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The EEG of the Olfactory Bulb of the Rabbit and its Reaction to Psychopharmacological Agents*

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Introduction

Neurophysiological studies have shown that the olfactory bulb is an integral part of the brain (Kerr and Hagbarth, 1955; Lavin *et al.*, 1959; Ottoson, 1963). The olfactory bulb was also reported to exhibit persistent electrical activity (Gerard and Young, 1937), which was studied using different animal species, experimental set-ups and modes of approach.

In the unrestrained, chronically electrode-implanted, intact rabbit a rhythmic regular discharge of potentials was recorded in the awake state. According to Hernandez-Péon *et al.* (1960), it seems that this rhythmic pattern of electrical activity of the olfactory bulb reflects a state of alertness which changes with the physiological or behavioural state of the animal. It is intensified in the aroused state and in augmented olfaction, as in sniffing (Lavin *et al.*, 1959; Penaloza-Rojas *et al.*, 1964), and is slowed down and suppressed during relaxation and sleep (Faure and Vincent, 1964). On the other hand, with acute electrode preparations, Adrian (1950) found no rhythmic discharge of potentials but rather a spontaneous intrinsic activity of the olfactory bulb which persists after the olfactory bulbs have been disconnected from the brain or from the olfactory mucosa.

It is obvious that a state of alertness is reflected in the olfactory electrical potential and that mesencephalic centrifugal mechanisms of the central nervous system can influence the olfactory bulb (Lavin *et al.*, 1959). We have therefore reasoned that different psychopharmacological agents should affect olfactory bulb activity through their effect on the central nervous system. Our present experiments were designed to investigate the olfactory bulb potentials in acute and chronic electrode

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preparations of rabbits under different physiological conditions, and to elucidate the effect of different psychotropic agents on the olfactory potentials in terms of EEG and behaviour.

Material and Methods

Experiments were performed on twenty adult female rabbits, weighing 2.5 –3.5 kg each, with five acute and fifteen chronic electrode preparations. In the acute preparations, the animals were tracheotomized under ether and local procaine anaesthesia. Gallamine triethiodide (Flaxedil) was added to keep the animal immobile. Artificial respiration was provided at a continuous rate of 30 strokes per minute. Electrodes were implanted in the brain stereotaxically through burr holes in the exposed skull, according to the method of SAWYER *et al.* (1954). Coordinates were as follows: frontal cortex—(FC) A 5, L 2; olfactory bulb (OB)—A 22, L 2; an indifferent screw electrode served for grounding. After surgery, ether was discontinued at least two hours prior to EEG recording.

Chronic electrode-implanted rabbits were prepared by the method described in earlier reports (KHAZAN and SAWYER, 1963, 1964; KHAZAN and MCCASH, 1965). Animals were anaesthetized i.v. with 30 mg/kg pentobarbital and placed in a stereotaxic implantation instrument. The skull was exposed and holes were drilled into it with a dental burr. Two kinds of electrodes were used: a) two monopolar epidural silver electrodes on the frontal cortex and on one of the olfactory bulbs; b) bipolar concentric electrodes in the second olfactory bulb and in the dorsal hippocampus (DHPC), the coordinates being—P 3, V 5, L 4.5. The outer pole of these electrodes was made of stainless steel tubing and the inner pole of coated stainless wire. The electrodes were insulated, except at the tips, which were separated by 0.5 mm of insulation. The electrodes were cemented together by a dental cement "Grip", and the ends of the electrodes were then soldered to a 7-contact female miniature connector. The skin was drawn tightly around the mould and sutured; antibiotics were administered i.m. after surgery, and the rabbits were allowed to recover for one week before experiments were carried out.

For the experiments the rabbits were placed in a rectangular cage 17 × 50 × 35 cm, equipped with water and food supply, with one side made of square wire gauze which permitted observation of behaviour. Injections of the drugs used were given i.v. into the ear veins. The following drugs were administered: 1. pentobarbital sodium 5 and 30 mg/kg; 2. chlorpromazine HCl 2.5 and 5 mg/kg; 3. DL-amphetamine sulphate 2 and 4 mg/kg; 4. LSD-25 50 and 100 mcg/kg.

