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On the Effects of Cutaneous Stimulations upon EEG, Respiratory and Muscular Activities of Frog

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The effects produced in the average frequency-patterns of EEGs from the olfactory bulb and forebrain of frog (*Rana nigromaculata*) by cutaneous pressure stimulations that are spatio-temporally constant in intensity and by repetitive tactile stimulations which are variable in intensity were observed. The changes in the respiratory movements and muscle activity produced by these stimulations were also observed and correlated with the EEG changes.

In correspondence with the "inhibitory" and augmentative effects in movements, muscle tone and respiration, decrease and, respectively, increase of the EEG potentials in both the olfactory bulb and forebrain were observed during tne application of the two types of stimulation. EEG potentials in the forebrain were augmented more effectively by contralateral than by ipsilateral tactile stimulation.

INTRODUCTION

It has been demonstrated that cutaneous pressure stimulus to a resting frog inhibits its voluntary and reflex movements and produces a state of *animal hypnosis* (TAKAGI 1953, 1954; WATANABE 1953, 1955), whereas a cutanetus tactile stimulus causes the animal to jump. In addition, the respiratory and cardiac movements of the frog are also inhibited and, respectively, augmented by these stimuli (SATO 1954, 1955; YAMAMOTO 1955, 1956).

In the present study, an attempt was made to determine whether the EEG led from the olfactory bulb and forebrain of the frog is also influenced by these stimuli in relationship to the modifications of voluntary and respiratory movements.

METHOD

Fourty three unanesthetized frogs (*Rana nigromaculata*) were used. To prevent movement artefacts during EEG recordings in seventeen

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frogs, nerves to the limb muscles were sectioned as many as possible.

After the brain was exposed under mild ether anesthesia or without anesthesia, the tip of the electrodes was placed on the surface of the olfactory bulb or the forebrain. Recording was usually monopolar, with the indifferent electrode placed on the bone; when bipolar, the interelectrode distance was about 2 mm. In most cases, silver wire electrodes, 0.25 mm thick and coated with polyethylene except their tips, were used, but in some cases a spiral electrode about one mm in diameter, made of silver wire 0.04 mm in thickness, was used with the monopolar method. Records were traced on bromide paper through a four-stage push-pull amplifier and an electromagnetic oscillograph (YEK, Tokyo) with an H-type vibrator.

Respiration was monitored by recording the throat movements. These were converted by air-transmission method to fine movements of a mirror pasted onto the rubber membrane of MAREY's tambour and the light beam reflected from the mirror was projected onto the bromide paper in a magnetic oscillograph to be recorded simultaneously with the afferent nerve impulses. Recording electrodes were placed on the cutaneous nerve moistened with RINGER-solution beneath an isolated skin fragment (Fig. 3, A), which was connected to the body by a single cutaneous nerve.

Pressure stimulus was applied through a clip on a 2 cm area of the skin of the back or lateral abdomen (Fig. 2). The pressure from the clip was equivalent to about one kilogram weight. Consequently, not only cutaneous pressure receptors but also pain receptors were stimulated. To observe the change in the respiratory movements, FREY's hair was occasionally used to deliver cutaneous pressure stimulus.

Repetitive tactile stimulation was delivered to the skin of the back by repetitive air jet flowing through a small tip of a glass tube, obtained by channeling a part of the air current from a rotary pump turning at about 2.5-3 c/sec. Its intensity was controlled to avoid eliciting any slight body movement. To observe the effects of tactile stimulation upon the respiratory movement and cutaneous nerve afferent discharge, tactile stimuli were delivered also by lightly touching the previously mentioned isolated skin with a feather of a cock (Fig. 3-C).

To obtain the average frequency-pattern of the EEG tracing, 120 (8×15) ordinated double harmonic analysis of KOBAYASHI (1953, 1955) or the instant frequency analyser of UEMURA et al. (1961) were used. The principle of this analyser (see Fig. 1) is as follows: the waveform to be analyzed is recorded magnetically on a 1/2 inch magnetic tape, using a pantagraph specially designed by UEMURA for boundary displacement type magnetic recording (BD recording). Next, the recorded magnetic tape (T) is placed in contact with a rotary magnetic play back head (RH), which is turned by an electric motor. As the head (H) rotates at the velocity of v cm/sec, it rubs against the lower

