

VISCERAL AND METABOLIC CHANGES ON STIMULATION OF LIMBIC SYSTEM OF BRAIN OF MONKEYS*

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The control of the limbic system of brain over visceral and metabolic activities in the body has attracted the attention of many workers in the past few years (Anand, 1957). Various visceral and metabolic effects of stimulations of different limbic structures have already been reported (Kaada, 1951 ; Turner, 1954 ; Magnus & Lammers, 1956 ; Anand & Dua, 1956 & 1956a). Disturbances in these have also been reported as a result of destructive lesions involving the limbic structures (Anand, Dua & Chhina, 1957 ; Anand, Chhina & Dua, 1959). The results reported lack unanimity and these probably are due to differences in the techniques used and restricted studies.

The previously reported studies in response to stimulation (Anand & Dua, 1956a) were conducted mainly with the earlier available implements. With the availability now of modern electronic devices for recording different visceral responses, the study has been conducted more precisely. Secondly, the present responses have been recorded in anaesthetised animals, as against the previous study carried out in unanaesthetised animals, to observe the differences in results, if any, of stimulations of central nervous structures in anaesthetised states.

MATERIALS AND METHODS

These results are from 21 monkeys (*macaque*) in whom stimulations of the limbic system were carried out. In each of them, generally two to four multilead electrodes were implanted stereotaxically in different regions with the help of Horsley-Clark instrument, by the technique described elsewhere (Anand, 1955).

Stimulations were carried out with a Grass model S4 square-wave stimulator. The stimulations and recordings were carried out under Dial anaesthesia (0.5 cc./kgm. of a 10% solution injected intraperitoneally). Biphasic pulses with the following characteristics were employed for stimulation: pulse duration 0.1 to 10 milliseconds ; frequency 5 to 500 cycles/sec. ; and intensity 1-15 volts (peak to peak). The parameters found most suitable for eliciting blood pressure and respiratory responses were of frequency 50 c/sec.; pulse duration 2 m.sec.; and intensity 3-5 volts.

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Simultaneous records of blood pressure, respiration, skin temperature, hand volume, and electrocardiogram were taken during stimulation on a Grass model III D 3 channel, and an Offner type T eight channel electroencephalograph machines.

Blood pressure was recorded with a Statham 23 A pressure transducer attached to the E. E. G. machine through a demodulator unit. The transducer was connected to the femoral artery through a closed fluid system filled with 10% citrate solution. Respiratory movements were recorded by a strain gauge attached to an aluminium chest piece which was fastened by a strap around the chest and connected through a bridge to the E. E. G. machine. Smith (1938) has reported that for recording respiratory movements a pneumograph applied outside the chest is preferable to the tracheal cannula method, as that merely measures alterations of the intratracheal pressure and does not give accurate information regarding the respiratory phase in which the alterations occur. Blood flow through the hand was measured by a hand plethysmograph attached to a statham transducer which in turn was connected to the E. E. G. machine through a bridge. Skin temperature was recorded with a multilead thermistor bridge attached to the E. E. G. machine. Heart rate was calculated from the E. E. G. recordings.

Changes in the sugar, sodium, potassium and reduced glutathione contents of blood in response to each stimulations were also noted. One hour stimulations were carried out in unanaesthetised animals and samples of blood for these estimations taken before and immediately after the stimulation period. Only one region was stimulated on any day. Blood sugar was estimated by the method of Nelson and Somogyi (Hawk *et al.* 1954) and blood reduced glutathione by the method of Woodward and Fry (1932). Sodium and potassium were estimated with the Beckman direct reading flame photometer.

