dose of 20 μg/kg caused the appearance of low voltage fast waves indicative of cortical activation (Fig. 7B). A similar response was obtained with three 2 ml puffs of cigarette smoke (Fig. 7C). Thirty minutes after the injection of mecamylamine (1 mg/kg), six 2 ml puffs of smoke caused low voltage fast waves interspersed with bursts of spindle activity (Fig. 7D). 20 min after a further injection of mecamylamine (2 mg/kg) nicotine 20 μg/kg failed to affect cortical activity (Fig. 7E). Three minutes later (not illustrated) nine 2 ml puffs of smoke caused complete cortical desynchronization. The most usual effect of mecamylamine, however, was to reduce the cortical response to smoke, from complete desynchronization to low voltage fast waves interspersed with bursts of spindle

activity as illustrated in Fig. 7D. In one experiment in which smoking was continued in the presence of a nicotine block by mecamylamine, complete desynchronization and also behavioural arousal occurred. Mecamylamine did not prevent the cortical activation caused by amphetamine (0·5 mg/kg).

Pentobarbitone sodium

In six experiments, the cortical activation caused by nicotine (2 μg/kg per 30 s) and amphetamine (0·5 mg/kg) was prevented by the injection of sodium pentobarbitone (2·5–10·0 mg/kg). In one experiment in which nicotine (2 μg/kg per 30 s)

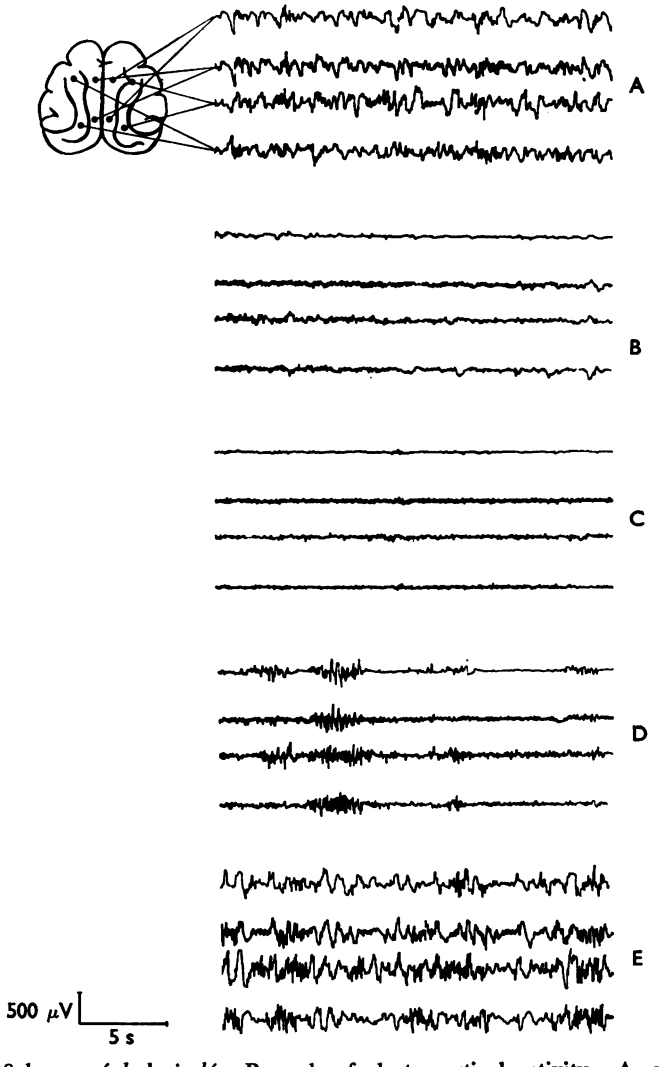

FIG. 7. Cat, 2·8 kg, *encéphale isolé*. Records of electrocortical activity. A, control activity (asleep); B, low voltage fast waves after nicotine 20 μg/kg injected intravenously; C, a similar response after three 2 ml puffs of cigarette smoke introduced into the lungs; between C and D, mecamylamine (1 mg/kg) was injected intravenously; D, low voltage fast waves interspersed with spindle bursts after six 2 ml puffs of cigarette smoke; E, no change in control activity following injection of nicotine 20 μg/kg, 20 min after a further injection of mecamylamine (2 μg/kg).

caused behavioural arousal, this response was also abolished by pentobarbitone. The effects of pentobarbitone on the changes in cortical activity caused by cigarette smoke were studied in three further experiments. The nostrils were taped and both vagi sectioned. Before the injection of pentobarbitone (2·5 mg/kg), 2 ml puffs of smoke caused complete cortical desynchronization or cortical activation. After pentobarbitone, 2 ml puffs of smoke caused a reduction in amplitude of the high voltage slow waves and spindle bursts became more prominent. The changes in cortical activity caused by smoke were therefore not as great as those observed in the presence of mecamylamine.