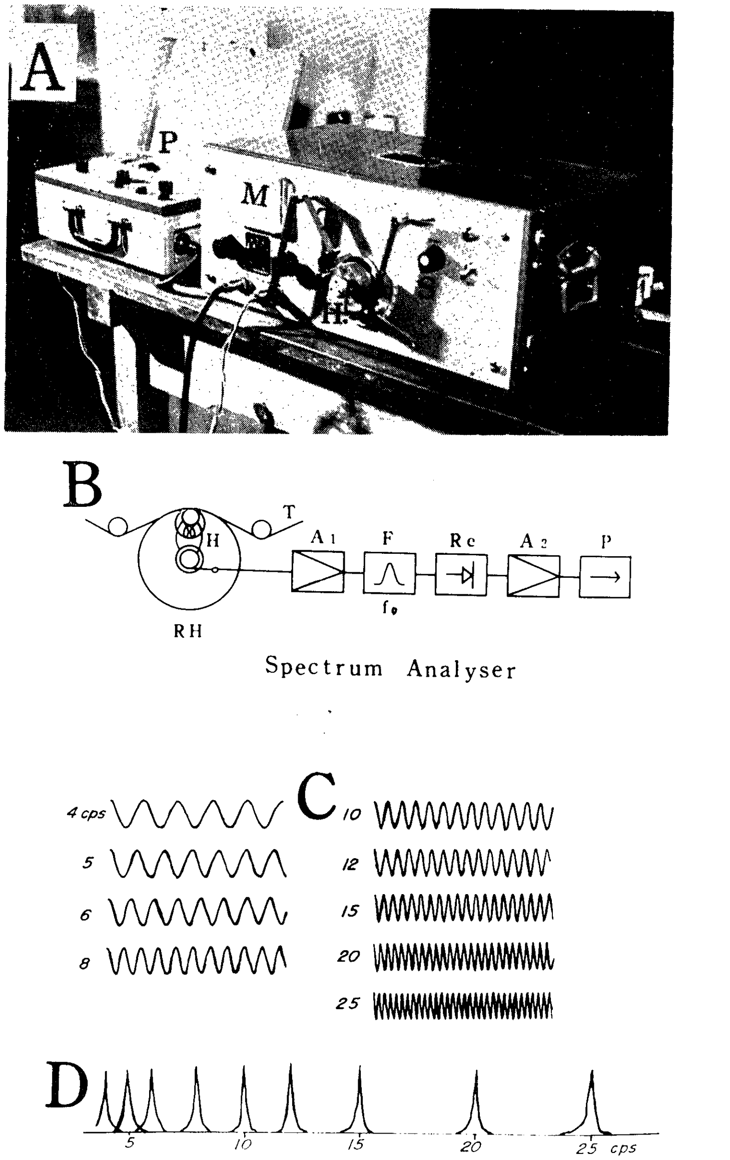

Fig. 1. The instant frequency analyzer for EEG.

A : Front view of the instant frequency analyzer for EEG.
M : galvanometer. S : Electric switch to rotate the rotary head H.
P : Pen recorder to trace the frequency spectrum of the wave form recorded on the tape T. (See text).
B : Block diagram of the analyzer. T : 1/2 inch magnetic tape, onto which the EEG is recorded for analysis. RH and H : Rotary head.
A_1 : First amplifier. F : Filter. R_e : Rectifier. A_2 : Second (DC) amplifier.
C : Sinusoidal waves of various frequencies (in c/sec). D : Analyzed peaks obtained from the various waves in C.

surface of the tape to produce an oscillating electric current with a frequency of f c/sec according to the following relationship:

(1) $f = vf_r$

where f_r is the frequency of the oscillation in the EEG recorded onto the tape. The oscillating current of f c/sec is amplified by the amplifier (A_1) and the output current of A_1 is passed through the filter (F). If the frequency of F is fixed to f_0 c/sec, then the frequency f in (1) becomes f_0, so that from the relation:

(2) $f_r = (1/v_0) f_0$ (f_0 : const.)

an oscillating current with a frequency of f_r c/sec, which is inversely proportional to the rotation velocity v of the head (H), is exclusively passed through F. Thus, the oscillation with this frequency in the EEG recorded magnetically on the tape (T) will be analyzed. The faster the rotation velocity of the head is, therefore, the slower the EEG oscillation to be analyzed and vice versa. The analyzed oscillating current is rectified by the rectifier (R_e) to obtain its amplitude. The rectified current is then amplified by the D. C. amplifier (A_2) for recording on paper by the heated stylus type pen recorder (P). Here, the rotary head H is set in motion by pushing the switch button (S). After the maximum rotation velocity is attained the head gradually loses its velocity at a constant rate. The galvanometer (M) gives a constant indication of the amount of outflowing current from the D.C. amplifier (A_2). During the period in which the head (H) increases its velocity, the sensitivity of the analyzer is checked and adjusted, and during the period in which the velocity decreases, the frequency spectrum of the EEG is traced by the recorder (P). By using this analyzer, a continuous frequency spectrum of the EEG recorded onto magnetic tape may be readily traced in about half a minute. In addition, signals to show the frequency used as the abscissa of the spectrum are also simultaneously marked along with the peaks in the spectrum being recorded.

RESULTS

Effects of Cutaneous Pressure Stimulation.

Prior to the operation, the effect of cutaneous clipping upon the muscular activity of the intact normal frog was observed first in an unfixed state, as illustrated in Fig. 2-b and c. Upon application of the clips, quickly reaches its maximal strength. In this brief period one can assume that the stimulus is mainly of a tactile type. This stimulus would predominantly elicit extensor reflexes which correspond to the jumping movement often caused by a tactile stimulus in normal frogs. Soon afterwards the stimulus becomes constant, continuous, of a pressure type and the frog assumes a posture characterized by

