

## CARBACHOL-INDUCED AGONISTIC BEHAVIOR IN CATS: AGGRESSIVE OR DEFENSIVE RESPONSE?

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*Abstract.* The effects of intrahypothalamic carbachol microinjections were investigated in unprovoked cats. The carbachol evoked mydriasis, attention, vocalization, and piloerection, i.e. features of a typical defence were usually concomitant in evoked response, while the clear-cut aggressive or escape patterns appeared only once. No basic differences were observed in the set of manifestations induced by low (1–2.5  $\mu$ g) and high (20–40  $\mu$ g) doses of carbachol, and from left and right hypothalamus as well as from medial and lateral portion of the hypothalamus. It is concluded that carbachol-induced response does not represent an aggressive pattern but corresponds to the cat's defence and threat behavior.

### INTRODUCTION

Acetylcholine, carbachol, or other cholinergic compound with muscarinic effect injected into cat's hypothalamic area, and especially into its medial and anterior parts, induces emotional behavior (1, 16, 34, 35). A similar response pattern has been obtained from rather large areas of the hypothalamic-midbrain axis, and from some other parts of the brain (2, 7, 15, 22, 34). Although the descriptions of basic gross behavioral changes are concordant, some somatic and behavioral components brought on various interpretations of the total response induced cholinergically from the hypothalamus. The biological character and direction of this response might be understood as: (i) fear-like "go off"

state (6, 8, 18, 33), (ii) "awaiting" state described as a threat or defensive response with fear and/or rage elements (28, 41), or (iii) "go on" state corresponding to aggressive response with directed and even savage attacks (1, 9, 21, 35, 40). The authors usually described components of all these states together.

The discrepancies in the behavioral patterns and in the assessment of their character may be partly due to the fact that different brain regions were stimulated (21, 27); various cholinergic drugs (9) and different forms of drugs (7) were used, which necessitated special investigations. Moreover, different solvents of cholinergic drugs could be responsible for different effects (42). One of the essential causes of the variety of responses, however, is probably due to different experimental conditions, and particularly to different conditions of provocation of the animals. An intended or unintended presentation of distinct categories of stimuli applied in various ways may have a substantial influence on the response. Appearance of a man (16), pushing forward a hand or a stick (36), or a glove (6), grabbing at the animal (6), use of another attacking cat (25) are effective stimuli. It is known (5, 12, 32) that the perception of external stimuli is changed in emotional states following the hypothalamic stimulation, and therefore an altered meaning of sensory stimuli in carbachol-induced emotional state may modify the primary pattern of the centrally elicited response. The presence of the experimenter near the animal is itself a strong stimulus, and that is why it has been suggested (16, 41) that the experiments should be carried out in a sound-proof chamber, isolated from any stimuli. However, to induce responses corresponding, to those under natural conditions, the animal should not be completely isolated from the environment.

The purpose of the experiments was: (i) the description of the response elicited in cat by intrahypothalamic carbachol injection under established and clearly specified conditions in which, the animal is not isolated from the environment, but it is not purposefully stimulated; (ii) the description of behavioral patterns occurring spontaneously under given experimental conditions; (iii) an attempt to determine the biological character of this response and indicate its possible natural equivalent in cat's behavior.

#### MATERIAL AND METHODS

The subjects were 36 adult cats of both sexes (24 females, and 12 males) of 1.8–3.2 kg body weight. The animals were kept in separate cages in natural illumination, fed once a day with a mixture of cereal

and meat in proportion 2:1. They received milk at the same time. The animals were adapted to laboratory conditions for 3 wk prior to operation. They ingested food normally, and allowed the experimenter to handle them.

Stereotaxic operations were performed in semi-sterile conditions, under hexobarbital anesthesia (80–90 mg/kg i.p.). The animals got penicillin i.m. to prevent infection. The guide cannulas used to introduce the injecting cannula were made of stainless steel (1 mm outer and 0.7 mm inner diameter) and were fixed to the skull with self-polymerizing methacrylate resin (Duracryl Special, Spofa, Prague). Two guide cannulas were bilaterally implanted in anterior hypothalamic region according to stereotaxic coordinates of Jasper and Ajmone-Marsan's atlas (24):  $F = 13.5$ ;  $L = 1.5$  or  $2.0$ ;  $H = -3.0$ . Sixteen cats got intracerebral unipolar electrodes in order to control EEG activity in the anterior hypothalamus and amygdala region. The electrodes of 0.1 mm diameter were made of stainless steel wire (Medwire), with teflon coating, except for 1 mm tip. The convalescence period following the operation was 10–14 days.

