STUDIES ON N-MONOMETHYLTRYPTAMINE

Report III. A tranquilizing action of N-monomethyltryptamine hydrochloride in chronic electrode-implanted cats

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N-monomethyltryptamine (NMT) and its derivatives possess a strong contractive action on the rabbit, guinea pig, rat and cat uteri in vivo and in vitro, and a slight tranqilizing action both in humans and in rats. Serotonin (5-hydroxytryptamine) also has a contractive action on animal uterus and has a calmative action. There is a close similarity not only in their structures possessing an indole nucleus but also in their functions between the NMT derivatives and serotonin. Therefore, the tranquilizing effects of NMT-derivatives—NMT hydrochloride (NMT-HCl)—on the neo-, paleo, and archicortex (and hypothalamus dorsalis) were studied as compared with serotonin in chronic electrode-implanted cats, and the following results were obtained:

1. *NMT-HCl has a calmative action which might be caused by lowered activation of amygdala. During the lowered activated state, the threshold of brain activity was raised about 170% after the administration of NMT-HCl.*
2. *After the injection of serotonin, calmness also appeared in the cats.*

INTRODUCTION

A series of our previous experiments indicates that N-monomethyltryptamine (NMT) and its derivatives possess a strong contractive action on the rabbit, guinea pig, rat and cat uteri in vivo and in vitro[1–4], and on the other hand, a slight tranquilizing action both in human beings and rats[5–6].

Serotonin (5-hydroxytryptamine) closely resembles NMT in the chemical structures i.e. possessing an indole nucleus, therefore, it is interesting to study whether or not they have similar physiological actions. In particular, recently the study of the function of indole nucleus has become a center of attraction. In the present study, we have tried to make it clear whether NMT and serotonin possess a tranquilizing action due to the presence of an indole nucleus, which is common to both of them.

MATERIALS AND METHODS

The experiments were made on 8 chronic cats by the Latin-square method at an interval of two weeks[7].

(1) Preparation of chronic cat

The cat anesthetized with pentobarbital sodium 30 mg/kg I. P. was placed on the stereotaxic apparatus. The purpose of the present study is to determine the responsive site of the brain to methyltryptamine, therefore we selected three cortexes. They are the neocortex (ant. sigmoid gyrus: the sensory motor area was usually chosen for recording electrical activity of the neocortex, because of its invariable sensitiveness to activations by various stimulations), paleocortex (pyriform lobe: instead of the pyriform lobe, the electrical activity of the amygdaloid nucleus was usually recorded because of their remarkable similarity) and archicortex (hippocampus). The nucl. hypothalamus dorsalis was also added for recording.

Paralleled bipolar recording and stimulating electrodes insulated except for their tips (polar distance: 0.5 mm) were inserted stereotaxically to both sides of the nucleus of hypotnalamus dorsalis, hippocampus and amygdala with the aid of a stereotaxic apparatus, and the atlas of SNIDER and NIEMER[8].

During the preparation, recruiting response in hypothalamus dorsalis and injury discharges in hippocampus and amygdala were used for determination of the depth of the electrode.

Recording EEG from the neocortex was taken by small wooden screws 1 cm apart.

After all the electrodes were in position, they were connected to the connector with the insulated lead wire, then the connector was fixed on the frontal bone by mounting with dental cement.

Three weeks after recovery, the prepared cats were placed into the soundproof cage and used for the experiment.

(2) Stimulation and recording

EEG were recorded by 4-channel ink-writing multiple polygraph (NIHON KHODEN RM-150). For the determination of the threshold level of brain activity, the electrical stimulation to the hippocampus was delivered from the electronic stimulator (NIHON KHODEN MSE-3).

The intensity of stimulus was 15 c/s, 1 msec wide, 5 second duration and 3.5 to 7.0 volts.

(3) Drug application

The NMT-hydrochloride (NMT-HCl), from our biochemical laboratory, and serotonin (Daiichi pure chemicals) were dissolved into the Ringer solution and applied intraperitoneally. 3-4 mg/kg of the two substances could cause a characteristic EEG pattern appearing on each lead. The location of inserted electrode tips was histologically verified after the experiment.

To adjust the cats to the experiment and to minimize emotional disturbances throughtout the whole experiment, each prepared cat was placed into the soundproof experimental cage one hour every day. After the cats had become emotionally stable, the experiments commenced.