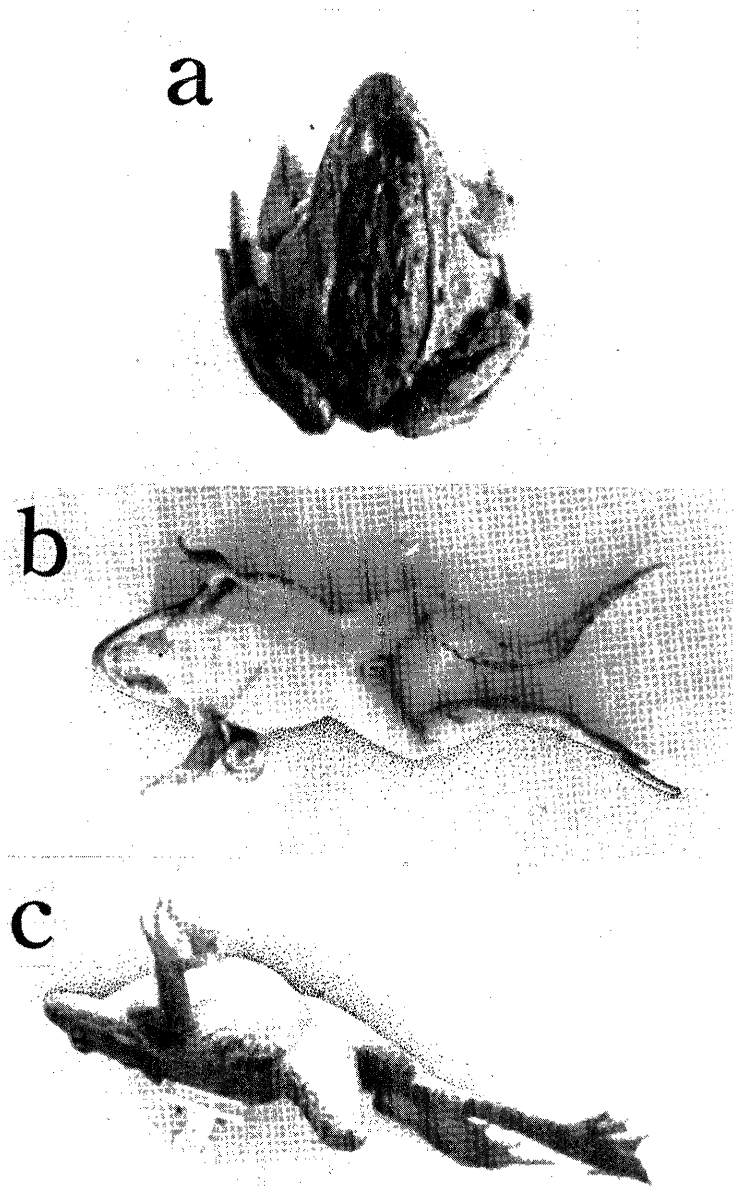

Fig. 2. Effects of cutaneous pressure stimulus upon the muscular activity of the normal and unfixed frog.
a : Control, resting state. b and c : Loss of muscular activity produced by cutaneous clipping.