The animals were put into the experimental chamber half an hour before injection. Injections were performed on a table nearby and cats were replaced into the chamber. The injections were given through a cannula with 0.5 mm outer and 0.2 mm inner diameter introduced into the brain so that it protruded from the outer cannula by 1.0 mm. A microinjector (E. Zimmermann, Leipzig) was connected directly with the injecting cannula. Carbachol (carbamylcholine chloride, Fluka-Buchs, CCh) was dissolved in sterile and aprotogenic 0.9% NaCl solution, and pH measured, its mean value being  $7.1 \pm 0.2$  (SD,  $n = 10$ ). If pH value was lower than 7.0, 0.003 M solution of buffer  $\text{Na}_2\text{HPO}_4 - \text{NaH}_2\text{PO}_4$  was added. Carbachol solution at room temperature (20°C) was injected into the brain unilaterally in a volume of  $2.0 \pm 0.1 \mu\text{l}$  at a rate of about 0.1  $\mu\text{l/s}$ , and about 10 s after the injection had been terminated the injecting cannula was removed.

The animals were divided into two groups. The first group (25 cats) got CCh in doses of 5.0–10.0  $\mu\text{g}$  and the other (11 cats) – in doses 1.0–40.0  $\mu\text{g}$ . The intervals between successive CCh injections were never shorter than 7 days, and no more than 10–15 injections were given into one point of the brain. Before the last injection of CCh all the cats were submitted to control experiment: a sham injection, and an injection of 2  $\mu\text{l}$  0.9% NaCl.

The experimental situation is shown in Fig. 1. The animal was under observation in an oval shaped chamber  $110 \times 80 \times 60$  cm, with a plexiglass pane  $43 \times 52$  cm in the front wall. A similar pane was in

the ceiling of the chamber, which was illuminated from above by an electric 60 W bulb. The temperature inside the cage oscillated between 23–26°C. During the observation the experimenter remained in front of the pane, about 1–1.5 m from the cat, performing only what was necessary to attend to the apparatus. No other person approached the chamber, and the cats were not provoked in any way. The permanent presence of the experimenter in the animal's range of vision and the lack of sound-proof isolation secured a full flow of information from the cat's outside surrounding.

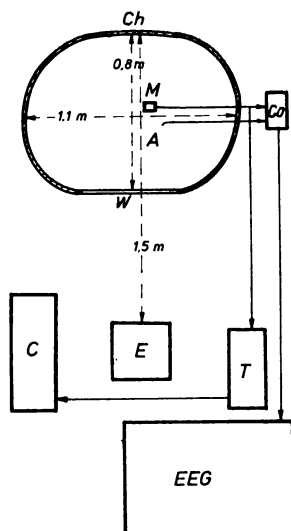


Fig. 1. Schematic draft of the experimental situation; Ch, elliptic chamber; W, window (42 × 52 cm) in the front wall; A, animal; M, microphone fixed up under the chamber ceiling; E, experimenter's place; Co, connector; C, electromechanical vocalization counters; T, taperecorder; EEG, vocalization and EEG recorder.

EEG activity was registered by 8-channel electroencephalograph (EEG 8.111 VEB Messgerätewerk, Zwönitz). The cat's vocalization was registered on one of the channels with the use of a microphone fixed up under the ceiling of the cage. Additional recording of the vocalization was also performed by means of electromechanical counters, counting and summing up the number and duration of the vocalization episodes (growling and hissing) according to Várszegi and Decsi's method (41). In order to facilitate the assessment of locomotor activity, the floor of the experimental chamber was divided into four fields. The latency period was measured with 5 s accuracy from completion of the injection to the appearance of the first manifestation. All the measurements and recordings were carried out for 30 min ± 5 s since the response onset. If during observation period some epileptic EEG discharges or convulsions occurred (3% of experimental sessions), the data concerning the relative session were rejected.

After the completion of the experiment the cats were killed by an

overdose of anesthetics, their brains fixed in 10% solution of neutralized formalin, frozen, and cut into 40–60  $\mu\text{m}$  thick sections. Photomicrographs were made from unstained slices on photosensitive paper according to Guzman-Flores' et al. method (19). The localization of the injection points was determined on this base. A part of the slices was stained with cresyl violet for histological analysis.

The  $2 \times 2$  contingency table and the chi-squared test with the Yates correction (3) were used for statistical evaluation.

## RESULTS

### *General behavior*

Carbachol at all the dose levels evoked clear-cut changes in the animals' behavior. The full set of manifestations created a defensive character of the response. The characteristics accompanying the elicited response were always the following: strongly increased cat's attention, widely opened eyes, careful watching all the surrounding details, and orienting response to all stimuli of the environment, as well as pupils dilatation and steady vocalization manifested by regular growling, and, rarely, hissing (Fig. 2).