EEG recordings were taken with a Fritz Schwarzer EEG apparatus. For slow wave low frequency (S) records of the olfactory bulb, the filter

setting was adjusted to reject activity over 15 c/sec, and for high frequency (F) records it was adjusted to register activity as high as 70 c/sec (Fig. 1).

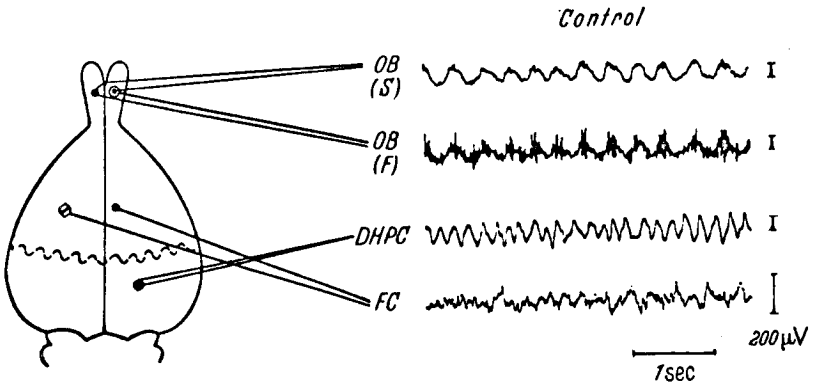


Fig. 1. EEG tracings in the rabbit with chronic indwelling brain electrodes. The upper two tracings are from the olfactory bulbs: *OB (S)*—Olfactory bulb slow frequency, filter setting is adjusted to reject activity over 15 c/sec to show the "slow" rhythmic activity of the *Respiratory Waves*. *OB (F)*—Olfactory bulb fast frequency, filter setting is adjusted to register activity as high as 70 c/sec to show the "superimposed" rhythm of the *Respiratory Waves*. *DHPC* = dorsal hippocampus, *FC* = frontal cortex. Time basis and voltage calibration are as indicated. Symbols and signs used apply to all subsequent figures

Results

The alert EEG pattern of the olfactory bulb in the rabbit can be classified into two main categories:

I. *Respiratory Waves* which are elicited in the EEG tracings of the olfactory bulb in the chronically implanted animal. These waves are illustrated in Fig. 1., and are of two types:

a) "Slow" rhythmic activity characterized by high amplitude low frequency waves of 2—4 c/sec corresponding to the rate of respiration,

b) "Superimposed" monomorphous sinusoidal rhythm superimposed in batches on the "slow" waves, having a frequency ranging between 55—65 c/sec. Their potential varies with the depth of respiration and state of alertness, and ranges between 100—400 μV.

II. *Intrinsic Waves* which appeared in the acute preparation, devoid of the rhythmic "slow" wave activity, presumably due to the absence of inspiration. They are characterized by low amplitudes ranging between 50—100 μV, and a frequency of 50—60 c/sec (Fig. 2).

In the acute experiments, the EEG tracing of the olfactory bulb was totally different from that presented by the chronically implanted intact rabbit. The electrical activity recorded here is mainly the *intrinsic activity* of the bulb. It can easily be made to adopt the *Respiratory Waves* a) and b) in an augmented form, by blowing air through the

nostrils of the animal's nose, when both potentials appeared and corresponded to the rate and amount of air blown in (Fig. 2).

During the awake state (Fig. 3), the cortex elicits a desynchronized EEG with high frequency waves. This predominant desynchronization of the cortex, together with the *Respiratory Waves* of the olfactory bulb, are