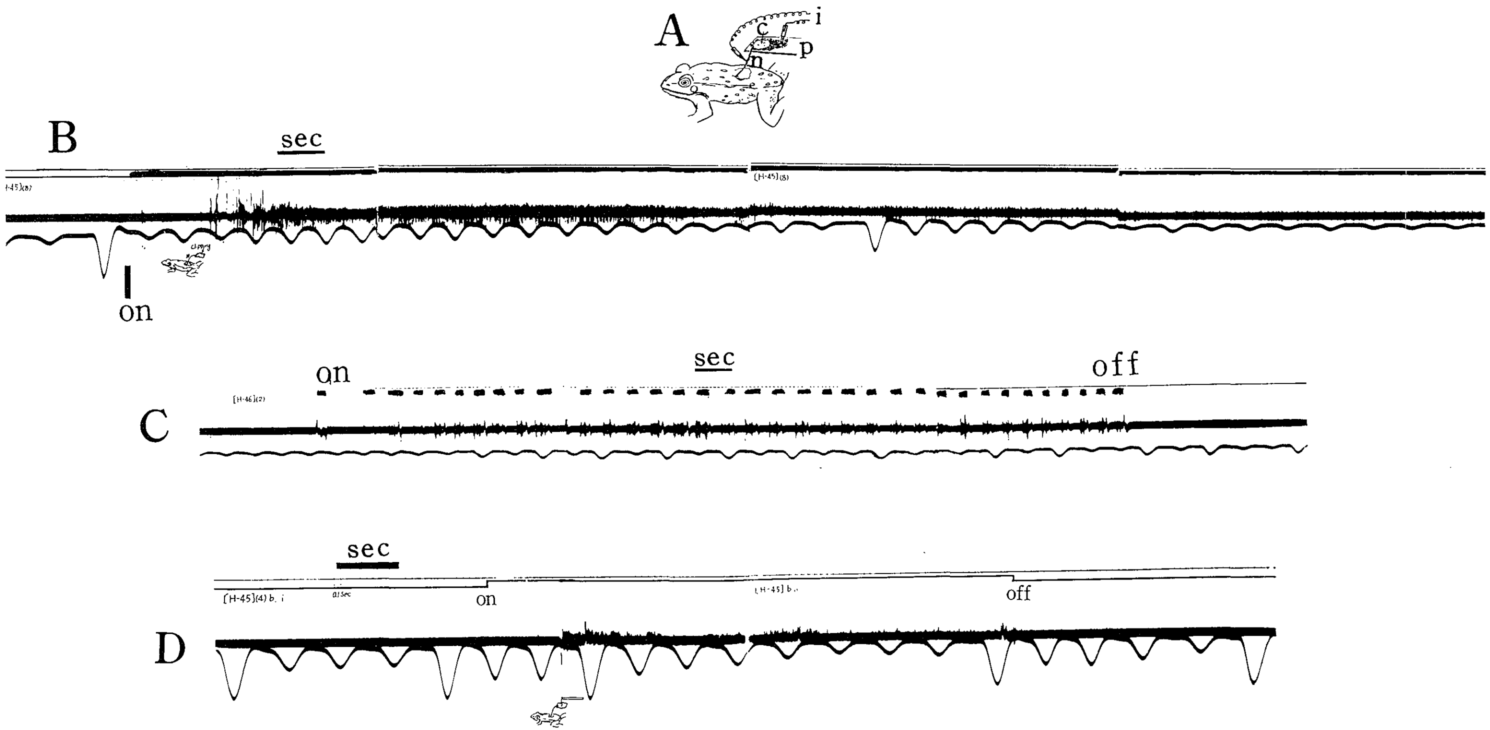

Fig. 3. Changes in respiratory movements produced by cutaneous pressure and repetitive tactile stimulations.
A : A piece of dorsal skin 'c', which belongs to the receptive field of cutaneous nerve 'n' and is connected to the body by this nerve only, was placed on absorbant cotton with Ringer's-solution on a polystyrole plate 'p'.
'i' : electrodes for recording cutaneous impulses.
B : Effects produed by cutaneous pressure stimulus (clipping applied at the 'on' signal).
C : Effects of repetitive tactile stimuli (light touch of skin 'c' with a cock's feather).
Upper tracing : nerve impulses. Lower tracing: respiration in B,C, and D.

extended limbs and showed no voluntary movements. In addition, the muscle tone was also decreased considerably (Fig. 2.b, c). When lightly touched, in this state, the frog is unable to react with movement (see TAKAGI, 1953). Augmentation of respiratory movements and trains of cutaneous afferent impulses were elicited immediately after the initiation of cutaneous clipping of the isolated skin fragment, which was connected only to the body by a single cutaneous nerve