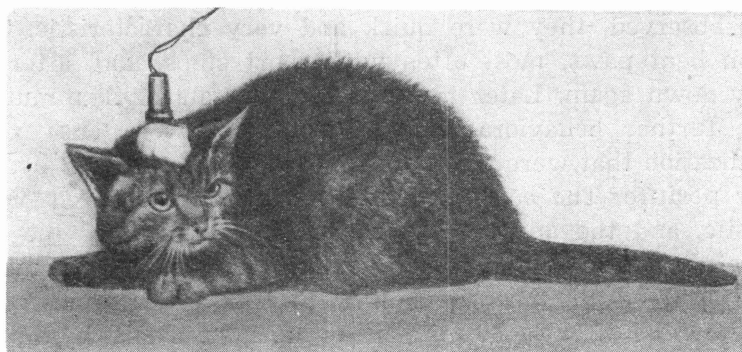


Fig. 2. Typical posture of the cat 10 min after unilateral intrahypothalamic injection of CCh (10  $\mu\text{g}$ ). Note widely open eyes with marked mydriasis, slightly backward contraction of the ears, a lowered head, and piloerection along the back. The cat is observing an experimenter, paying at the same time no attention to the wires connected to its head socket. The photograph was taken during one of the growling episodes.

In all the cases the position the cats took up in the chamber was as if danger was coming from the window and thus from the experimenter, although the experimental situation in the laboratory was identical before the injection and after it. The locomotor activity most often

declined. The cats lay near the wall with mildly arched backs, and markedly dropped down heads, especially during growling episodes. The ears were more or less drawn to the back of the head, but not flattened, and a piloerection could be observed. Most often the animals remained crouched, and they frequently retreated to the farthest "corner" of the chamber. All the above mentioned characteristics always appeared jointly and none of them was observed to manifest itself independently from the others (except for the pupils dilatation). The remaining vegetative and motor patterns supplemented the above set, but they were of various intensity and did not seem to be so closely connected with the above described response.

The presented set of characteristics gave the cats' behavior an air of "cowardly looking round", as if the animals "were expecting some danger", or were "seeking the source of danger", and simultaneously they took an attitude of threat (Fig. 2). The cat's response to slightest environmental change proved that all the stimuli acquired some aversive properties. The animals' attention was chiefly concentrated on the experimenter, as the largest subject nearby.

After 15-20 min of the response, the cats became more "relaxed". The animals raised their heads, looked around more intensely examining the walls and ceiling of the chamber. If any locomotor movements could be observed, they were quick and very characteristic. The cats moved on bent paws, most often with short steps, and, after a short walk, lay down again. Later between the 25th and 30th minute of the response, further behavioral changes were observed. The excitement and vocalization that were still persisting were accompanied by a different body posture. The constriction of the ears disappeared or became less drastic, and the animals approached the window of the chamber very carefully, sniffed, and sometimes tried to get out. In most cases the cats resumed the posture similar to that prior to injection and stopped growling after 30-60 min, sitting upright, usually by the window. Summing up, the aversive and agonistic attitude to all environmental stimuli at first increased rapidly, then declined till it finally reached the pre-injection state.

We consider the course of the cats' general behavior as emotional-defensive response.

#### *Frequency of response components occurrence*

The specification of components listed according to the frequency of their occurrence (Fig. 3) and compiled from a great number of experimental sessions proves that the manifestations that appear most frequently (48-100% of sessions) are those most typical for emotional-

defensive state: arousal response and attention, permanent vocalization (growling and hissing), piloerection most often along the spine and tail, tremor and mild arching of the back. Vegetative manifestations were less frequent (28–47%): salivation (the saliva being at first thin

