CENTRAL DEPRESSANTS AND EVOKED CLICK RESPONSES WITH SPECIAL REFERENCE TO THE RETICULAR FORMATION IN THE CAT

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The present authors (1-3) have reported that the evoked potentials and ensuing rhythmic after-discharges in the auditory cortex of the unanesthetized cat caused by click stimuli are easily affected by change of environmental conditions. Furthermore, they have found that pentobarbital sodium, chloralose and chlorpromazine in small doses increase the magnitude of the cortical click responses without facilitating the evoked click responses in the inferior colliculus. Urethane and ethyl alcohol are quite unique in their actions, producing only a progressive decrease in the cortical responses.

Since the demonstration of central role of the reticular formation (RF) in the brain mechanisms by Moruzzi and Magoun (4), the ascending inhibitory and facilitatory functions have been discussed by many investigators. Domino (5) and Killam (6) have emphasized that the potentiation of the recruiting response by barbiturates is due to a release phenomenon of the recovery cycle in the thalamic relay through the ascending influence of the brain stem RF. In the previous papers (1, 3), small doses of pentobarbital sodium, chloralose and chlorpromazine are likely to facilitate the transmission of the auditory ascending impulses to the auditory cortex by inhibiting the activity of RF. The essential role of the RF in modification of the evoked auditory responses has been described by Hernandez-Peon et al. (7, 8), Desmedt et al. (9) and Chin et al. (10). However, the complex nature of interaction between the auditory responses and the RF still remains to be settled in detail.

In the present experiments the effects of electrical stimulation of the RF on the click responses in the auditory cortex and the relay nuclei were studied before and after the administration of central depressants in the cat.

METHODS

The encéphale isolé preparations of about 75 adult cats of either sex were used. The fixation of the animals, operative procedures and delivery of click stimuli were similar to those reported previously (1-3). The recording of the evoked potentials was not started at least earlier than 3 hours after the termination of ether inhalation in a sound-proof
room at the room temperature of 28±1°C. The potentials recorded by oscilloscope (San’ei Sokki, Type UB-203) were photographed using a long-recording camera. The EEG was monitored on ink-writing oscillograph (San’ei Sokki, Type IR-102).

The RF was stimulated with square waves delivered from an electronic stimulator (Nihon Koden, Type MSE-3) through paired dental brooches, insulated except the tip with about 2 mm interval. An interval of each click was 5 seconds. RF stimulation (1.0 msec, 300/sec) for period of about 7 seconds was started 1 second before certain clicks. Accordingly, one set of two click responses was obtained during a period of RF stimulation. The sites of stimulating electrodes in the RF (F: 4 to -12, L: 3, H: 1 to -10) were determined according to the topographical map of Snider and Niemer (11), and were confirmed histologically after the termination of the experiments by fixing the brain with intracarotid injection of formaldehyde.

Pentobarbital sodium, chloralose, urethane, ethyl alcohol and chlorpromazine hydrochloride dissolved in Ringer or saline solution were infused into the ulnar vein at a speed of 100 ml/hr.

RESULTS

1. Click responses during arousal and resting states

The auditory evoked potentials were simultaneously recorded from the left ectosylvian gyrus of the auditory cortex and one of the relay nuclei of auditory pathway such as the medial geniculate body of thalamus, inferior colliculus or cochlear nucleus. As

![Fig. 1. Evoked click responses during arousal and resting states.](image)

AC: Auditory cortex (middle ectosylvian gyrus), MG: Medial geniculate body of thalamus, IC: Inferior colliculus, CN: Cochlear nucleus.
shown in Fig. 1, the evoked click responses in the auditory cortex and medial geniculate body decreased usually in amplitude, when the spontaneous EEG in the motor cortex manifested an arousal pattern. During exhibition of a synchronized pattern of the cortical EEG intermingled with spindle bursts, the click responses in the auditory cortex and medial geniculate body showed rather an increase in magnitude. On the other hand, the click responses in the inferior colliculus and cochlear nucleus were not modified in connection with the pattern of the cortical EEG (Fig. 1).