The areas stimulated were confirmed postmortem, both macro and microscopically. The different central nervous areas stimulated in all the animals are listed below:—

Areas Stimulated.	Number of animals.
1. Anterior cingulate gyrus.	12
2. Orbital surface of frontal lobe.	15
3. Tip of temporal lobe.	10
4. Amygdaloid nuclei.	12
5. Pyriform cortex and periamygdaloid region.	2
6. Hippocampus.	9
7. Hippocampal gyrus.	6
8. Supra-optic region of hypothalamus.	2
9. Lateral thalamus.	2
10. Head of the caudate nucleus.	4
11. Lateral temporal cortex.	2

TABLE 1

S. No.	Areas Stimulated.	Respiration	Blood Pressure	Heart Rate
1.	Anterior Cingulate Gyrus.	Increase in depth and in rate also.	Fall or no change.	No change.
2.	Posterior Orbital Surface of Frontal Lobe.	Marked slowing & arrest with stronger stimulation.	Fall, or fall followed by rise.	Increase, associated with arrest of respiration,
3.	Tip of Temporal Lobe.	Slowing and decrease in depth and arrest.	Mostly fall, followed by rise in some.	Variable.
4.	Amygdaloid Nuclei.	Slowing and arrest.	Mostly fall, or fall followed by rise in few.	Mostly increase.
5.	Pyramiform Cortex and Periamygdaloid Region.	No change, or some slowing at high intensity.	Very slight fall at 2 m.sec. pulse duration; fall at 5 m. sec. & 8 volts intensity.	No change.
6.	Hippocampus.	Mostly no change, or slight slowing at high intensity.	Slight fall at 7 volts & 10 volts only.	No change.
7.	Hippocampal Gyrus.	Arrest and slowing in majority, occasionally there was quickening.	Rise on stopping the stimulation.	Variable.
8.	Supraoptic Region of Hypothalamus.	Mostly increase in rate.	Fall.	No change.
9.	Lateral Thalamus.	Variable.	Fall or fall followed by rise.	No change.
10.	Head of the Caudate Nucleus.	Mostly slowing and increase in depth.	Fall, rise in some cases on stopping stimulation.	Variable.
11.	Lateral Temporal	Slight increase in rate and	No change.	No change.

RESULTS

Some of the cardiovascular and respiratory responses generally obtained on stimulation of different regions of the limbic system are summarised in Table I.

(1) **Circulatory Changes.**

(a) *Blood Pressure* : Fall in B. P. of varying degrees was observed on stimulation of most of the regions of the limbic system (Fig. 1, 5, 7, 10). In addition to the fall, in some animals fall followed by rise was noted on stimulation of posterior orbital surface (Fig.3), temporal tip, amygdaloid nuclei and lateral thalamus. Stimulation of hippocampus produced a fall only with high voltage of stimulation. Stimulation of the hippocampal gyrus, on the other hand, produced a rise in B. P. (Fig. 8). Stimulation of head of the caudate nucleus also gave this response in some cases. No change in B. P. could be produced on stimulation of the temporal cortex (lateral and inferior temporal gyri).

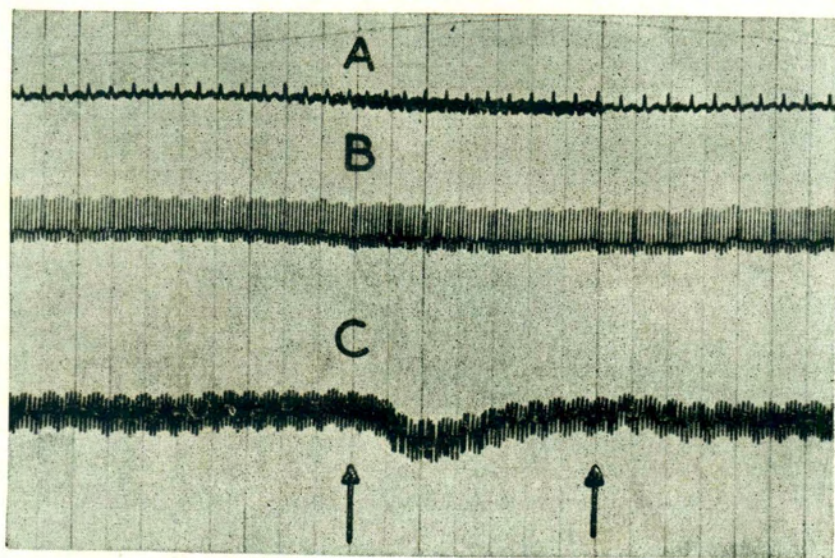


Fig. 1. Records of respiratory movements (A), electrocardiogram for counting heart rate (B), and blood pressure (C) in a monkey having electrodes implanted in the right anterior cingulate gyrus. Stimulation of this (between the two arrows) led to a fall in blood pressure, slight increase in depth of respiration, and no change in heart rate.