Before the experiment, to ensure the cats were emotionally stable, they were taken into the cage again for about half an hour, until drowsy EEG pattern was obtained, i. e. 12-14 c/s, 80-100 μv with low voltage fast waves (25-30 c/s, 30-40 μv) in the sensory-motor area, 15 c/s, 20-30 μv wave in hypothalamus, mainly 8-10 c/s with a few 15-30 c/s, 20-30 μv wave inhippocampus, 20-25 c/s, 20-30 μv waves in amygdala (Fig. 1A. 2A).

RESULTS

(1) NMT-HCl administration

After injection of NMT-HCl 2-3 mg/kg, the cat went into a drowsy posture lasting 4-5 hours. EEG patterns characteristic of the sensory-motor area appear in the form of an increase of a slow wave, a few spindles (9-10 c/s) and irregular sharp waves[9] were derived from the hypothalamus and low voltage and fast waves from amygdala after 30 min (Fig. 2B). During 300 min after NMT-HCl injection, there were regular spindle bursts in the sensory-motor area, low voltage and fast waves in the hypothalamus, irregular decreased frequency (10 c/s) interspaced with a few fast waves (25-30 c/s) in hippocampus. In amygdala, a few regular slow waves (12-15 c/s) also intercurrently occurred (Fig. 2C).

Also after injection of NMT-HCl (3-4 mg/kg), the cat often defecated, urinated (in 6 cases) and vomited (6 cases) (Fig. 1B) and showed more attention to circumstance and detective movements (in all cases) (Fig. 1C, D), scratching the cage (in 2 cases), salivating (in all cases), glooming (in 4 cases), biting the lead wires (in 3 cases).

Some of these abnormal behaviors, which were called "rage-like behavior" (e. g. biting the lead wires, scratching the cage etc.) and "searching reaction" (detective reaction) lasted for 3-36 min, then the cats relapsed into the drowsy state, which was maintained for about 3-5 hours.

During the drowsy state, the external behaviors that we observed were the cats took a "sphynx type" or dropped their heads on their paws, eyes half-closed, and was oblivious to environmental changes (Fig. 1E, F.) (e. g., calling out her name, observer's movement etc.) If a strong or sharp stimulus was given, (e. g., clapping, flashing etc.), the EEG changed into the awake pattern immediately (some times one drowsy type would change to another) but the same drowsy pattern reappeared. In order to compare the drowsy state before and after administration of NMT-HCl, the experiment of the electrically repeated stimulations was performed and the thresholds of "searching reaction" were determined. The time interval of each stimulus applied on hippocampus was 30 min or more, the stimulation was given only in the drowsy state. During the stimulation, if adequately intensive (threshold voltage), the cat's eyes were fully opened, and "searching reaction" appeared (Fig. 1G). Even when the cats showed no reaction, we had to wait for 15 min in expectation of the recovery of brain activity[10] before a new experiment was attempted.

Electrical stimulation to the hippocampus in the drowsy state, produced a typical after-discharge of EEG at the opposite side of the sensory-motor area, hippocampus and amygdala.

In our experiments, because the intensity of the repeated stimulus was at the level of the threshold of hippocampal activity, the after-discharge did not spread to the whole brain but was only localized[10], and the "searching reaction" appeared. If the supra-threshold stimulus was given, the after-discharge would spread to the whole brain and severe "epileptical reaction" would appear. (Fig. 1H)

On electrical stimulation, we took EEG from the opposite side of the sensory-motor area and hippocampal for determination of the brain level threshold. The experimental results obtained are shown in Fig. 3.

As shown in figure 3C, the threshold of the brain activity was elevated after injection of NMT-HCl, and such high thresholds lasted as long as 5 hours.

(2) Serotonin

After administration of serotonin 3-4 mg/kg I. P., vomiting and "searching reaction" appeared in 5-23 min in all cats. About half an hour after injection, the cats showed drowsiness, Slow rhythmical waves appeared in the sensory motor area and in the hypothalamus. And regular low voltage, slow waves (10 c/s) with a few fast waves (18-20 c/s) appeared in the hippocampus. In the amygdala, 12 c/s slow waves appeared. After 5 hours, fast waves appeared again in all leads, and a few desynchronized spindles appeared in the amygdala (Fig. 4).