2. Influence of RF stimulation on click responses in the auditory cortex

An amplitude of the click responses in the auditory cortex was changed according to the sites of stimulation in the RF. Fig. 2 illustrates a series of the responses before, during and after RF stimulation. In many of the cases, the click responses in the auditory cortex were reduced in amplitude by RF stimulation. However, stimulation of certain areas of the RF increased significantly the cortical click responses in amplitude. Fig. 3 shows influence of RF stimulation on the EEG recorded from the motor and auditory corticies. In accord with the manifestation of the EEG arousal pattern in the motor cortex, the spike potentials in the auditory cortex evoked by click stimuli decreased usually in amplitude and ensuing rhythmic after-discharges disappeared during and after RF stimulation. In some cases, however, the click spike potentials and the ensuing after-discharges were facilitated by RF stimulation, although the EEG in the motor cortex turned to the desynchronized pattern. In these cases, increasing the intensity of RF stimulation resulted in a reduction of amplitude of the click responses and a disappearance of the rhythmic after-discharges.

Repeated click stimuli at a frequency of 8/sec produced a series of the repetitive responses in the auditory cortex, as reported in the previous paper (2). RF stimulation also modified the repetitive click responses (Fig. 4). The duration of RF stimulation was about 10 seconds and the repetitive click stimuli started at 2 seconds delayed to the onset of RF stimulation. Then, both click and reticular stimuli were
FIG. 3. Influence of RF stimulation on EEG recorded from the motor and auditory cortices. MC: Motor cortex. AC: Auditory cortex; Time scale: 1 second.

interrupted simultaneously. Mostly, RF stimulation decreased the repetitive click responses in amplitude. The facilitation of the responses was also observed in response to stimulation of certain areas of the RF.

Therefore, the influences of RF stimulation on the cortical responses evoked by single click stimuli were assessed in correlation to the stimulating sites in about 20 animals. Fig. 5 is a sagittal section of the brainstem 3 mm apart from the median plane, and shows the sites of stimulation producing the inhibitory (filled circle) or faci-
litative (open circle) effect. In general, the inhibitory sites were diffusely distributed in the RF. On the other hand, the facilitatory sites were localized mainly in the pontine RF and rarely in the rostral and caudal parts of RF. Fig. 6 demonstrates the histological placement of stimulating electrode in the RF.

3. Influence of RF stimulation on click responses in the auditory relay nuclei

Fig. 7 illustrates the effects of stimulation of the inhibitory area of RF (F: 2, L: 3, H: -1) on the click responses in the auditory cortex and the relay nuclei of the auditory pathway. During RF stimulation the amplitude of the click responses recorded from the auditory cortex and medial geniculate body was decreased by 30.1 and 25.6%, respectively (Table 1). On the other hand, the magnitude of the click responses in the inferior colliculus was not changed by RF stimulation. The click responses in the cochlear nucleus showed a slight decrease in amplitude (15.3%) during RF stimulation.

**Fig. 7.** Influence of RF stimulation on single click responses in the auditory cortex (AC), medial geniculate body (MG), inferior colliculus (IC) and cochlear nucleus (CN).

<table>
<thead>
<tr>
<th>Recording site</th>
<th>No. of animals</th>
<th>% change</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auditory cortex</td>
<td>17*</td>
<td>-30.1</td>
<td>± 3.1</td>
</tr>
<tr>
<td>Medial geniculate body</td>
<td>3*</td>
<td>-25.6</td>
<td>± 7.5</td>
</tr>
<tr>
<td>Inferior colliculus</td>
<td>3*</td>
<td>1.5</td>
<td>± 2.2</td>
</tr>
<tr>
<td>Cochlear nucleus</td>
<td>4*</td>
<td>-15.3</td>
<td>± 5.3</td>
</tr>
</tbody>
</table>