Carbon monoxide

In four experiments, 2 ml samples of 5% carbon monoxide caused changes in cortical activity after mecamylamine, similar to the changes that occurred with cigarette smoke. This is illustrated in Fig. 8. Before the introduction of carbon monoxide, or smoke, the electrocorticogram consisted of spindles and high voltage slow waves (A). Electrocortical activity became completely desynchronized after fifteen 2 ml puffs of smoke (B) and behavioural arousal also occurred. Thirty minutes after the injection of mecamylamine (1 mg/kg), sixteen 2 ml puffs of smoke

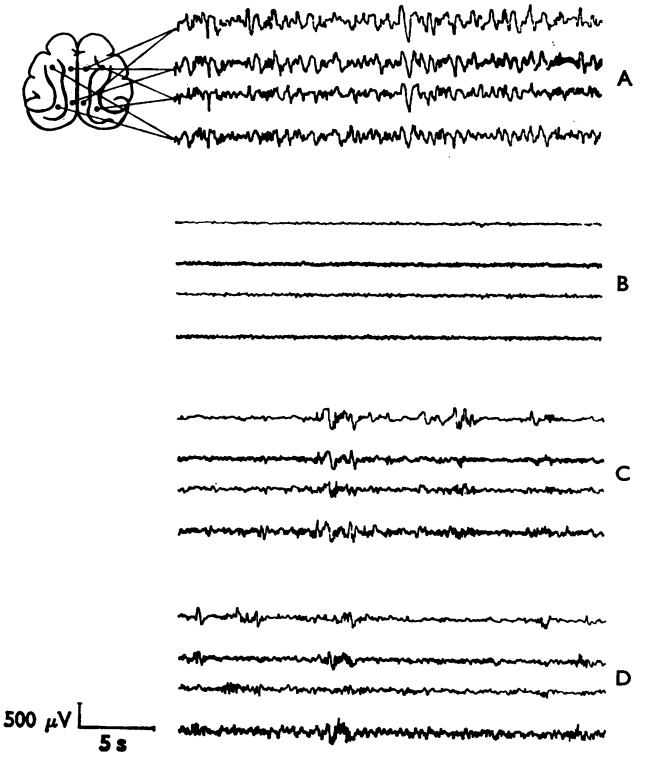

FIG. 8. Cat, 2·8 kg, *encéphale isolé*. Records of electrocortical activity. A, control activity (asleep); B, low voltage fast waves after fifteen 2 ml puffs of cigarette smoke introduced into the lungs; between B and C, mecamylamine (1 mg/kg) was injected intravenously; C, low voltage fast waves interspersed with bursts of spindle activity and occasional slow waves, after sixteen 2 ml puffs of cigarette smoke; D, a similar response after thirteen 2 ml samples of 5% carbon monoxide introduced into the lungs.

caused only low voltage fast waves interspersed with bursts of spindle activity and occasional slow waves (C). An additional four 2 ml puffs did not further desynchronize cortical activity. Thirty minutes later, thirteen 2 ml samples of 5% carbon monoxide caused changes in cortical activity similar to the changes observed with smoke (D). An additional seven 2 ml samples of carbon monoxide did not further modify cortical activity. In one of the four experiments, cortical activation and behavioural arousal occurred after the introduction into the lungs of fifteen 2 ml samples of carbon monoxide. Carbon monoxide also caused similar changes in cortical activity in the absence of mecamylamine. Likewise in the presence of pentobarbitone (2·5 mg/kg), changes in cortical activity similar to those observed with cigarette smoke again occurred. In comparison with mecamylamine, however, more smoke or carbon monoxide was required to produce the same response.