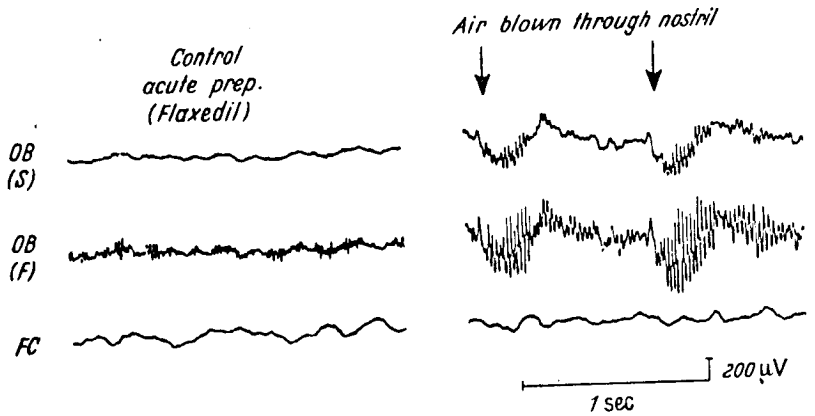


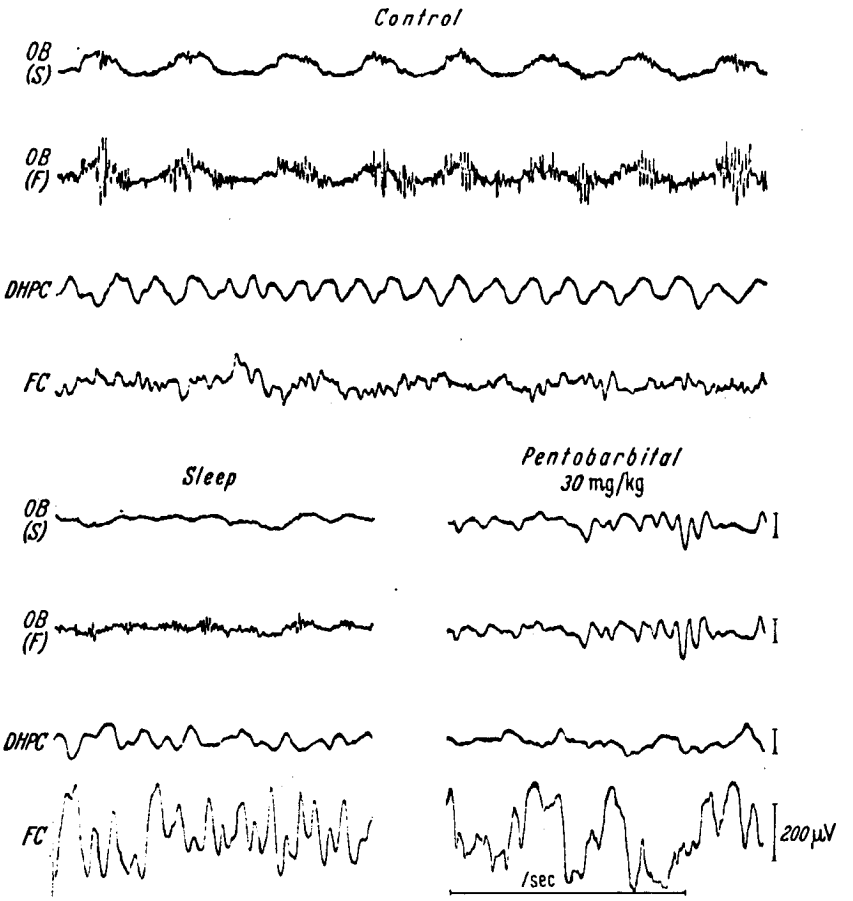
Fig. 2. The record on the left shows the EEG of the olfactory bulb in the acute immobile rabbit preparation. Note the lack of the rhythmic "slow" activity in *OB (S)* and Intrinsic Waves in *OB (F)*. On the right, both of the *Respiratory Waves* are shown as a result of artificial inspiration by blowing air through nostril, while *FC* activity remains unchanged

considered to be a typical pattern of alertness. During the relaxed state, the frequency of the "slow" waves, as well as the amplitude of both the "slow" and the "superimposed" waves, are diminished. This relaxed pattern would revert to the alert pattern, or even augmented alert pattern, through any sensorial or non-olfactory stimulus, where desynchronization is seen in the cortical EEG together with rhythmic hippocampal theta waves.