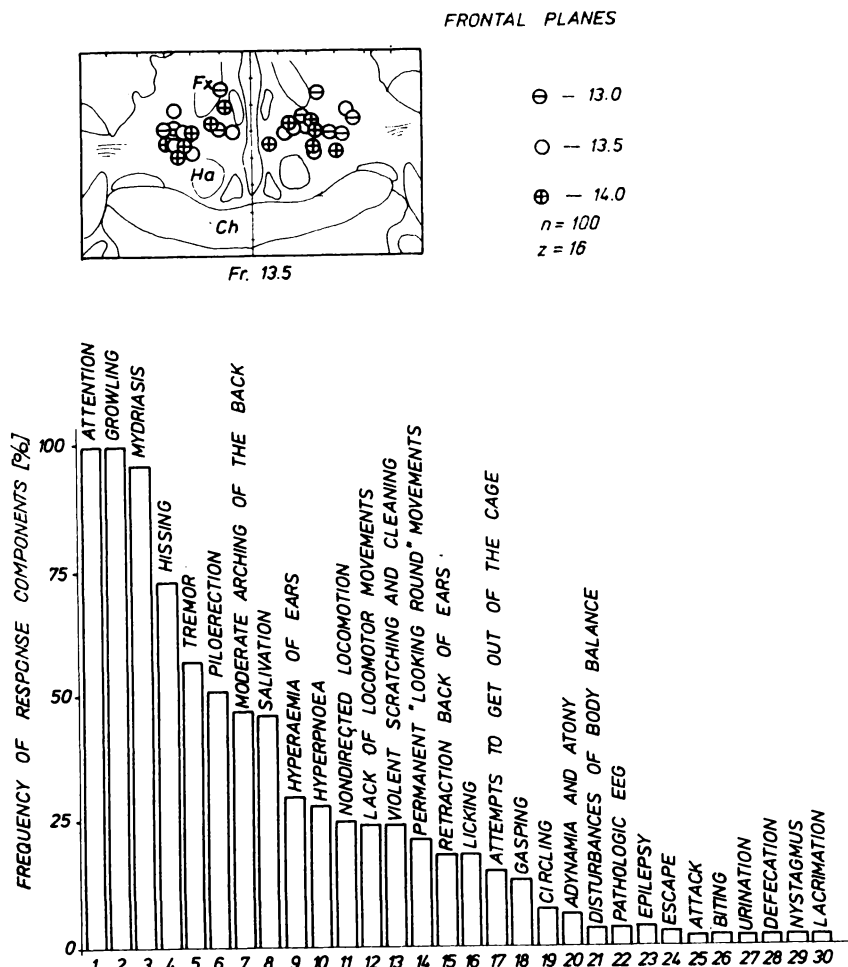


Fig. 3. Response components observed after unilateral intrahypothalamic injection of CCh (10  $\mu$ g) in unprovoked freely moving cats. The response components are ranged according to the proportional frequency of their appearance in 100 experimental sessions. Numbers below the columns (1-30) refer to respective components. Localization of all involved injection points is indicated at the top of the Figure in frontal plane 13.5 according to Jasper and Ajmone-Marsan's stereotaxic atlas (24). Points located rostrally or caudally to this plane are marked with different signs. Abbreviations:  $n$ , number of experimental sessions;  $z$ , number of animals; Ch, optic chiasm; Fx, fornix; Ha, anterior hypothalamus.

then thick), licking the mouth, ear congestion, hyperpnoea, rarely turning to gasping. Motoric manifestations appeared in 6–25% and consisted of non-directed locomotion, violent and persistent scratching, mostly of the head, being evidence of itching or hypersensitivity. The cats' motor responses were evidently bi-modal. On the one hand an increased number of locomotor movements was noted, like running in different directions, circling, frequent attempts to get out of the chamber; on the other hand there was a decline of locomotor movements in comparison with the control period, up to a complete absence of locomotor movements (24%) during 30 min of observation. In exceptional cases very strong vegetative excitation was followed by complete adynamy and atony, similar to slumber or even deep sleep, which occasionally interrupted the response course. The cats displayed an increased locomotor level in 32.5% of all experimental sessions while in 67.5% the level decreased, or the locomotor movements vanished.

A strong general excitation was always accompanied by looking round movements connected with watching the surroundings. In some cases these movements became very strong, rhythmical, and sometimes revealed attributes of stereotypy (permanent looking round movements).

Occasionally (1–3%) cats revealed behavioral patterns with pathological character: disturbances of body balance during locomotion, discharges in EEG activity (pathologic EEG), epileptic fits and nystagmus. No more than one or two cases of furious escape, spontaneous attacks against the experimenter, biting the microphone and cables inside the chamber, urination, defecation and lacrimation were observed.

### *The response latency*

The latency period of emotional-defensive response evoked by intrahypothalamic microinjections of 5–10  $\mu\text{g}$  of CCh was in 85% of experimental sessions 1–3 min, in 2.5% it was shorter than 1 min, and in 12.5% it exceeded 6 min (general range: 0.5–16 min). The distribution of the response latencies in 50 s intervals is shown in Fig. 4A.

Most components of the response appeared concurrently, or in short intervals, and only some of them (e.g. intense looking round, locomotor movements, hyperaemia of ear and nose skin) could be observed later, after the whole response had developed. In almost all the cases, the

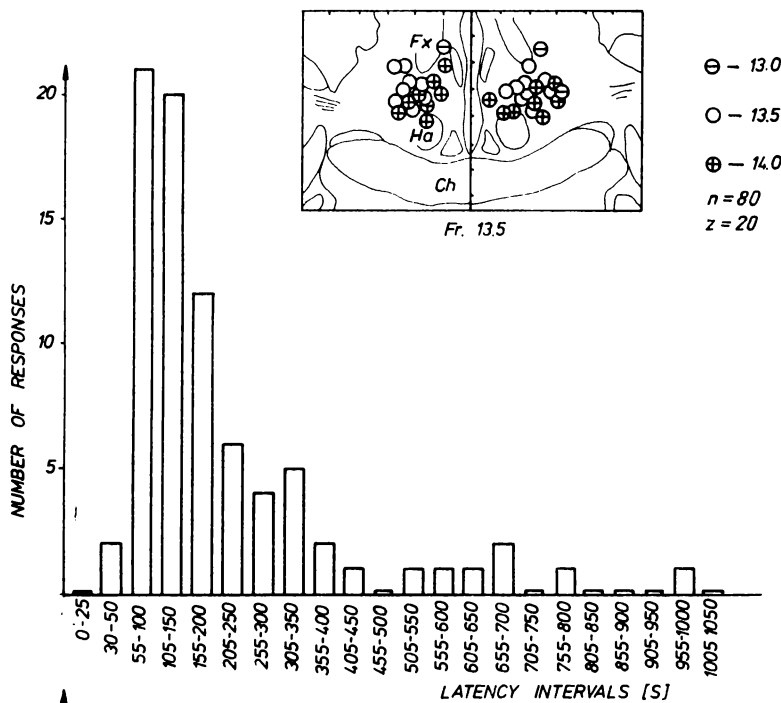
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Fig. 4. Latency and duration of the response evoked by unilateral intrahypothalamic injection of CCh (10  $\mu\text{g}$ ); A, distribution of response latencies in 50 s intervals. Latencies were estimated in 5 s steps. The first interval is divided into two parts in order to indicate the shortest latency (30 s). B, distribution of response duration in 5 min intervals. Localization of involved injection points is common for both distributions. Other denotations as in Fig. 3.