DISCUSSION

From appearances, the behaviour of the cats was the same before and after NMT-HCl administration. Actually, however, from the EEG, (lead from neo-, paleo, -and archicortex) the drowsy pattern, after administration of NMT-HCl, was different from that of non-treated cats. If we observe the brain activity, the threshold of hippocampal activities is higher by about 170% than non-treated brain activity, and such higher threshold can be maintained more than 5 hours.

Our unpublished data indicates this higher threshold can continue 9 hours, and beyond the threshold gradually lowered to its original level. (in 1 case).

As mentioned above, the threshold of response to electrical stimulation after administration of NMT-HCl was raised, and the cats showed little concern about their surroundings. At that time, the amygdala showed a low level activating pattern.

It may be assumed that the animal with amygdala destroyed and the animal treated with NMT-HCl showed similar behavior.[11,12].

Serotonin is one of the normal physiologial substances in the brain tissue. It has a synaptic inhibitory action and is considered to be one of the "inhibitory cerebral neurohumoral transmitters"[13] In our experiment, after the administration of serotonin, the abnormal behaviors of the experimental animals appeared together with EEG of low activity patterns in the hippocampus and amygdala lasting 30-40 min.

These changes resemmble those after NMT-HCl administration. It seems very likely that such calmative action of the serotonin and NMT-HCl was caused by the effect of the indole neucleus common to both substances on the amygdala, but definite conclusion must await further investigation.

Indole ethylamine is structurally close to NMT-HCl and serotonin. After the injection of indole ethylamine 20 mg/kg into the cats, because of temporary stimulation of the central nervous system, the animals were convulsive and extremely excited[14]. There have been clinical repor s that after oral administration of indole 2 gm, patients developed anxiety, headache and vertigo[14].

After the injection of 3-4 mg/kg of NMT-HCl or serotonin, the "vomiting," "searching reaction," "biting the lead" and "scratching the cage" appeared. These reactions may be explained as the results of temporary excitation of the central nervous system.

Although no definite conclusion can be drawn from such inadeguate experimental materials, it seems very likely that NMT-HCl, similar to serotonin, can cause a long lasting tranquilizing effect on the cats after short-lived excitation of the central nervous system possibly by indole ring.

SUMMARY

Tranquilizing effects of NMT-HCl on the neo-, paleo-, and archicortex (and hypothalamus dorsalis) were studied as compared with serotonin in chronic electrode-implanted cats, and the following results were obtained:

1. NMT-HCl has a calmative action which might be caused by lowered activation of the amygdala.
2. 3-36 min after administration of NMT-HCl, the cats exhibited abnormal behavior possibly resulting from severe excitation of the central nervous system.
3. The threshold of brain activity was raised about 170% after the administration of NMT-HCl as compared with that before its adiministration.
4. 3-23 min after the injection of serotonin, calmness also appeares in the cats. Vomiting and searching reaction appeared before calmness was produced.