Chlorpromazine

The effects of nicotine (2 μg/kg per 30 s) and cigarette smoke (2 ml puffs) on cortical activity and behaviour were unaffected by chlorpromazine (2·0–4·0 mg/kg). The similar effects of intravenous amphetamine (0·5 mg/kg) were, however, prevented by chlorpromazine.

Atropine

In six experiments, atropine (0·3–0·4 mg/kg) injected 30–160 min before nicotine modified the electrocortical response but not the behavioural response caused by nicotine 2 μg/kg injected at 30 s intervals for 20 minutes. In one experiment, seven injections of nicotine caused behavioural arousal, without affecting cortical activity, which continued to show high voltage slow waves. The behavioural arousal occurred for a total time of 8 min during the 20 min injection period. In five further experiments nicotine caused behavioural arousal, whilst only transient periods of desynchronization, sometimes interspersed with bursts of spindle activity, were recorded on the electrocorticogram.

Blood pressure

The resting blood pressure in sixty-two of the *encéphale isolé* preparations varied between 70 and 100 mm Hg. In the remaining six preparations, resting blood pressure was between 50 and 70 mm Hg. Blood pressure was not affected by the injection of nicotine (2 μg/kg per 30 s), the introduction of cigarette smoke or carbon monoxide.

Discussion

Nicotine 2 μg/kg given intravenously to anaesthetized cats every 30 s for 20 min caused cortical activation and an increased release of cortical acetylcholine (Armitage *et al.*, 1969b). 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. There is a parallelism between these findings and the results now obtained with the *encéphale isolé* preparation. The " stimulant " or " activating " effects of the smaller dose of nicotine (2 μg/kg) given more frequently were maintained, whereas those of the larger dose (4 μg/kg) given less frequently, although initially more pronounced, were generally not maintained. Similarly when

behavioural arousal also occurred, the effects of the smaller dose of nicotine were usually more prominent.

The present experiments have demonstrated changes in cortical activity and behaviour, caused by small puffs of cigarette smoke introduced into the lungs, which are similar to the effects of small amounts of nicotine injected intravenously. In addition, cigarette smoke also caused transient desynchronization of cortical activity when applied to the nostrils. That smoke caused " induced " waves in the olfactory bulb, without necessarily affecting cortical activity, suggests either differing thresholds for the two responses, or the involvement of a second neural pathway. The second suggestion receives support from the observation that a typical cortical " arousal " response to an odour stimulus can be prevented by blockade of the trigeminal nerve (Stone, Williams & Carregal, 1968). Either a separate mechanism or the interaction of the olfactory and trigeminal systems may therefore be responsible for the cortical response. Although nicotine can excite sensory nerve endings (Brown & Gray, 1948 ; Douglas & Gray, 1953), it probably does not contribute to the changes in olfactory activity induced by smoke. Hughes & Mazurowski (1962) reported eleven smoke derivatives that triggered " induced " waves in the olfactory bulb of the rabbit, whereas nicotine was ineffective. Effects of smoke on olfactory mechanisms may, however, contribute to the overall central response following the inhalation of cigarette smoke. A similar discharge of " induced " waves, from the human olfactory bulb during the inspiration of cigarette smoke, has recently been described (Hughes, Hendrix & Wetzel, 1968).

The effects of cigarette smoke on cortical activity were generally not abolished but only reduced by the administration of mecamylamine. The effects of nicotine, however, were blocked by mecamylamine, thus confirming a similar observation made by Yamamoto & Domino (1965). These authors showed that the e.e.g. and behavioural effects of nicotine were central in origin, for they were blocked by mecamylamine, which readily passes into the central nervous system, but were not blocked by the quaternary blocking agent trimethadinium, which does not easily penetrate the blood-brain barrier. If cigarette smoke can modify cortical activity in the presence of a nicotine block by mecamylamine, other agents capable of exerting a pharmacological response must be present in the smoke. Removal, by Cambridge filter, of the particulate phase of cigarette smoke, which includes nicotine, did not always prevent cortical desynchronization. This finding suggests that a constituent of the vapour phase of smoke must sometimes contribute to the response. Lambiase & Serra (1957) considered that alterations of electrical activity of the cortex, caused by smoking, could be attributed to actions of nicotine and carbon monoxide, the formation of carbon monoxide from the burning tobacco leading to slight hypoxia. The smoke from the standard cigarettes used in the present studies contained by analysis 4·5–5·5% carbon monoxide. Cigarette smoke raises carboxyhaemoglobin levels, and it has been shown that in the inhaling cigarette smoker approximately 80% of the carbon monoxide is retained in the body (Bokhoven & Niessen, 1961). The present studies, with which carbon monoxide caused changes in cortical activity almost identical to those caused by smoke, support the original suggestion by Lambiase & Serra (1957). Dell, Hugelin & Bonvallet (1961) studied the effects of hypoxia on the reticular and cortical diffuse systems of the vagotomized *encéphale isolé* preparation and described an initial excitatory response, which consisted of generalized cortical activation similar to the