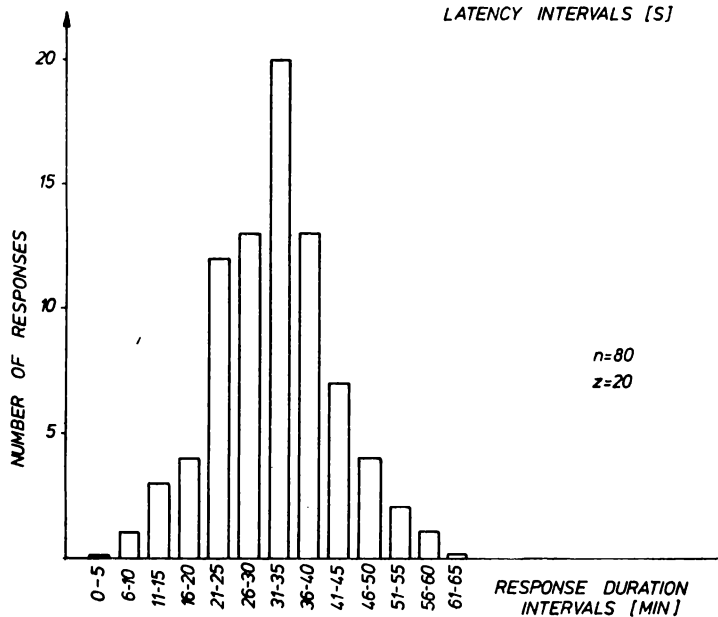


A

FRONTAL PLANES



B



vocalization was the first clear-cut manifestation consisting of regularly repeated growls, and accompanied by a general change of behavior. Pupils dilatation and attention pattern initiated the response less frequently. Other signs, like salivation, tremor, increased respiration rate, as well as pupils dilatation appeared as first signs, usually independently of each other, only in responses in which the vocalization was very poor or non-existent, and the cat's general behavior did not change at all, or revealed some changes hard to assess (no more than 5% of sessions). Such signs were never an integrated behavior, and were not considered to be emotional-defensive response. The control injections never induced any clear-cut manifestation or change in the cat's behavior.

#### *The response duration*

While the onset of a violently increased response is easy to determine, it is very difficult to define accurately the end of emotional-defensive response which is slowly dying away. The first to vanish were the changes of the cat's general behavior together with vocalization, whereas the vegetative manifestations (mydriasis, tremor, etc.) persisted sometimes as long as 1–2 h following the injection. The changes in the cat's aversive emotional-defensive attitude to environmental stimuli were in amazing accordance with the vocalization changes. The longer the animals remained in the above-described emotional state, the longer their spontaneous growling persisted. After abatement of vocalization, the cats' behavior was similar to the pre-injection state, and hard to evaluate under conditions when the animals were not provoked. Thus it may be assumed that the duration of spontaneous vocalization is the index of duration of CCh induced response.

The average duration time of emotional-defensive response measured by the duration of vocalization was, for the dose 10  $\mu\text{g}$  of CCh  $32.1 \pm 9.6$  min (SD,  $n = 80$ ) and the minimal and maximal values were respectively 10 and 60 min. Figure 4B shows the distribution of duration of the response at 5 min intervals.

#### *Effects of the CCh dosage on response components*

The comparison of responses induced by injections of 1.0–2.5  $\mu\text{g}$  CCh (low doses) and 20.0–40.0  $\mu\text{g}$  of CCh (high doses) from the same sites of the brain (Fig. 5) clearly shows that the main components of emotional-defensive response (increased attention, growling, mydriasis, hissing, piloerection and tremor) occur with similar frequency, irrespective of CCh dose. However, the occurrence of some vegetative and motor signs depended on the administered CCh dose. Low CCh doses only spora-

dically elicited salivation, ear hyperaemia and hyperpnoea, while these signs appeared with considerably greater frequency in responses evoked by high doses. The motor manifestations also were slightly more frequent. The statistical significance was reached only for salivation ( $\chi^2_c = 7.29$ ,  $P < 0.01$ ) and ear hyperaemia differences ( $\chi^2_c = 6.53$ ,  $P < 0.02$ ).