During sleep state, there is a progressive disappearance of the rhythmic "slow" waves, which are replaced by slow irregular waves having no relation to the respiratory rate. Suppressed activity of the "superimposed" waves is also seen. This pattern of olfactory bulb activity during sleep state shows a good correlation with the synchronized EEG of the cortex, characterized by high voltage slow waves accompanied by frequent "spindle bursts" (Fig. 3).

The administration of a sedative dose of pentobarbital (5 mg/kg), or of an anaesthetic dose (30 mg/kg), or of chlorpromazine (2.5—5 mg/kg) in the "chronic" rabbits, provoked synchronization of the cortical EEG. On the other hand, these agents exert different effects on olfactory bulb activity. A sedative dose of pentobarbital (5 mg/kg) induced suppression of both types a) and b) of the *Respiratory Waves*. The tracing regained

its pattern of alertness when the animal was aroused. Treatment with a hypnotic dose of pentobarbital (30 mg/kg) causes the disappearance of the *Respiratory Waves*, and the appearance of new non-rhythmic low-amplitude waves showing batches of rhythmic activity of higher



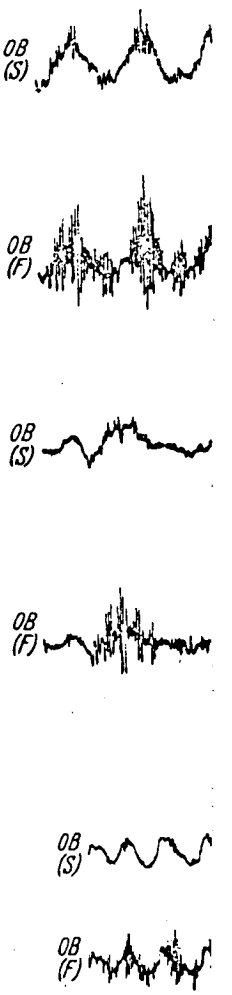


Fig. 3. EEG tracing of the rabbit during natural sleep and pentobarbital sodium sleep (30 mg/kg). Note the difference in the olfactory bulb's pattern of activity during sleep, and under pentobarbital anaesthesia. While in *OB (S)* high frequency "superimposed" waves are filtered off, *OB (F)* demonstrates the effect of pentobarbital in depressing frequency from 55—65 c/sec down to 12—16 s/sec. *DHPC* and *FC* are unchanged

Fig. 4. EEG effect of chlorpromazine on the olfactory bulb electrical activity *OB (S)* and *OB (F)*. Note the suppression of the "slow" waves and of the "superimposed" waves of the control by chlorpromazine corresponding to the behavioural depression of the rabbit

Fig. 5. EEG changes of the olfactory bulb in chronically implanted rabbits 15, 30, and 60 min after i.v. administration of 50 mcg LSD-25. Note the dramatic change in the tracing after 30 min, marked by an increase in frequency of both potentials of the *Respiratory Waves* in *OB (S)* and *OB (F)*. One hour later, there is an increase in the frequency of the "superimposed" waves by 15—25%, and of the "slow" rhythmic activity by about 100%, combined with a decrease in their amplitudes. This pattern continues for several hours