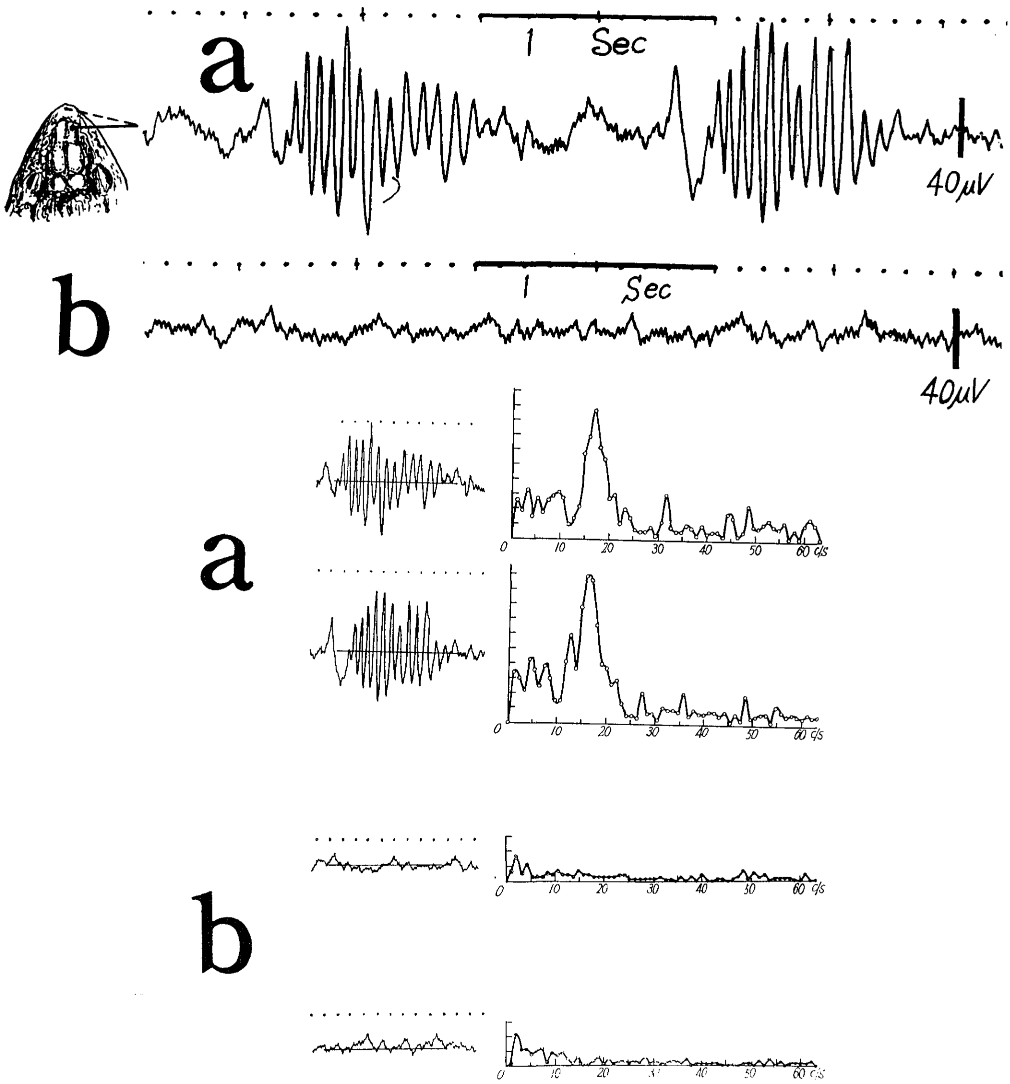

Fig. 4. Effect of cutaneous clipping upon the electrical activity of the frog olfactory bulb.

a : Control before stimulation. b : During cutaneous clipping.

On the top are the actual tracings, on the bottom right their respective frequency spectra. Base lines in EEGs on the left of the spectra indicate the range of double harmonic analysis.

(Fig.3-B). Progressively, the frequency of the cutaneous afferent nerve impulses decreased slowly and the amplitude of the respiratory movements gradually decreased (Fig. 3-B). Even with a very light pressure stimulus delivered by a FREY's hair, a similar effect could occasionally be observed (Fig.3-D).

The decrement in the frequency of cutaneous afferent impulses was more prominent during respiratory augmentation than during respiratory depression. Thus the augmentative effect of cutaneous discharges seemed to de related to rapid adaptation to a stimulus of progressively increasing intensity, whereas the depressive effect appeared related to slow adaptation to stimuli of spatio-temporally constant intensity.

One example of the most prominent effects of cutaneous pressure stimulation upon the activity of the olfactory bulb is shown in Fig. 4. The spindle like oscillations present in the control EEG tracing disappeared following pressure stimulation. In the frequency spectra, before stimulation, a predominant peak was located at about 17 c/sec, with other minor peaks in the lower and higher frequency ranges. All these peaks remarkably diminished during pressure stimulation.

From the forebrain, more irregular oscillations than those observed in the olfactory bulb were seen in the control state (Fig.5-A). In the

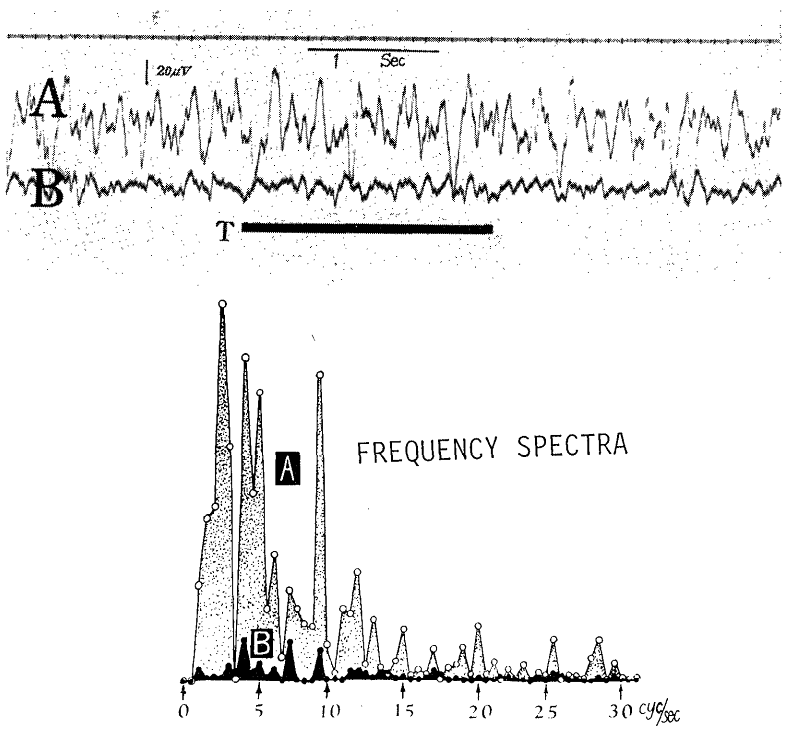

Fig. 5. Effects of cutaneous clipping upon the forebrain FEG of the frog.
A : Control. B : Cutaneous clipping. The black horizontal line 'T' indicates the portion of record analyzed (see respective frequency spectra underneath).

corresponding frequency spectrum, several peaks were seen in the frequency ranges of about 1-3, 3-7, 8-13 and 14-30 c/sec. In many instances the peaks in the frequency range below 7 c/sec was predominant and these around 10 c/sec were subdominant (Fig.5 and 8). Under the influence of pressure stimulation these EEG oscillations were considerably diminished (Fig. 5-B), the tracing assuming a pattern reminiscent of the *low voltage fast activity* of adult man and mammal. In its frequency spectrum, all peaks, predominant in the contorl state, were considerably lowered.

The Effect of Repetitive Tactile Stimulation.