* : Per cent change of the click responses in each animal is mean value of 10 trials.
4. Effects of central depressants on the modified click responses in the auditory cortex by RF stimulation

It was reported previously (1) that pentobarbital sodium, chloralose and chlorpromazine in the small doses increased the amplitude of the click responses in the auditory cortex and that these depressants in the large doses depressed the responses. These results

![Fig. 8](image1)

**Fig. 8.** Effect of pentobarbital sodium on the inhibition of cortical click responses caused by RF stimulation.

![Fig. 9](image2)

**Fig. 9.** Effect of chloralose on the inhibition of cortical click responses caused by RF stimulation.
were also confirmed in the present experiments. The intravenous infusion above 4 mg/kg of pentobarbital sodium abolished apparently the inhibitory effect of RF stimulation on the cortical click responses, as shown in Fig. 8. In this respect, chloralose and chlorpromazine behaved similarly with pentobarbital sodium. Chloralose in the infused dose of 30 mg/kg (Figs. 9 and 10) and chlorpromazine in the infused dose of 2 mg/kg (Fig. 11) abolished completely the depressing effect of RF stimulation on the cortical click responses.

On the other hand, urethane and ethyl alcohol behaved quite differently from pentobarbital sodium, chloralose and chlorpromazine. The present experiments confirmed

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**FIG. 10.** Effect of chloralose on the inhibition of cortical click responses caused by RF stimulation in three preparations.

Each point in the figure represents mean of 10 recordings.

**FIG. 11.** Effect of chlorpromazine on the inhibition of cortical click responses caused by RF stimulation.

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Fig. 11. Effect of chlorpromazine on the inhibition of cortical click responses caused by RF stimulation.
the previous findings (1) that the infusion of urethane and ethyl alcohol produced only a progressive decrease in the amplitude of the cortical click responses. Furthermore, the inhibition of the click responses caused by RF stimulation was invariably observed even the intravenous infusion of 400 to 1,000 mg/kg of urethane (Fig. 12). The effect of ethyl

![Fig. 12. Effect of urethane on the inhibition of cortical click responses caused by RF stimulation.](image)

![Fig. 13. Effect of ethyl alcohol on the inhibition of cortical click responses caused by RF stimulation.](image)
alcohol on the inhibition of the cortical click responses caused by RF stimulation showed a close resemblance to that of urethane, and the amplitude of the responses was apparently depressed by RF stimulation even after the infusion of 400 to 800 mg/kg of ethyl alcohol (Fig. 13).

**DISCUSSION**

The click responses recorded from the auditory cortex and medial geniculate body of thalamus were smaller in amplitude during the arousal state than during the resting one. However, no significant difference between both states was observed in amplitude of the click responses recorded from the inferior colliculus and cochlear nucleus. In another series of experiments using the unanesthetized, unrestrained cat with chronically implanted electrode, the present authors (12) showed that the cortical click responses were larger in size and more stabilized during the resting and drowsy states than during the alert one, and that the responses were smaller in the deep sleep state than in the alert one. These results coincided well with the findings reported by Chin et al. (10) and Huttenlocher (13). Therefore, it is considered that the resting state in the acute encéphale isolé preparations corresponds with the drowsy state, but not with the deep sleep state in the unrestrained animals.