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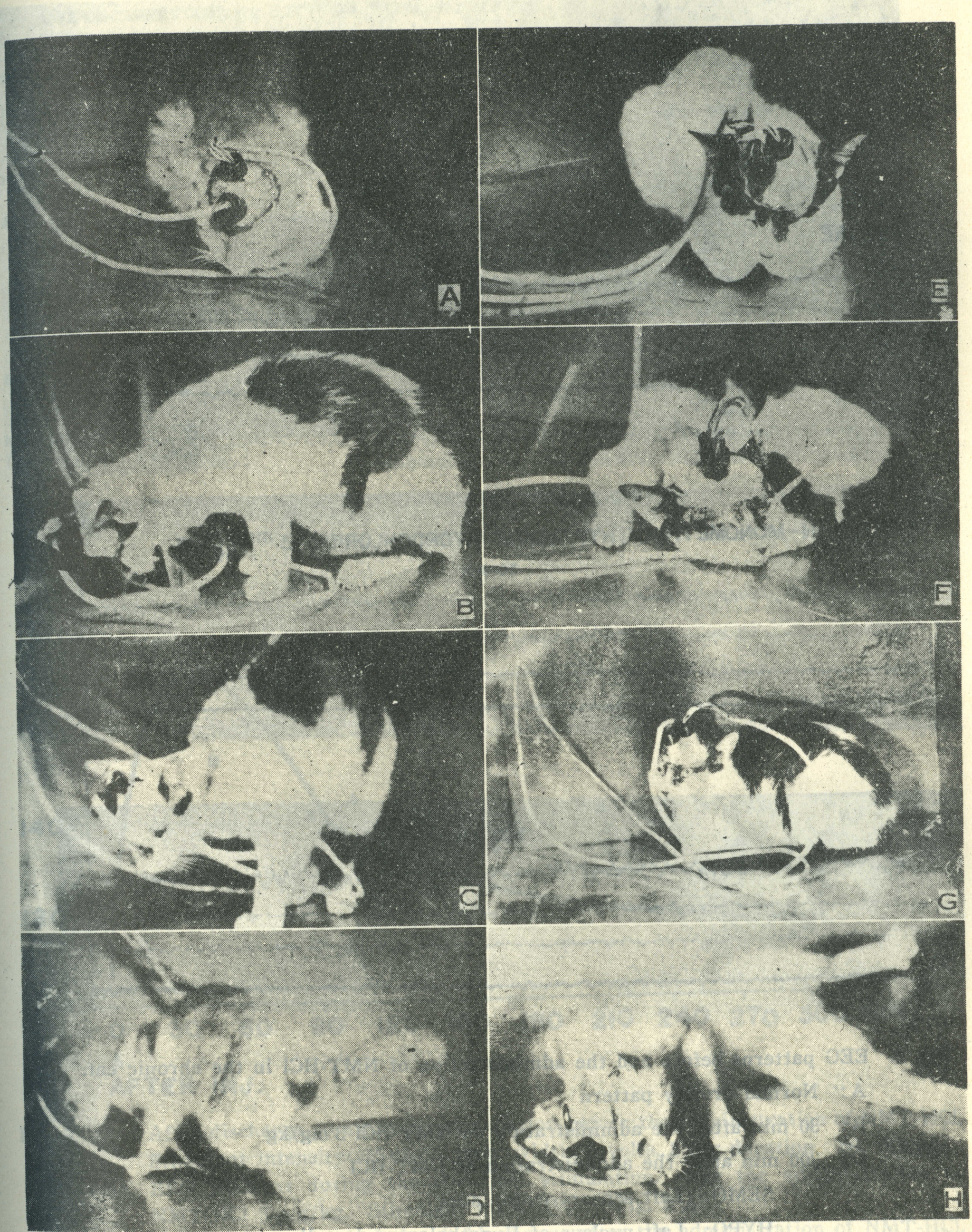


Fig. 1. A: The Cat has the emotional stable EEG pattern before the administration of NMT-HCl. #2. 2.7 kg.

B: Vomiting 17 min after the administration of NMT-HCl 4 mg/kg. #2. 2.8 kg.

C & D: Searching reaction 15 min after the administration of NMT-HCl. 3–4 mg /kg. C: #3. 2.8 kg. D: #2. 2.7 kg.

E & F: Types of drowsy state at 35–280 min after the administration of NMT-HCl. 3 mg/kg. #2. 2.7 kg.

G: Some attentive postures caused by adequate electrical stimulation (15 c/s, 1 msec width, 5 volts, 5 sec duration to contralateral Hippocampus). #2. 2.7 kg.

H: Like the "Epileptical reaction" caused by supra-threshold stimulation (15 c/s, 1 msec width, 15 volts, 5 sec duration to contralateral Hippocampus). #5. 3.2 kg.