During repetitive cutaneous tactile stimulation with either a feather (Fig, 3C) or air jet (Fig. 6), the respiratory movements would readily augment in amplitude and/or frequency (Fig. 6). Immediately after the onset of very feeble air jet stimulation it was rather difficult to detect any change in the activity of the olfactory bulb (Fig. 7). After about 1.3 sec, however, the amplitude of the oscillations would increase and this effect would persist after the end of the stimulation (Fig. 7). A large biphasic potential was observed between the spindle-like oscillations at intervals of about 2.5-1.5 sec in the control record obtained from the olfactory bulb but not in that from the forebrain (see Figs. 7 and 8). This potential was appeared repetitively at similar intervals of respiratory rhythm observed in Fig.6 to suggest electrical activity related to respiration. In the frequency spectrum three or four peaks were observed (at about 7.5, 9.5, 11-12 c/sec) in the control state: during cutaneous tactile stimulation the six peaks at about 2.5-3.5, 6.5, 9, 11.5, 13 and 25 c/sec were enhanced while the two peaks at 7.5 and 9.5 c/sec were lowered. The enhanced peak in the frequency range of 2.5-3.5 c/sec might reflect the above-noted biphasic potential. This potential (Fig. 7) was not included in the analysis of the control spectrum but only in the analysis during the stimulation, since it was not occured in the analysed area in the control EEG (Fig. 7A).

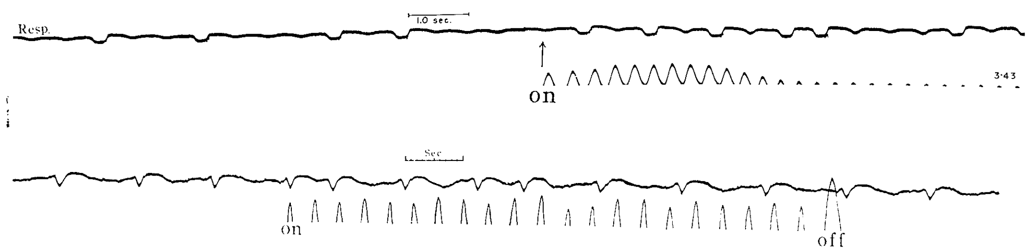

Fig. 6. Augmentative changes in the respiratory throat movements brought out by repetitive air jet stream to the skin of the frog.
Upper curves: Respiratory movements. Lower curves : Strength of air jet stimulation, lower half of which are scaled out.

The forebrain EEG showed similar effects from tactile stimulation through the feeble air jet after a period of about one second (Fig. 8). As illustrated in Fig. 9, the changes would depend upon the area of cutaneous tactile stimulation. From the back at the midline (M_p), no definite effects could be observed in the tracings themselves (M_p-A and M_p-B), but their frequency spectra showed a slight enhancement of several peaks (3.5-4, 6-7 and 10 c/sec) and a diminution of others (4.6-6 c/sec).

Contralateral stimulation of the posterior back (L_p) produced an incremental effect appreciable in both the EEG tracing and its frequency spectrum(L_p-B). The enhanced peak in the lowest frequency range was at about 2.7 c/sec (probably corresponding to the stimulation frequency). The same stimulation to a more proximal portion (L_a) caused enhancement of the main EEG rhythms (tracings L_a-A and L_a-B) and in their frequency spectra; all peaks between 3 and 13 c/sec were increased in height.

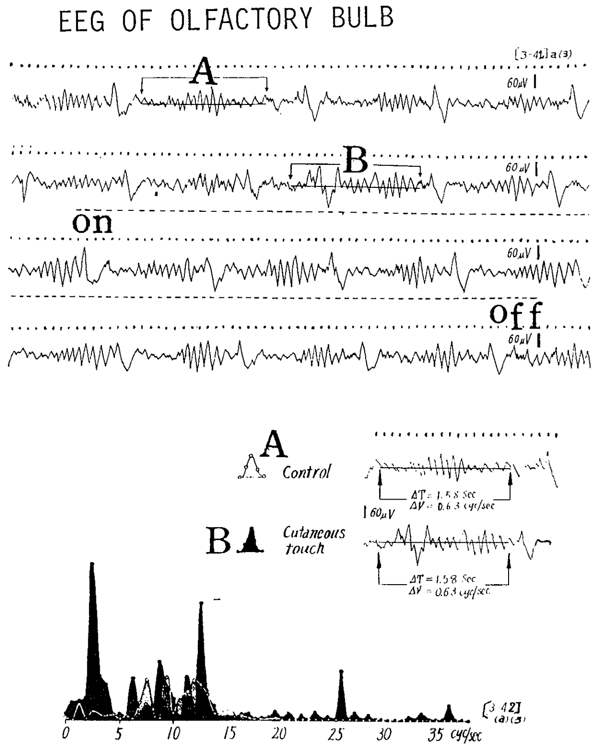

Fig. 7. Enhancement of electrical activity of the frog olfactory bulb produced by repetitive cutaneous tactile stimulation.
Very weak repetitive air jet stream of about 3/sec was delivered to the dorsal skin during the period indicated by the broken line between 'on' and 'off'. The dotted line on top of each tracing represents 0.1 sec intervals. The frequency spetra were obtained by double harmonic analysis from the portions 'A' and 'B', respectively.