classical cortical arousal produced by a sensory stimulus. These authors considered the excitatory effect to be due to activation of the ascending reticular activating system and described the first stage of hypoxia as a " state of enhanced vigilance ".

If carbon monoxide in smoke is responsible for the change in cortical activity by an action on the reticular formation, then the cortical response following the introduction of smoke into the lungs should be abolished by pentobarbitone, which blocks e.e.g. arousal following reticular stimulation (Bradley & Key, 1958). Although the effects of smoke in the presence of pentobarbitone were not as pronounced as the effects observed in the presence of mecamylamine, some cortical desynchronization was evident. Carbon monoxide may therefore exert a central action additional to that on the reticular activating system. This action could be on the cerebral blood vessels. Very low concentrations of carbon monoxide (0·2–0·5%) in the inspired air cause an increase in cerebrospinal fluid pressure (Maurer, 1941) and dilate the pial arteries (Sjöstrand, 1948), an indication of substantial cerebral vasodilatation and blood flow acceleration. Ingvar, Baldy-Moulinier, Sulg & Horman (1965) have provided evidence for a significant correlation between cerebral blood flow and the e.e.g., both variables being dependent on the oxidative metabolic activity of the nervous tissue.

Chlorpromazine blocks the arousal produced by a sensory stimulus, but has little effect on the similar response caused by direct stimulation of the reticular formation (Bradley & Key, 1958). The e.e.g. alerting response produced by adrenaline is depressed by chlorpromazine (Martin, Demaar & Unna, 1958), and Bradley, Wolstencroft, Hösli & Avanzino (1966) have shown that chlorpromazine can antagonize the excitatory actions of noradrenaline at the brain stem level, when both drugs are applied by iontophoresis. In the present experiments, chlorpromazine prevented the cortical activating effects of amphetamine, but did not antagonize the cortical activation or behavioural arousal caused by nicotine or cigarette smoke. This suggests that nicotine does not act by direct stimulation of central adrenergic neurones, or that its site of action in the reticular formation is rostral to that at which chlorpromazine exerts its effects.

In the *encéphale isolé* preparation, bilateral lesions in the posterior hypothalamus augmented irregular slow wave activity and spindle bursts in the cortex (Torii & Wikler, 1966). These changes were similar to those produced by atropine, and led to the conclusion that effects of atropine are mediated by actions on the posterior hypothalamic-thalamic diffuse projection system. The finding that atropine applied topically to the cerebral cortex in low concentrations can elicit pronounced slow wave activity (Armitage *et al.*, 1969b) and the evidence of Krnjević & Phillis (1963) that the excitatory effects of acetylcholine in the cerebral cortex are muscarinic in character, suggests that atropine may act at different levels within the central nervous system. The question of whether atropine modifies the central actions of nicotine indirectly at a subcortical or cortical level therefore remains unsolved. The fact that atropine did not prevent the behavioural arousal caused by nicotine, however, demonstrates that the neural pathways involved in the behavioural response are either non-cholinergic, or that the cholinergic synapse is insensitive to atropine.

The effects of cigarette smoke on cortical activity were not modified by vagotomy, suggesting that the response was not mediated partly by stimulation of sensory receptors in the lungs. Although a possible contribution from sensory mechanisms

in the nasal epithelium should be considered, it seems likely that when smoke is introduced into the lungs, nicotine is primarily responsible for the changes observed, although carbon monoxide may play a secondary role.

It is remarkable that small amounts of nicotine or cigarette smoke cause cortical and behavioural arousal in the presence of chlorpromazine, a drug which produces "a state of indifference and unresponsiveness to the environment and to sensory stimulation" (Bradley, 1968). This observation suggests that the pharmacological effect the inhaling cigarette-smoker may subconsciously be attempting to achieve is likely to be an extremely subtle one.

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