(b) *Heart Rate* : Changes produced in the heart rate on stimulation of different limbic regions, were quite variable. On most of the occasions stimulation did not produce any change in heart rate, at other time there was some

increase or decrease. Generally an increase in heart rate was produced on stimulation of amygdaloid and periamygdaloid regions (Fig.7), and an increase, accompanying respiratory arrest, was observed on stimulation of posterior orbital surface of the frontal lobes (Fig. 3).

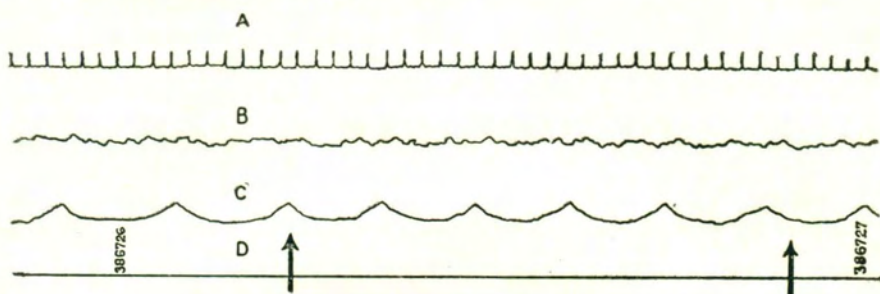


Fig. 2. Records of electrocardiogram (A), hand volume changes (B), respiratory movements (C), and skin temperature (D), in a monkey with electrodes implanted in the right anterior cingulate gyrus. Stimulation of this produced quickening of respiratory rate, slight increase in heart rate, and a slight decrease in hand volume indicating vasoconstriction. No change produced in skin temperature.

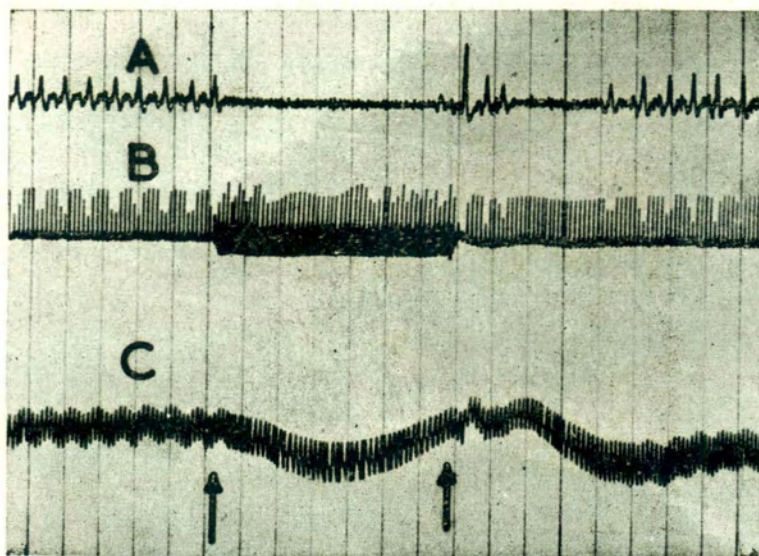


Fig. 3. Records of respiratory rate (A), electrocardiogram (B), and blood pressure (C), fall followed by rise in blood pressure, and some increase in heart rate in response to stimulation of the orbital surface of frontal lobe between the two arrows.

(c) *Changes in the Blood Flow Through the Hand:* Stimulation of the temporal tip, amygdaloid nuclei and hippocampus produced increased blood flow

through the hand (vasodilatation), as judged by increase in volume of the hand (Fig 6). Vasoconstriction was observed in majority of animals on stimulation of the orbital surface of the frontal lobes (Fig. 4), and in some after stimulation of the anterior cingulate gyrus and hippocampal gyrus (Figs. 2 & 9). Variable results were produced on stimulation of the head of the caudate nucleus. Stimulation of other regions did not produce any appreciable change in the volume of the hand.