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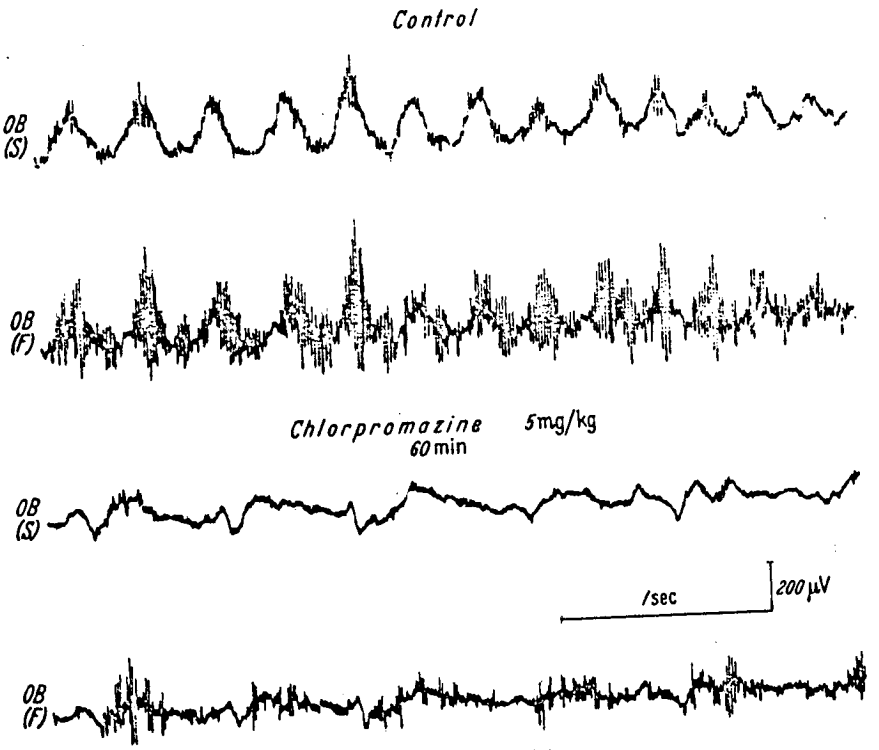

Fig. 4 (Legend see p. 230)

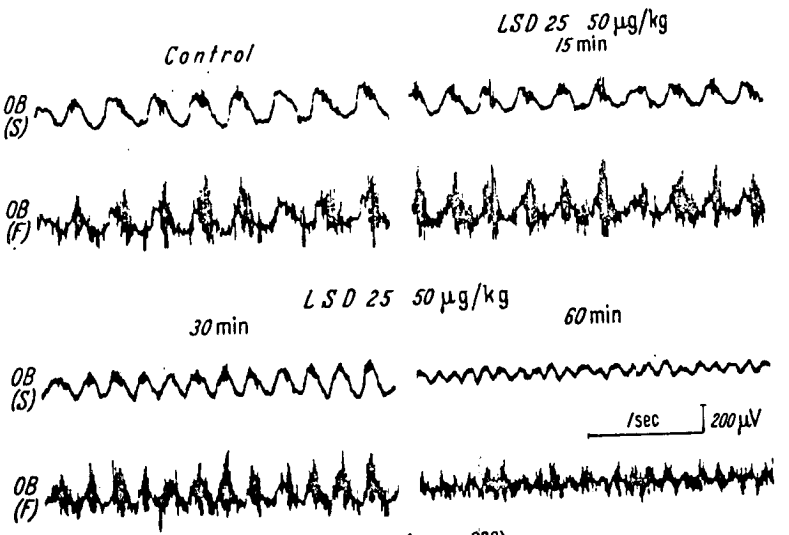

Fig. 5 (Legend see p. 230)

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frequency (12—16 c/sec). These batches can be regarded as the suppressed form of the "superimposed" waves which appeared along with the inspiration (Fig.3). Chlorpromazine induced suppression of the olfactory bulb electrical activity in a similar way as that induced by a sedative dose of pentobarbital, denoted by a marked reduction in the amplitude of the *Respiratory Waves*, bringing the voltage of the "superimposed" waves down to approximately 30—100 μV (Fig.4). Moreover, the frequency of the "slow" waves was also reduced to approximately 1 c/sec.