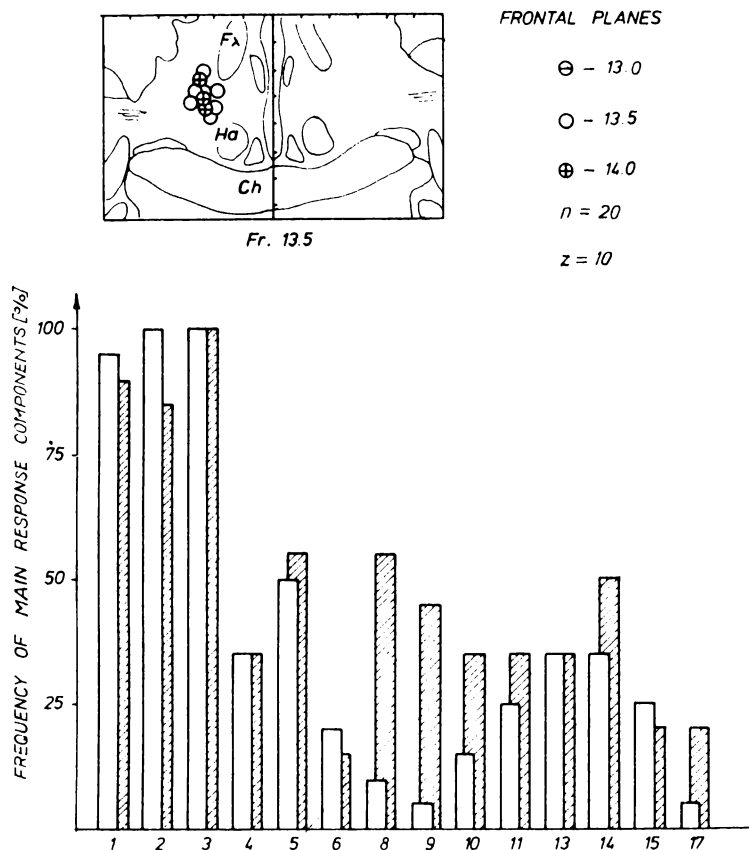


Fig. 5. Effects of CCh dosage on the set of response components; white columns, frequency of response components following low doses (1.0-2.5 µg); hatched columns - following high doses (20.0-40.0 µg). Numbers below the pairs of columns refer to respective components described with other denotations in Fig. 3.

#### *Effects of the cannula localization on response components*

Emotional-defensive responses were evoked every time by unilateral CCh injections. CCh injections into the right or left hypothalamus evoked a fully developed and qualitatively similar response. The comparison of frequency of response components elicited from injection points situated symmetrically on the left and right side of the brain

(6 cats) shows that responses evoked from both sides of the hypothalamus are equivalent and have almost the same set of manifestations (for  $n = 22$ ).

Some cats, in whose hypothalamic areas the cannulas have been unintentionally localized asymmetrically, were used to investigate the differences between responses elicited from medial and lateral hypothalamic regions. The whole injection point pool was divided into medial points situated about 0.2–1.9 mm from the midline, and lateral points situated ca 2.0–3.8 mm from the brain midline. The frequency particular to manifestations elicited from the medial region differed to some extent from those evoked from the lateral area (Fig. 6). Medial region stimulat-