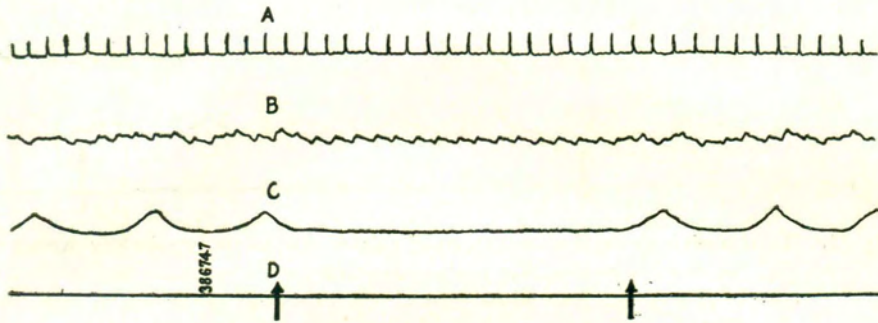


Fig. 4. Records of electrocardiogram (A), hand volume (B), respiratory movements (C), and skin temperature (D), Stimulation of orbital surface of frontal lobe led to respiratory arrest and vasoconstriction, but no change in heart rate and skin temperature.

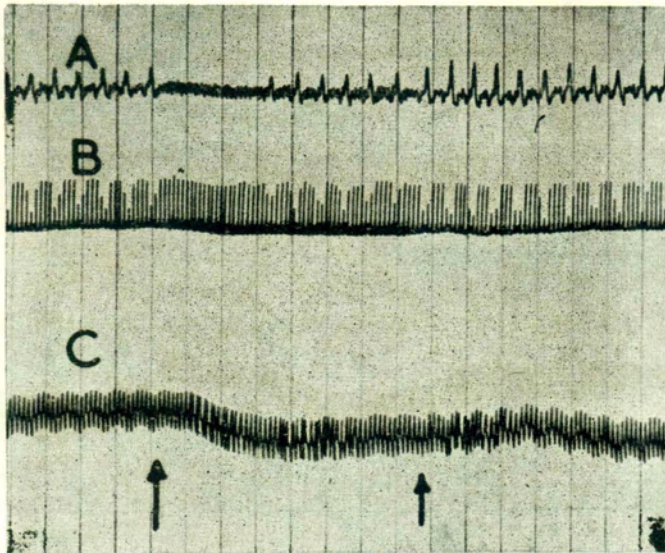


Fig. 5. Stimulation of the tip of temporal lobe in a monkey produced arrest of respiration (A), fall in blood pressure (C), and no change in heart rate (B).

(d) *Skin Temperature*: Skin temperature mostly registered no change on stimulation of the various structures, except on stimulation of caudate

nucleus there was generally some rise. Very slight increase or decrease was also occasionally produced after stimulation of other regions.

(2) Respiratory Changes.

Slowing of respiration, and its arrest on stronger stimulation, was obtained on stimulation of posterior orbital surface, temporal tip, amygdala, and hippocampus (Figs. 3,4,5,6,7 & 9). Some slowing and decrease in amplitude

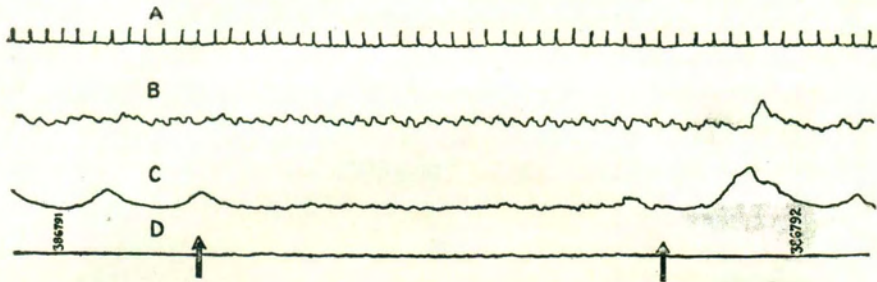


Fig. 6. Stimulation of tip of the temporal lobe produced arrest of respiration (C), slight vasodilatation (B), but no change in heart rate (A), and skin temperature (D).