The reticular formation is known to be not a homogenous structure in its function. Magoun and Rhines (14, 15) demonstrated the reticular descending inhibitory and facilitatory influences. A dual ascending mechanism of the RF was demonstrated by Cordeau and Mancia (16) and Magnes et al. (17), who pointed out the EEG synchronization produced by low-frequency stimulation of the brain stem RF. The present experiments also revealed the existence of both ascending inhibitory and facilitatory mechanisms of the RF in the auditory cortical responses. High-frequency stimulation of the inhibitory area which was diffusely distributed in the RF resulted in a decrease in amplitude of the cortical responses to single and repetitive click stimuli as well as in a desynchronization of the EEG in the motor cortex. However, stimulation of the certain areas of the pontine RF facilitated the cortical click responses in amplitude, although it resulted in the similar desynchronization of the cortical EEG, and increasing the stimulation intensity caused the decrease in amplitude of the cortical click responses. The contrary findings between the increase in the cortical click responses and the EEG desynchronization caused by stimulation of the facilitatory area in the RF suggested that the distribution of the EEG activating system in the RF might be somewhat different from that of the ascending inhibitory system to the auditory cortex.

Demetrescu and Demetrescu (18) reported that high-frequency stimulation of the pontine RF produced variable effects on the thalamocortical evoked potentials in the visual cortex of encéphale isolé cats with lesions in the central gray; i.e. stimulation of the ventral RF inhibited the evoked potentials, while that of the dorsal RF facilitated them. Dumont and Dell (19) showed an increase in amplitude and a shortening of latency of the cortical visual evoked potentials during RF stimulation. The excitation
and inhibition of cortical neuronal firing in response to light by mild RF stimulation were described by Fuster (20). The present experiments demonstrated that the facilitation or inhibition of the cortical click responses varied according to the sites of stimulation in the RF.

In the present experiments, RF stimulation inhibited slightly the click responses recorded from the cochlear nucleus and medial geniculate body, while the responses in the inferior colliculus were not modified by RF stimulation. The existence of the efferent pathway from the insulo-temporal cortex to the cochlear nucleus was indicated by Desmedt et al. (9). If this indication is allowed, the slight inhibition of the click responses in the cochlear nucleus is assumed to pass through this efferent pathway.

The previous observation (1, 3) showed that the small doses of pentobarbital sodium, chloralose and chlorpromazine facilitated the auditory transmission only in the supratthalamatic level of the cat, and that urethane and ethyl alcohol inhibited both the evoked click potentials in the auditory cortex and inferior colliculus. The present experiments also demonstrated the abolition of the ascending inhibitory effect of RF stimulation on the cortical click responses by the small doses of pentobarbital sodium, chloralose and chlorpromazine but not by urethane and ethyl alcohol. These evidences serve to confirm the assumption that the facilitation of the auditory transmission by the small doses of pentobarbital sodium, chloralose and chlorpromazine results from the suppression of the reticular inhibitory mechanism, i.e. the release phenomenon.

**SUMMARY**

The modification of the click responses recorded from the auditory cortex, medial geniculate body, inferior colliculus and cochlear nucleus induced by stimulation of the various sites of the reticular formation (RF) were studied in the encephale isolé preparations of cats. Moreover, the effects of pentobarbital sodium, chloralose, chlorpromazine, urethane and ethyl alcohol on the changes of the cortical click responses caused by RF stimulation were investigated. The results obtained were summarized as follows:

1. The click responses in the auditory cortex and medial geniculate body were larger in the resting state than in the arousal one, while the click responses in the inferior colliculus and cochlear nucleus did not change during both states.

2. The amplitude of the cortical responses to single and repetitive click stimuli decreased by high-frequency stimulation of the reticular inhibitory area which was diffusely distributed in the RF. On the other hand, high-frequency stimulation of the reticular facilitatory area which was mainly localized to the pontine RF produced an increase in amplitude of the cortical click responses.

3. A slight decrease in the click responses caused by RF stimulation was observed in the medial geniculate body and cochlear nucleus, but the click responses in the inferior colliculus was not influenced by RF stimulation.

4. Small doses of pentobarbital sodium, chloralose and chlorpromazine abolished the depression of the cortical click responses induced by stimulation of reticular inhibi-
tory area. However, urethane and ethyl alcohol did not affect the reticular inhibitory effect on the cortical click responses.

REFERENCES

1) Nakai, Y.: This Journal 14, 235 (1964)