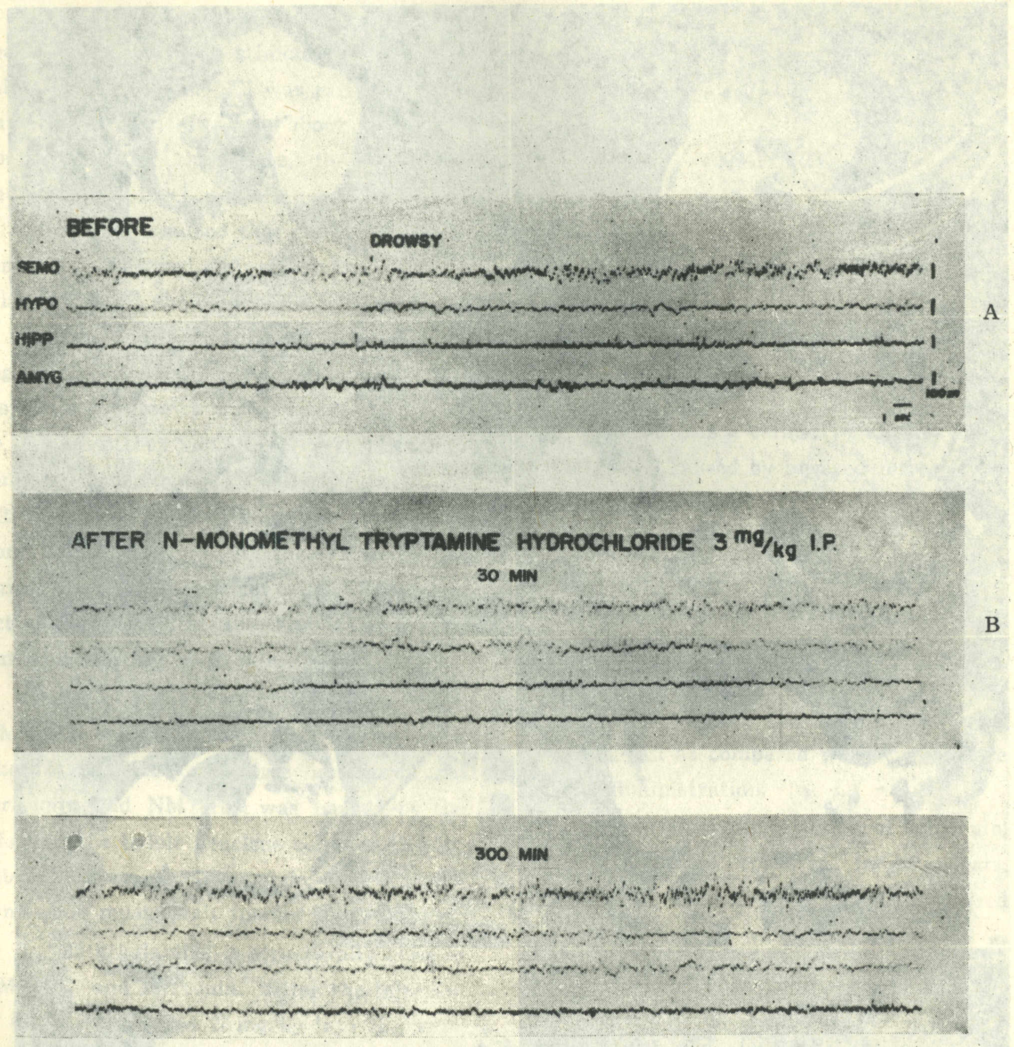


Fig. 2. EEG patterns before and the administration of NMT-HCl in the chronic cat.