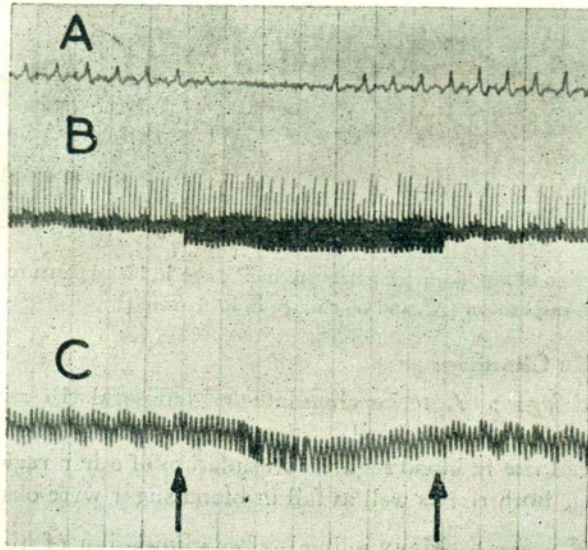


Fig. 7. Stimulation of amygdaloid nucleus in a monkey producing fall in blood pressure (C) arrest in respiration (A), and increase in heart rate (B).

was seen on stimulation of these regions with lower intensities. Increase in depth as well as in rate of respiration was produced on stimulation of the anterior cingulate gyrus (Figs. 1 & 2), and quickening was occasionally

observed on stimulation of the hippocampal gyrus (Fig. 8). Stimulation of the head of the caudate nucleus mostly produced some slowing with increase in depth of respiration (Fig. 10). Stimulation of supraoptic region of hypothalamus mostly increased rate of respiration while stimulation of lateral thalamus produced variable results.

Stimulations of pyriform and lateral temporal cortices, produced no change with lower intensities, while with very high intensities there was slowing or quickening of respiration respectively.

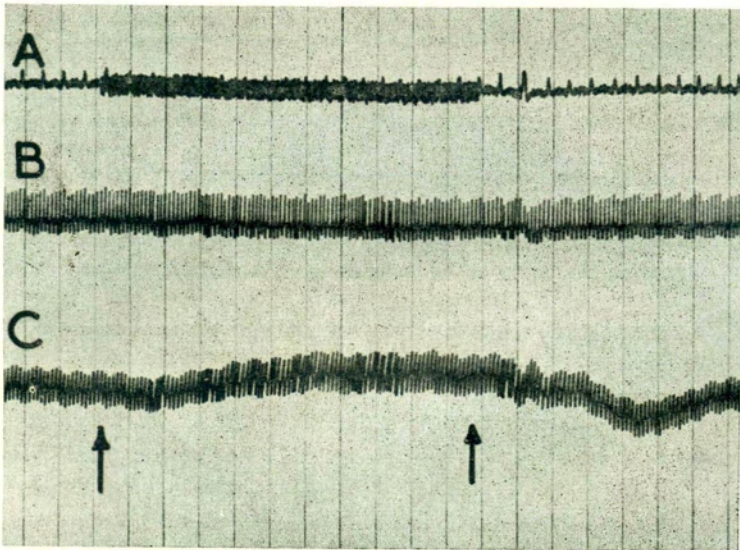


Fig. 8. Stimulation of hippocampal gyrus producing rise in blood pressure (C), slight quickening in respiration (A), and no change in heart rate (B).

(3) Metabolic Changes.

(a) *Blood Sugar*: Anterior cingulate and temporal tip stimulation produced a rise in blood sugar level. Stimulation of amygdaloid nuclei also mainly produced rise in blood sugar. Stimulation of other regions produced variable results, both rise as well as fall in blood sugar were observed.

(b) *Blood Sodium*: Many animals after stimulation of the orbital surface of the frontal lobe, anterior cingulate gyrus and temporal tip showed some fall in blood sodium. Other stimulations produced variable results.