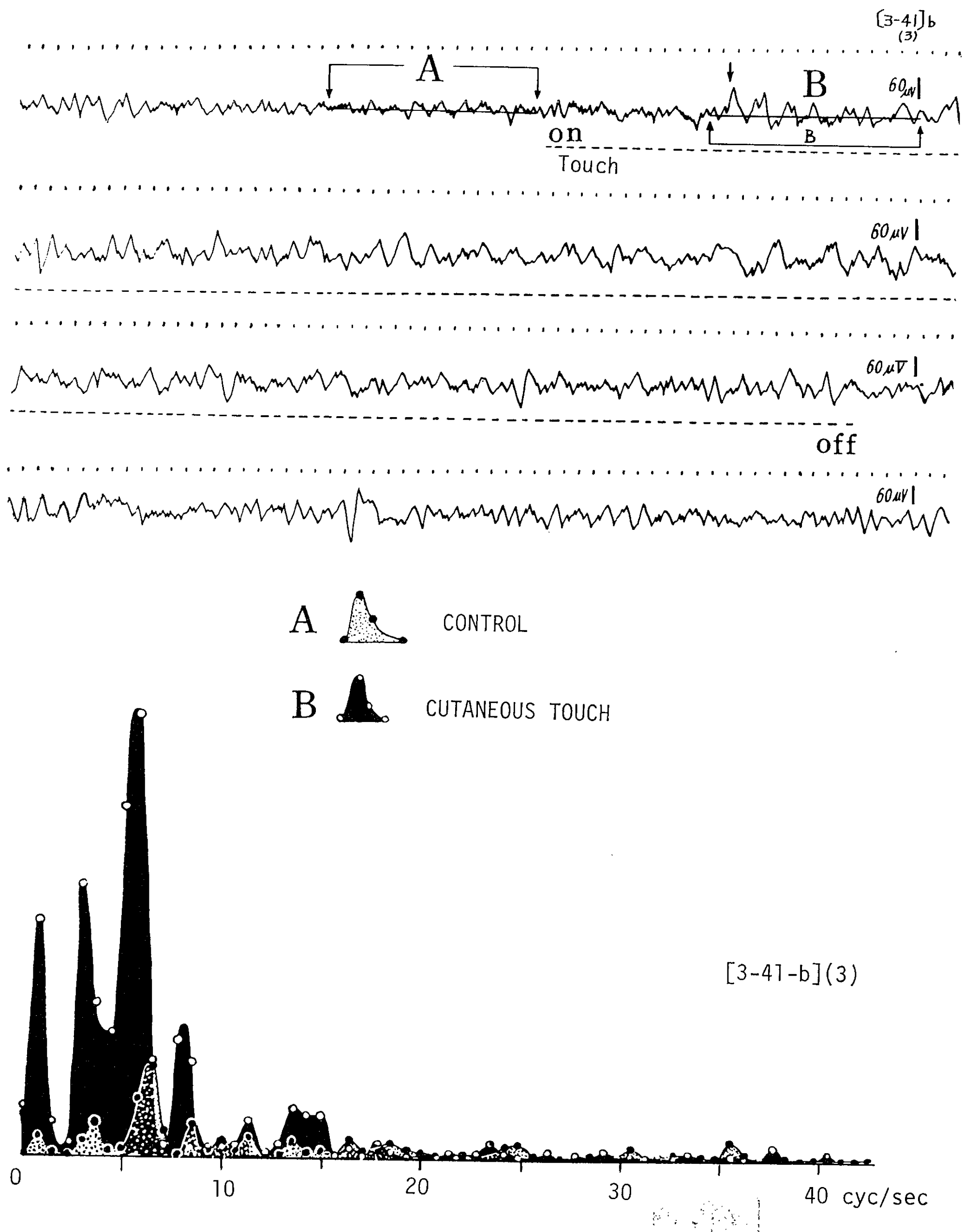

Fig. 8. Changes in the EEG from the forebrain of frog induced by repetitive cutaneous tactile stimulation.
Abbreviations see Fig. 7.