A : Normal drowsy pattern

B : 30 min after the administration on NMT-HCl 3 mg/kg

C : 300 min after the administration of NMT-HCl

SEMO: Left sensory-motor area

HYPO: Left nucleus of Hypothalamus dorsalis

HIPP: Left Hippocampus

AMYG: Left Amygdala

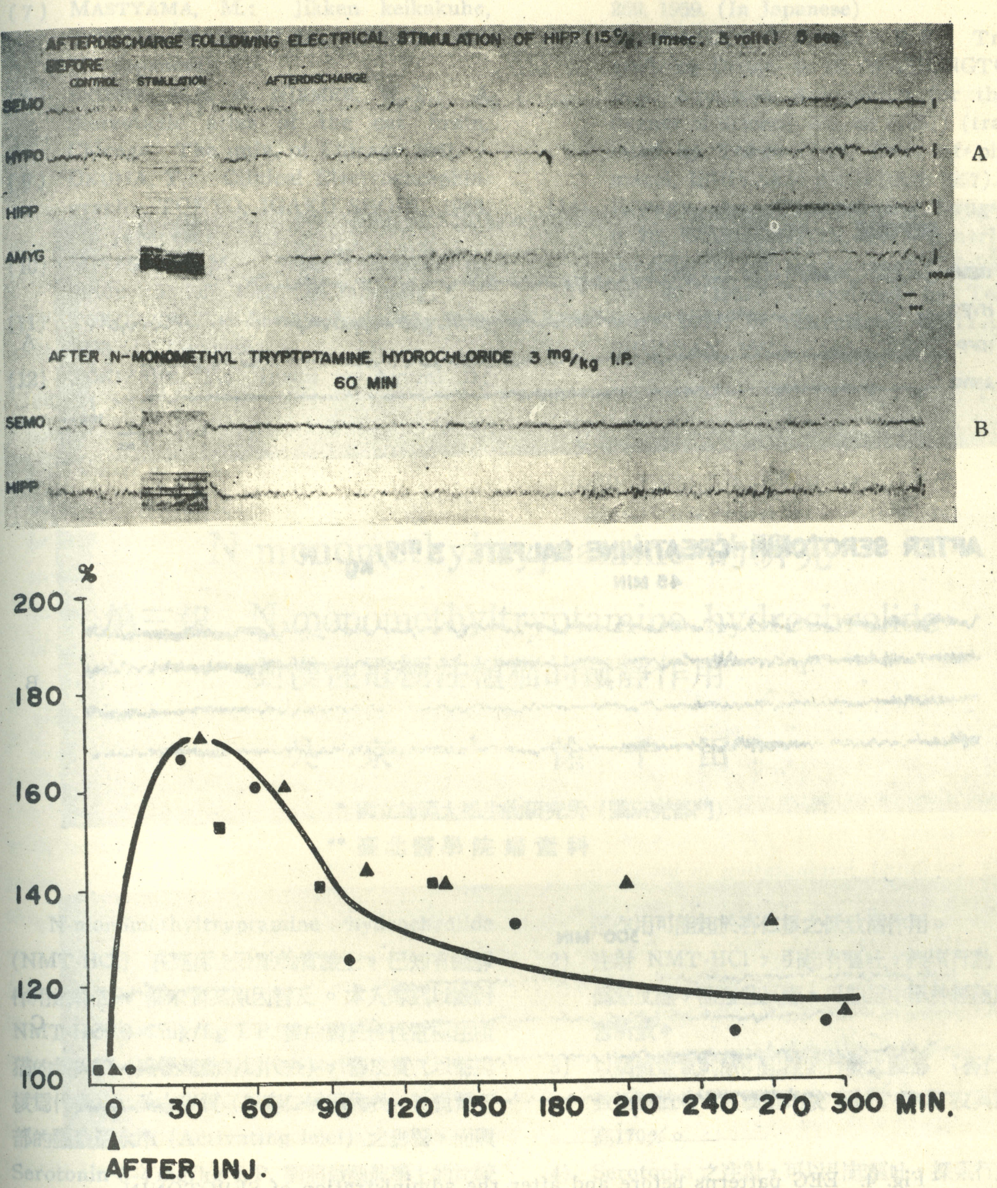


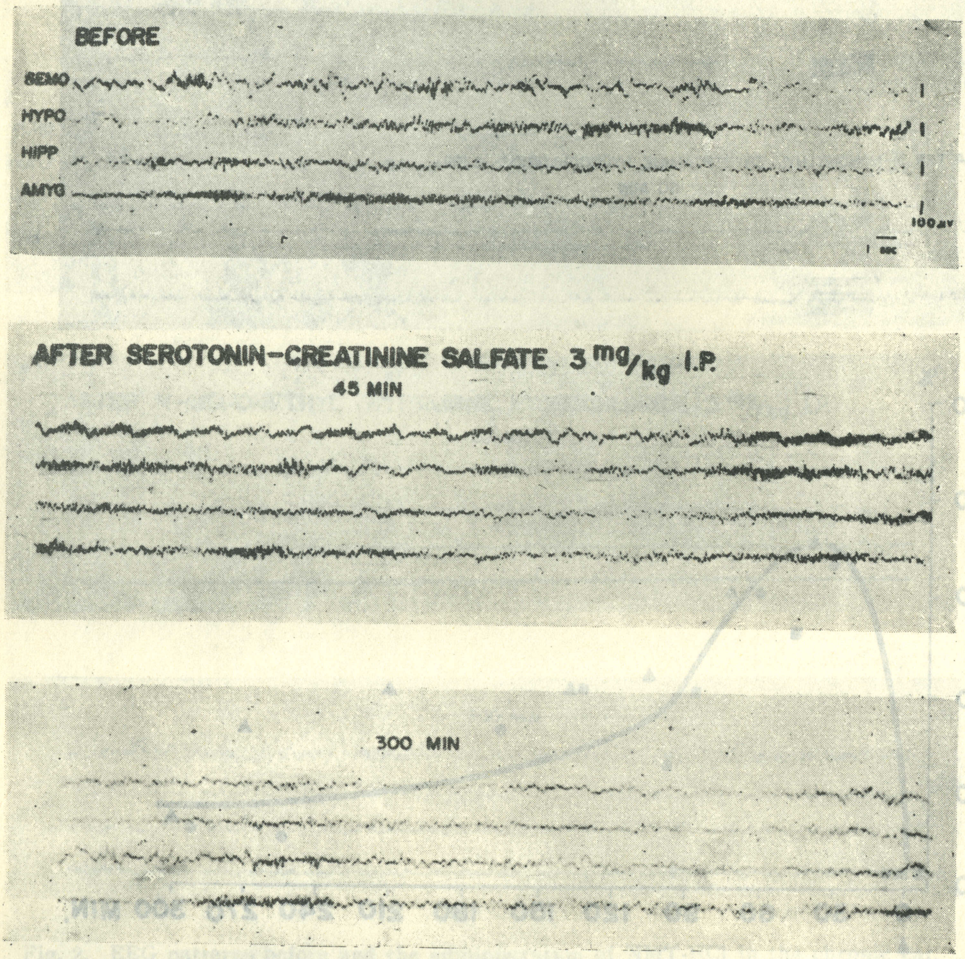
Fig. 3. A: After-discharge to electrical stimulation before the administration of NMT-HCl. Stimulant intensity: 15 c/s, 1 msec width, 5 volts, 5 sec duration. #6. 3.2 kg.

B: EEG patterns during the electrical stimulation, after the administration of NMT-HCl 3 mg/kg. Stimulant intensity: as Fig. 3 A.

C: Hippocampal electrical threshold change after the administration of NMT-HCl. Stimulant intensity: 15 c/s, 1 msec width, 3.5-5 volts, 5 sec duration to contralateral Hippocampus.

Abscissa: time minute after NMT-HCl administration.