(c) *Blood Potassium*: This was estimated only in two animals who had stimulation of the orbital surface and the anterior cingulate. There was some rise in it after these stimulations.

(d) *Blood Glutathione* : Reduced glutathione in the blood showed some fall after stimulation of the orbital surface. Anterior cingulate stimulation mostly produced some rise. Other stimulations produced mainly variable results.

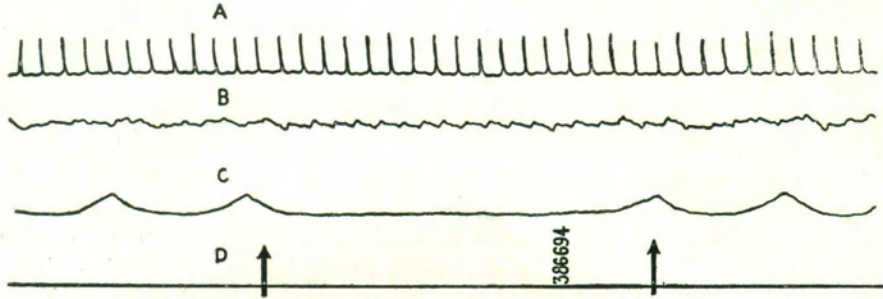


Fig. 9. Stimulation of the hippocampal gyrus leading to the arrest of respiration (C), some vasoconstriction (B), but no change in heart rate (A), and skin temperature (D).

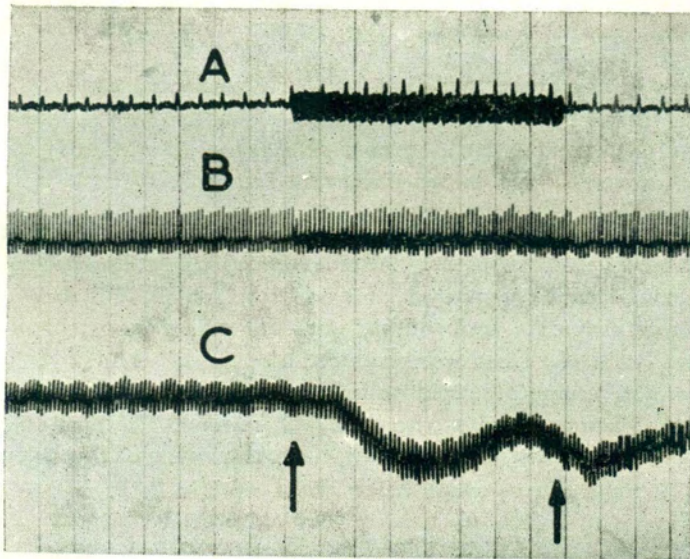


Fig. 10. Stimulation of the head of the caudate nucleus leading to fall in blood pressure (C), increase in depth of respiration (A), and no change in heart rate (B).

DISCUSSION

The parameters of stimulation found suitable for maximum respiratory and circulatory responses in this study were found to be different from those in conscious animals previously (Anand & Dua, 1956a). This possibly is due

to the effect of the anaesthesia used. The parameters of 10 m.sec. pulse duration found optimum by Kaada (1951), and also the optimum parameters reported by Koikegami *et al.* (1957), are also different from these. The differences may be due to different types of stimulators used giving different wave forms or due to different anaesthetic.

Blood pressure changes (mostly fall from different regions) obtained in the present study are different from the earlier report (Anand & Dua, 1956a) and similar to those reported by Kaada (1951). In unanaesthetised animals, stimulations of different frontal lobe structures produced a rise in B. P. In the present study on anaesthetised animals anterior cingulate stimulation did not produce any rise in B. P. The rise observed on stimulation of the orbital surface was also always preceded by an initial fall. Stimulation of head of caudate nucleus also produced a rise only on stopping the stimulation. These discrepancies are most probably due to the effects of anaesthesia, which thus favours the production of fall in B. P. Temporal lobe limbic stimulations both in the previous study (unanaesthetised) and the present one (anaesthetised) generally produced fall in B. P. These effects on B. P. are mostly opposite in character to those obtained by lesions in the frontal and temporal lobes (Anand, Dua, and Chhina, 1957; Anand, Chhina, and Dua, 1959).