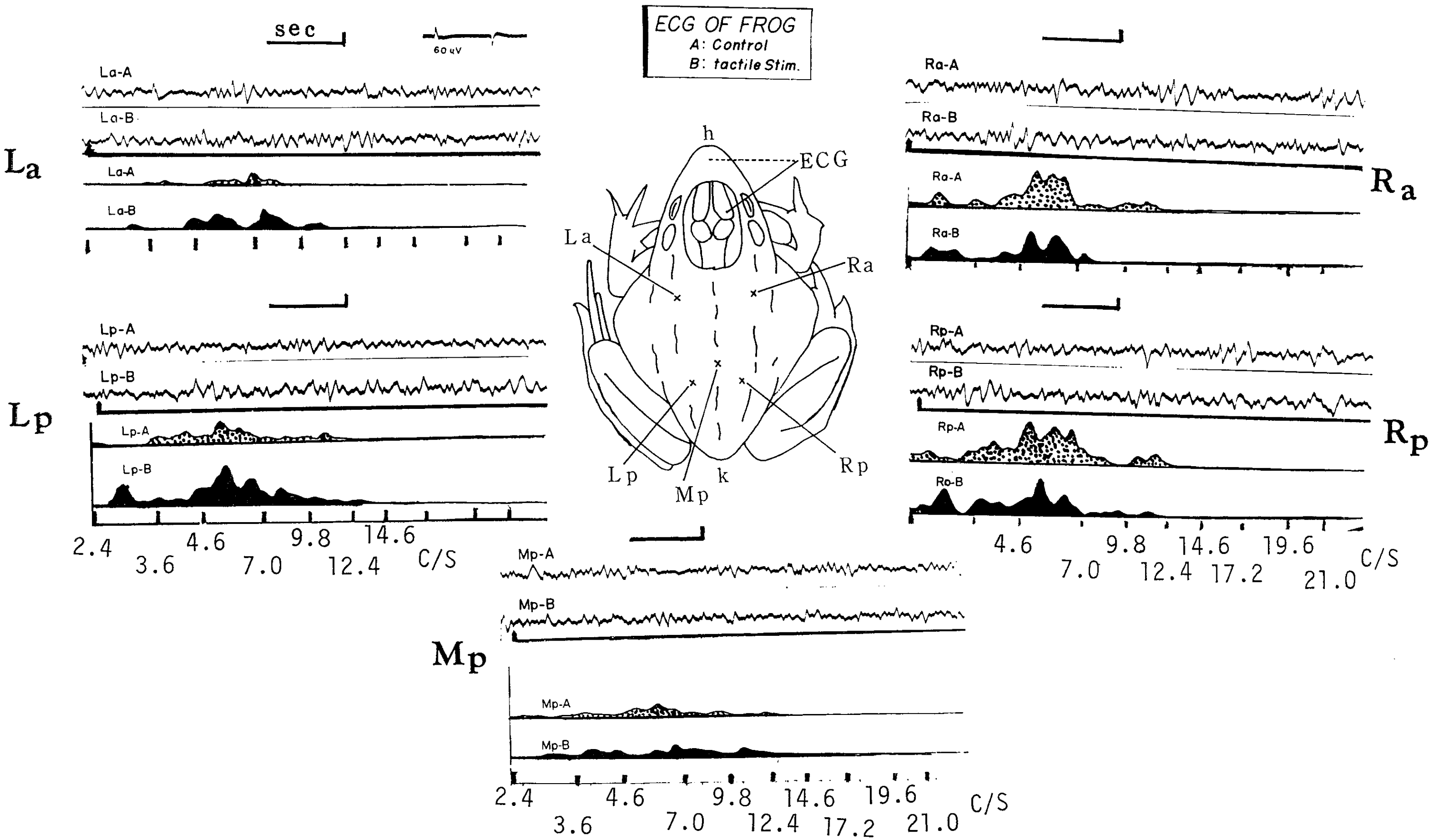

Fig. 9. Effects of cutaneous tactile stimulation by repetitive air jet upon the forebrain EEG of frog in relation to stimulus location. Middle; ECG : EEG recording electrodes. La, Lp Ra, Mp, and Rp represent the different locations for the application of the repetitive air jet streams.
Upper (A) and lower (B) EEGs are obtained before and during stimulation, respectively. Beneath the EEGs are corresponding frequency spectra.
Calibration: 1 sec and 60 μV.

Ipsilateral stimulation of the posterior back (R_p), tend also to enhance the frequency range of 4-7.5 c/sec (tracings R_p-A and R_p-B). In the frequency spectra, however, the peak at the stimulation frequency (about 2.7 c/sec) was definitely enhanced, while the dominant peaks of the control record were markedly lowered and only one peak of the first harmonic frequency (about 5.4 c/sec) was predominant. When ipsilateral stimulation was delivered to a more proximal area (R_a), similar effects were also observed in both the EEG tracings (R_a -A and R_a-B) and the peaks in their frequency spectra.

From these observations it appears that incremental effects will be caused by tactile stimulation following depressive effects in some instances.

DISCUSSION

The frog olfactory bulb, in situ, was found to exhibit higher frequencies in addition to the main 4-7 /sec rhythms described by LIBET and GERARD (1939).

The electrical activity of both olfactory bulb and forebrain changed into a low voltage fast pattern as a result of cutaneous pressure stimulation in correspondence with *animal hypnosis*, a state of hypomotility and atonia and respiratory inhibition. This change in the electrical activity may be corresponded to blocking of the *deactivation* due to supra-maximal pressure stimulation demonstrated dy KUMAZAWA (1963). On the other hand, the activity of the same structures would, in general, *augment* following repetitive cutaneous tactile stimulation which also increased muscular and respiratory movements.

It was demonstrated (SATO 1965; SATO et al. 1965) that repetitive algebraic summations of an average visually evoked response in a human occipital scalp elicited by a single flash stimulus result respectively a large oscillatory and sinusoidal wave form, when the summations were carried out at every intervals of a period of theta wave (1/5-1/7 sec) and that of alpha wave (about 1/10-1/14 sec). On the contrary, low voltage fast wave pattern was obtained by the repetitive summation at every time intervals of about 1/15-1/25 sec, in which not only an additive effect, but some occlusive effect was also verified by the summation of less than 0.1 sec intervals (SATO 1965; SATO et al. 19 65). As the cutaneous clipping generates high frequency and sustained trains of afferent impulses to cause respiratory inhibition and hypomotility (Fig. 3-B and D), so there may be such an possibility that the above occlusive effect in EEG to bring out low voltage fast contour may be corresponded to these depressions.

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