Ordinate: threshold change to the "serrching reaction" caused by stimulation of contralateral Hippocampus in per cent (control 100%) ▲ #2 2.7 kg NMT-HCl 3 mg/kg. ● #5 NMT-HCl 3 mg/kg. ■ #8 3.2 kg NMT-HCl 4 mg/kg.



A

B

C

Fig. 4. EEG patterns before and after the administration of SEROTONIN.

A: Normal drowsy pattern

B: 45 min after the administration of SEROTONIN 3 mg/kg

C: 300 min after the administration of SEROTONIN.

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N-monomethyltryptamine 的研究

第三報 N-monomethyltryptamine hydrochrolide 對慢性電極注植猫的鎮靜作用

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N-monomethyltryptamine hydrochrolide (NMT-HCl) 在臨床上及動物實驗上，已知有鎮靜作用之報告，惟未對其原因言及。本人等茲以檢討 NMT-HCl 3-4 mg/kg I. P. 後的對於慢性電極注植猫的新肉質（感覺運動野爲代表），舊皮質（以海馬核爲代表），及古皮質（以杏仁核爲代表）及視質下部的腦致活水準（Activating lelel）之影響，而與 Serotonin 3-4 mg/kg I. P. 對照做腦波學上的比較研究。所得結果如次：

1) NMT-HCl 確實有鎮靜作用能維持5小時，而這作用可能起於杏仁核之低致活作用。
2) 注射 NMT-HCl，引起有嘔吐，探索行動，及僞怒反應，而這些反應，可能爲中樞神經極度興奮所致。
3) 以閾值電氣刺激引起探索行動之腦部（杏仁核）致活閾值時，其閾值比較 NMT-HCl 投與前增高170%。
4) Serotonin 之注射，可以引起嘔吐，探索行動後也會引起鎮靜作用。