Changes in heart rate, which were quite variable, did not show any relationship to the blood pressure responses. Similar observations were made previously also (Anand & Dua, 1956a). Koikegami *et al.* (1957), on the other hand found, a correlation between heart rate and B. P.

Peripheral vascular responses, as judged by volume changes in the hand, have given interesting responses. After temporal lobe stimulations, there was generally vasodilatation produced, along with fall in B. P. But on frontal lobe (anterior cingulate and orbital surface) stimulations, generally some vasoconstriction in the hand was produced alongwith fall of B. P. This would explain how in unanaesthetised animals stimulation of anterior cingulate and orbital surface produces a rise in B. P., (Anand & Dua, 1956a), and in anaesthetised animals, inspite of this, there are other changes (dilatation of vessels in other situations) which bring about a fall in B. P.

No correlation was observed between skin temperature and other cardiovascular responses on limbic stimulation.

Respiration was increased in depth and in rate on anterior cingulate stimulation, just as in unanaesthetised animals (Anand & Dua, 1956a). But no acceleration was produced from the orbital surface stimulation in the present study, on the contrary slowing and arrest of respiration was obtained. Again this probably is due to the effect of anaesthesia and use of different parameters of stimulation. It appears that inhibition and arrest of respira-

tion can be more easily produced in an anaesthetised animal rather than in a conscious one. Temporal lobe stimulations mostly produced inhibition of respiration, as reported earlier in unanaesthetised animals also (Anand & Dua, 1956a). Koikegami *et al.* (1957) reported a rise in the rate of the respiration from stimulation of amygdala, but the present study does not confirm that.

Changes observed in the blood sugar (mostly rise) are similiar to those previously reported (Anand & Dua, 1956).

In contrast to the ablation studies which resulted in majority of the animals in a rise in blood sodium level (Anand, Dua & Chhina, 1957; Anand, Chhina & Dua, 1959), these stimulations produced in majority of the animals a fall in blood sodium.

Glutathione has been reported to fall in humans by Persky (1954) in psychological stress, while physical stress has no effect. In the present study orbital surface stimulations lowered its level in the blood.

Ablations and stimulations of the same regions do not always give opposite responses as one might expect, but sometimes may produce similar responses. After ablations of the frontal and temporal structures respiratory slowing has previously been reported. (Anand, Dua & Chhina, 1957; Anand, Chhina & Dua, 1959). Respiratory inhibition has also now been observed to result from stimulation of these regions.

This study also clearly brings out that responses obtained in conscious (unanaesthetised) animals are not comparable with those obtained in anaesthetised ones. Anaesthesia also changes the optimum parameters of stimulation and this may also explain many of the discrepencies between the results obtained by different workers under the effects of anaesthesia. It also brings into relief the importance of carrying out central nervous stimulations in unanaesthetised animals.

SUMMARY.

1. In twenty one monkeys stimulations of different limbic system structures were carried out in anaesthetised animals, and changes in blood pressure, heart rate, hand volume, skin temperature, and respiration recorded by electronic gadgets. On other days estimations of blood sugar, sodium, potassium and glutathione levels were carried out both before and after such stimulations.

2. A fall in blood pressure was registered in majority of the animals on such stimulations. Some of these responses differ from those reported earlier in unanaesthetised animals and their significance is discussed. It was noted

that although temporal lobe stimulations produced a fall in B. P. and vasodilatation in hand, frontal lobe stimulations produced vasoconstriction along with fall in B. P. Changes in heart rate and skin temperature were quite variable.

3. Respiration was also mostly inhibited on such stimulation, except anterior cingulate stimulation which increased its depth as well as rate. Discrepancies between responses obtained in anaesthetised and unanaesthetised animals are discussed.

4. Blood sugar in majority of the animals was raised, and blood sodium lowered, while changes in blood potassium and glutathione contents were variable.

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