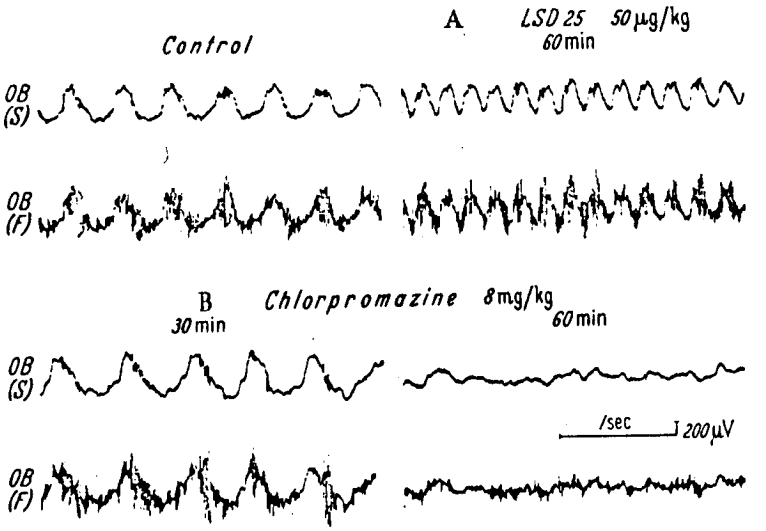

Fig.6. Blocking action of chlorpromazine (8 mg/kg i.v.) on the effect of LSD-25 (50 mcg/kg i.v.) in the EEG of the olfactory bulb, represented by retardation of the frequency of the "slow" waves almost to the pretreatment pattern (control) within 30 min, and successive suppression of the amplitudes of both "slow" and "superimposed" waves, at 60 min, as shown in Fig.4

Conversely, LSD-25 in doses of 50 and 100 mcg/kg induced a hyperalert pattern of the EEG of the cortex and of the olfactory bulb. There was a significant increase in the frequency of the "slow" waves reaching 6—7 c/sec within an hour. Batches of the "superimposed" waves seemed to be more compact, their frequency was increased by 15—25%, and this was accompanied by a remarkable and progressive reduction in the amplitude (Fig.5). However, amphetamine, in doses as high as 4 mg/kg, failed to induce the drastic change seen after LSD-25. The maximal increase of frequency of the "superimposed" waves was 5—15%, accompanied by an increase in frequency of the "slow" waves up to 4—5 c/sec.

The antagonistic effect of chlorpromazine in the LSD treated rabbit was obvious behaviourally and in the EEG (Fig.6).

Discussion

A study of the EEG-recordings of the olfactory bulb shows that a correlation exists between the pattern of its electrical activity and the behavioural or physiological state of the animal. The *Respiratory Waves* are induced by respiration as well as by the central nervous system state of arousal. As will be shown later, the states of relaxation, depression, or sleep on the one hand, or arousal on the other, bring about depression and augmentation of the *Respiratory Waves* respectively.

Wakefulness is regarded as a stage in which the reticular activating system plays an important role (Lindsley *et al.*, 1950; French and Magoun, 1952; Rossi and Zanchetti, 1957). The olfactory bulb activity has been considered to be influenced by the same system. The arousal discharges of the olfactory bulb activity were reported to be elicited by high frequency electrical stimulation of the mesencephalic reticular formation, and to disappear after lesions of that region (Kerr and Hagbarth, 1955; Lavin *et al.*, 1959; Hernandez-Péon *et al.*, 1960). Thus, physiological or pharmacological conditions which arouse, depress or block the physiological function of the reticular formation would elicit an essential change in the olfactory bulb electrical activity.

The olfactory bulb electrical activity becomes gradually suppressed by relaxation and sleep. During sleep, the rhythmic "slow" waves which are analogous to the slow potentials (Ottoson, 1954, 1959), and to electrical activity Type I described by Faure and Vincent (1964), are replaced by irregular flattened tracings; the "superimposed" waves which seem to be induced by the flow of the inspired air (Fig. 2) through stimulation of the receptors in the olfactory mucosa and which are seen as batches of 55—65 c/sec (Adrian, 1942, 1950; Faure and Vincent, 1964), are greatly suppressed (Fig. 3). This suppression reflects a central depression of the reticular activating system during the stage of sleep.

The lack of active inspiration through the nostrils in the tracheotomized animal, results in total disappearance of the *Respiratory Waves*. The remaining activity represents the *Intrinsic Waves* of the olfactory bulb (Adrian, 1950). It can be assumed that the "superimposed" waves of the olfactory bulb of the intact animal represent a stimulatory pattern of the *Intrinsic Waves* caused by the inspired air. Likewise, the appearance of the *Respiratory Waves* could be prevented by local anaesthesia of the olfactory mucosa, presumably as a result of the blockade imposed upon the transmission of the afferent impulses to the bulbs (Hernandez-Péon *et al.*, 1960). This assumption would also explain the augmentation of the *Respiratory Waves* of the olfactory bulb associated with sniffing, due to active olfaction (Gault and Leaton, 1963; Penaloza-Rojas *et al.*, 1964). The acute preparation, which exhibits only the *intrinsic*

activity of the olfactory bulb, can be made to show the *Respiratory Waves* a) and b) on blowing air through the nostrils. This would support the above assumption (Fig. 2).