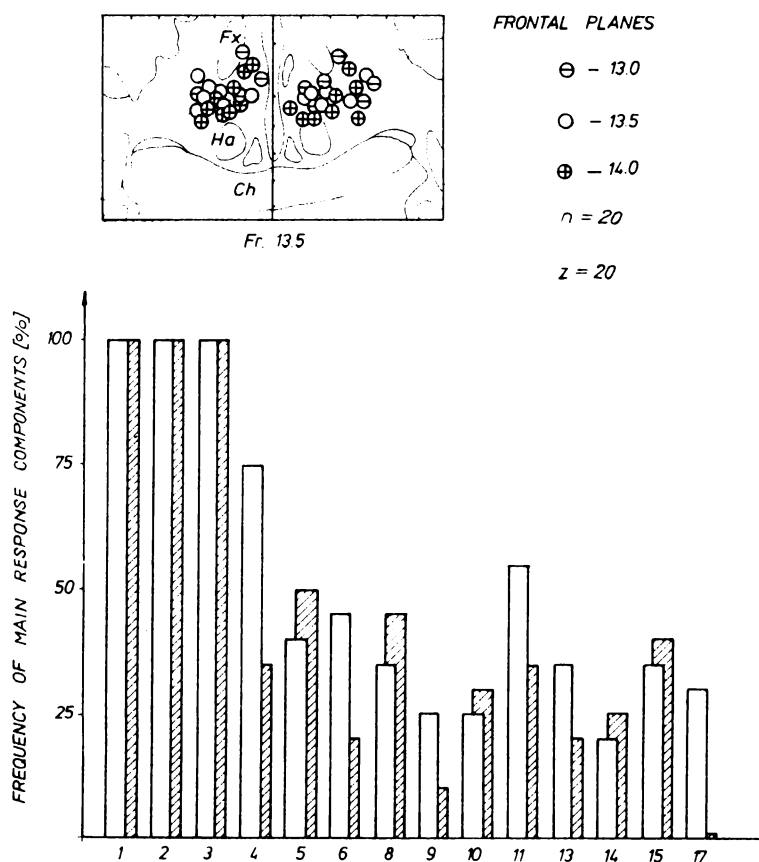


Fig. 6. Frequency of response components obtained from medial (0.2–1.9 mm from midline) and lateral (2.0–3.8 mm from midline) regions of the hypothalamus after unilateral injections of CCh (10  $\mu$ g). Numbers below the pairs of columns (white for medial and hatched for lateral parts) refer to respective components described with other denotations in Fig. 3.

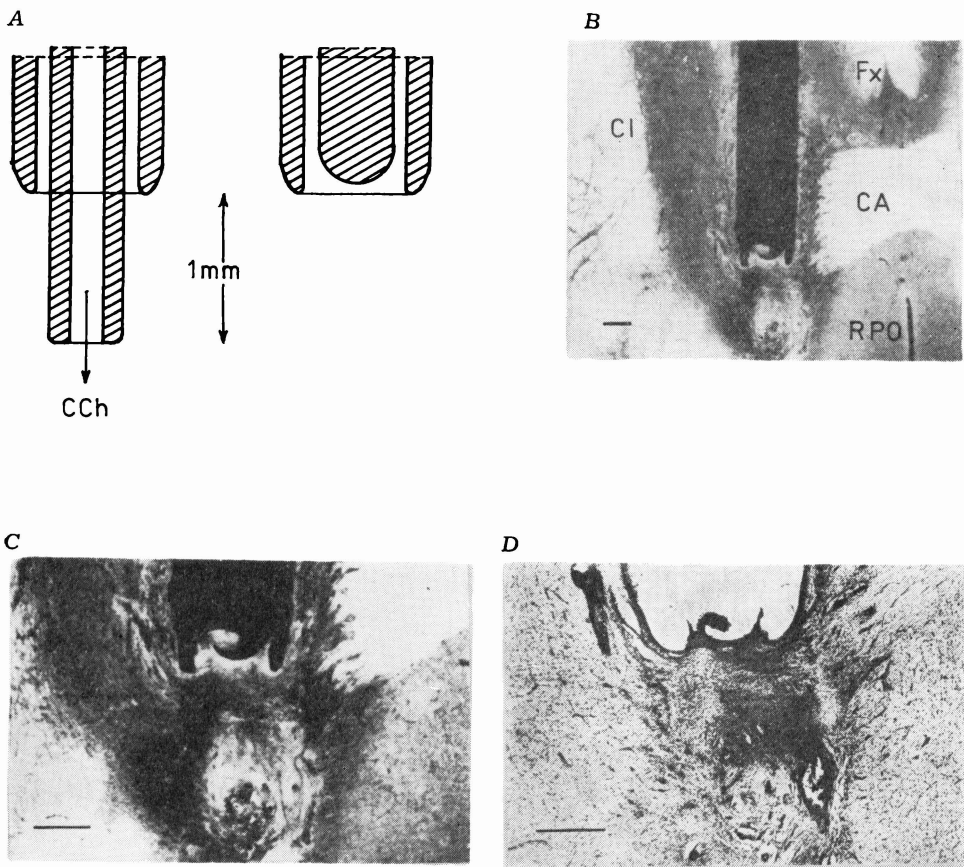


Fig. 7. Area of inner cannula penetration and CCh drop location after 15 injections of CCh ( $10\text{ }\mu\text{g}$  in  $2\text{ }\mu\text{l}$ ). The period of time, from the day of implantation until the animal has been sacrificed, was 164 days. *A, left*, the end of guide cannula during injection with an injecting cannula protruding from the outer one by  $1.0\text{ mm}$ ; *A, right*, the end of guide cannula after injection plugged up with stainless steel wire; *B*, unstained brain section illustrating the cannula localization at the caudal border of preoptic region; *C*, fragment of the unstained section showing necrosis under the guide cannula; *D*, the same section as in *C* stained with cresyl violet. CA, commissura anterior; CI, capsula interna; Fx, fornix; RPO, preoptic region.

The scale is  $0.5\text{ mm}$  in every case.

ion evoked more frequently hissing ( $\chi^2_c = 4.95$ ,  $P < 0.05$ ) with numerous attempts to get out of the chamber ( $\chi^2_c = 4.90$ ,  $P < 0.05$ ). The differences in piloerection and locomotion, although relatively large, did not attain the level of significance. In responses evoked from the lateral regions tremor and salivation were more frequently observed but the differences appeared not significant. Although the frequency of some manifestations from the lateral and medial part of the hypothalamus was somewhat different the character of the response was, in essence, the same.