Barbiturate anaesthesia (30 mg/kg) causes synchronization of the cortical EEG. The olfactory bulb activity was suppressed, leading to disappearance of the *Respiratory Waves* and the appearance of sinusoidal bursts of 12—16 c/sec which seem to represent a depressed pattern of the "superimposed" waves. The latter bursts of waves are correlated with the respiratory movements but did not seem to be comparable to the spindle bursts of the neocortex as suggested by HERNANDEZ-PÉON *et al.* (1960). The hippocampal activity characterized by its theta rhythm during arousal was also affected, resulting in an irregular tracing of slow waves. The change in the olfactory bulb electrical activity under barbiturate is presumed to be an indirect effect, emanating from the ascending reticular system of the midbrain and the limbic system which are both in a state of depression (FRENCH *et al.*, 1953; BRAZIER, 1963).

Under chlorpromazine, the rabbit is sedated but not sleepy. The tracing of the olfactory bulb observed after treatment with chlorpromazine (5 mg/kg) was more or less comparable to that seen during normal sleep (Fig. 4); in both cases, similar olfactory bulb activity is seen. This effect is likely due to the tranquillizing and depressant effect of the phenothiazine derivative on the reticular formation and on the hypothalamus (RINALDI and HIMWICH, 1955; BRADLEY, 1959), resulting in a suppressed CNS centrifugal activity.

In contradistinction to the chlorpromazine-treated rabbit, where the threshold for the sensory and environmental stimuli was elevated and olfactory bulb electrical activity suppressed, administration of LSD-25 (50 and 100 mcg/kg) caused behavioural excitation and activation of the EEG for as long as 5—6 hrs. The augmentation of the olfactory bulb electrical activity was expressed by a highly increased frequency of the "slow" waves by more than 100%, together with a 15 to 25% increase in the frequency of the "superimposed" waves. The pharmacodynamic antagonism of chlorpromazine and LSD-25 was clearly reflected in the olfactory bulb electrical activity (Fig. 6) in a way similar to their well-known clinical antagonism.

The physiological and pharmacodynamic findings obtained in these experiments would strongly reflect the effects of the drugs used on the CNS, conferred via the mesencephalic centrifugal system on the olfactory bulb.

Summary

The electrical activity of the olfactory bulb was studied on twenty adult female rabbits, weighing 2.5—3.5 kg each, in five acute and fifteen chronic preparations. The olfactory bulb, in the chronically implanted

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rabbit, elicited an electrical activity showing two types of potentials: a) "slow" waves of 2—4 c/sec and "superimposed" waves of 55—65 c/sec. Both of these potentials are induced by inspiration, and called the *Respiratory Waves*. The "superimposed" waves are regarded as a stimulated form of the spontaneous and persistent *Intrinsic Waves* of the bulb of the acute tracheotomized rabbit.

The *Respiratory Waves* are considered to be a normal pattern for the awake state of the animal. This pattern is depressed during states of relaxation and sleep, where the activity of the reticular activating system is suppressed. Likewise, pharmacological CNS depressants such as barbiturates and chlorpromazine, given intravenously to the animal, show a significant suppression of the *Respiratory Waves*, correlated to the degree of sedation and depression of the animal. On the other hand, amphetamine sulphate and LSD-25, known for their central stimulant effect, induced potentiation of the *Respiratory Waves* which is evident in the increase in frequency of both potentials.

The above physiological and pharmacodynamic findings come to emphasize the presence of a centrifugal CNS mechanism, playing an important role in the initiation of the electrical activity of the olfactory bulb. It may be concluded accordingly that the olfactory bulb electrical activity could serve as an additional tool in studying the possible mode of action of agents effecting CNS activity.

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