### *Reproducibility of the response*

The CCh elicited emotional-defensive response was reproducible to a great extent. The set of manifestations evoked by successive microinjections in a given cat was fundamentally the same regardless the violence of the response. The reproducibility was also characteristic of the latency period and was observed even in the order on which some signs appeared. The comparison of the responses following the first three and the further three microinjections (6th to 8th) from the same sites of the brain (in 10 cats,  $n = 30$ ) proved that neither the set of manifestations nor the animal's general behavior underwent any marked changes. Only tremor and salivation were somewhat higher in further repetitions of injections but the level of significance was not reached.

No relation was observed between the components of the emotional-defensive response and the cats' size, age, or sex, the hour of the day or the season of the year in which the experiments were carried out.

### *Histological verification*

All the injection points were localized in the frontal planes 13.0–14.0 (according to Jasper and Ajmone-Marsan's (24) stereotaxic atlas). The disposition of these points on lateral planes revealed a larger dispersion, but contained the planned injection area. A schematic specification of injection points was inserted in particular figures.

CCh injections into brain tissue produced a small necrotic area within the region of penetration of the injecting cannula. A histological analysis of the brain regions into which different numbers of microinjections were given revealed that the largest injury was caused by the first injection, while further ones changed the range of injury, but very slightly. A typical picture of the injection area after repeated administration of CCh is shown in Fig. 7. In a few cases when extensive damage was produced near the tip of the cannula, it was impossible to induce emotional-defensive response even by increased CCh dose.

## DISCUSSION

Our results prove that unilateral CCh injections into the hypothalamus elicit a fully developed, integrated and directed emotional-defensive behavior. The analysis of unprovoked cats' general behavior confirmed the earlier observation (36) that the most typical manifestations of defensive response such as pupils dilatation, attention response, vocalization and piloerection are the most frequent and clear-cut effects induced by CCh. Spontaneous acts of aggression and attack described in other papers (9, 21, 35, 40) were virtually absent from the set of manifestations that we obtained (1 attack in 100 experimental sessions). Similarly, the flight response, and even attempts to get out of the chamber were rather scarce (respectively 2% and 15% of sessions). These observations are in agreement with the results of a recent investigation (26) in which intraventricularly administered CCh enhanced fear-like behavior (withdrawal, crouching, backward flattening of the ears, "defensive hissing") without any aggressive action.

These results suggest that the response elicited by CCh, at least at the level of anterior hypothalamus and preoptic area, corresponds to a defence and threat response evoked by electrostimulation of the hypothalamus (13), and to natural forms of agonistic behavior (31), but is not an aggressive one. The main point does not consist in the change of terminology, but in differentiating two individual physiological states: offensive and sensu stricto defensive. Similar discrimination between aggressive and defensive behavior emerged in fighting situation in the rat (10).

According to external conditions the described response may shift either to withdrawal and flight, or to aggressive and attack response, as it has already been mentioned in early investigations with the use of electrostimulation (23). The fact that the offensive and defensive behavior are closely related might have caused some misunderstandings (17). When the cats were not provoked, the CCh injection induced a state of some anticipation: the animals assumed a crouched posture, the locomotor activity decreased, and, on the other hand, they were emotionally excited, and carefully searched for a potential source of threat and its localization, refraining, however from any resolute action. CCh induced state is in perfect conformity with the functions of the brain system postulated by Colpaert (14) that "suberves the animal's ability to adequately acquire fear as a secondary drive which purposefully enables it to adjust its behavioral output to the primary drive contingencies offered by its environment" (p. 38).

The data of investigations where the CCh or other cholinergic compo-

und was administered into the midbrain and mesencephalon areas and the obtained responses were blocked by means of anti-cholinergic drugs (4, 16, 28-30, 33, 37, 41) may corroborate the hypothesis that the cholinergic system is fundamentally responsible for eliciting and maintaining the above described behavioral state, although the mechanism of the central action of the administered cholinergic compounds is still not clear (20). CCh-induced response is thus a state of arousal which, depending on environmental stimuli, will turn to an adequate behavior, either to aggressive response (cholinergic trigger of aggressive reaction (1)), of flight, or will remain in the form of defensive and threat response as described in this paper. This defensive behavior with described characteristics is well conformed to the cats' natural behavior (31).

The induced manifestations show that CCh injected into anterior hypothalamic region triggers not only the defensive response, but also some components of other behaviors. The thermoregulatory patterns are particularly well manifested: tremor, hyperaemia of ears, and panting (11, 38). These results might indicate, according to Myers' suggestion (34), that the cholinergic system represents a common terminal neurochemical mechanism responsible for the transmission of all efferent signals. If these speculations are true, acetylcholine, CCh, or any other cholinomimetic injected into hypothalamus will, as a rule, trigger several different responses, or at least a few of their components together. In this situation a proper choice and evaluation of the characteristic manifestations specifically connected with the emotional-defensive response would be of prime importance. These problems are discussed in a separate paper.

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