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NEUROCHEMICAL BASES OF DEFENSIVE BEHAVIOR IN ANIMALS

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Abstract. This paper presents a literature review and the author's own investigations devoted to identifying the morphological organization of different subcortical structures and neurochemical processes involved in the regulation of defensive behavior. The effects of intrahypothalamic injections of two cholinomimetic substances, carbachol and d-tubocurarine, on rage and fear reactions were explored. Also the investigations were carried out with intrahypothalamic injections of anticholinergic substances, atropine and betamon or hexamethonium, which block selectively muscarinic and nicotinic receptors. The results indicate that carbachol injected into the hypothalamus after blocking the muscarinic receptors with atropine fails to elicit any defensive response; whereas carbachol injection administrated after nicotinic receptors had been blocked by betamon or hexamethonium induces full aggressive behavior. This indicated that carbachol acts, on the hypothalamic level, through the muscarinic receptors. The mechanism of d-tubocurarine action seems to be more complex because this compound induces fear response after either the muscarinic or the nicotinic receptors have been blocked. A possible mechanism based on chemically differentiated neuronal circuits at the hypothalamic level for two different defensive drives, fear and rage, is presented.

For many years much work has been devoted to identifying the morphologico-functional organization of sites in the central nervous system that regulate defensive behavior (5, 8, 10, 13, 15, 18, 19–21, 23, 26, 28, 29, 34, 35, 38, 39 and others). These investigations involved electrophysiological and neuroanatomical methods, and localized particular sites relevant for defensive behavior in animals. It has been shown that these sites occupy various levels of the central nervous system, and are, in principle, compatible with the structures which Papez (27) considered to be the total functional system regulating all the emotional responses, known in the literature as the "Papez circuit".

The investigations carried out hitherto by electrophysiological methods and, first of all, by electrical stimulation determined the localization of the sites taking part in the defensive behavior, but failed to explain in what way they participate in these processes. The possibilities of electrostimulation are limited because of nonspecific effects since various neuronal circuits lie within the stimulated region. It is known from numerous reports that the central nervous system does not contain a simple neuronal system representing only a single specific function, but, on the contrary, agglomerations of neurons involved in the regulation of various functions of the organism are found in the same location. A typical example of such a morphologico-functional mosaic is the hypothalamus in which neurons participate in the regulation of different functions such as: food and water intake, wakefulness and sleep, thermoregulation, and sexual as well as defensive behavior. Neuronal systems, interrelated and linked together as regards anatomy, are likely to possess their own specific chemical sensitivity underlying particular physiological mechanisms. It is possible that particular activities are started through the intermediary of various neurotransmitters. The above facts and observations prompted neurophysiologists to endeavor to find a more precise method which would allow use of stimuli more apt to exercise a specific influence, and to stimulate, or inhibit, selectively a particular type of neuron involved in the regulation of a definite function.

The development of biochemistry, and its ever increasing role in the investigations of the chemical processes in the brain, gave birth to speculations that some well defined chemical substances introduced into the central nervous system might, perhaps, stimulate selectively some definite neuronal systems. Thus, a very promising method of stimulating the brain of unanesthetized animals has been recently put to use. The method consists in injecting directly, into stricty defined loci, some pharmacological substances eliciting selective, well defined functional effects. By acting on chemically specialized central synapses chemical stimulation allows the selective activation of some functionally homogeneous neuronal systems, which respond specifically to a particular chemical compound. It is likely that there are receptive loci in neuronal systems for some group of chemical compound eliciting definite physiological effects. In order to understand better the physiological mechanisms these sites must be localized and their structure and chemical properties must be determined. The fact that various biogenic amines are differently disposed in particular brain regions (2, 7, 37) may be considered as a case for the conception of neurochemical specificity of particular functions. Although the importance of these amines has not been

precisely stated yet, many authors believe that they serve as modulators, or neurotransmitters in regulating various physiological processes (12, 14, 16, 17, 24, 25, 32).

In the literature there is relatively little information concerning defensive responses elicited by chemical stimulation of different brain structures (3, 4, 6, 9, 11, 25, 33, 36). However, it has been stated beyond any doubt that cholinomimetic substances introduced intracerebrally elicit defensive responses, and it is worth mentioning that different chemical compounds injected at the same loci, and acting selectively, evoke different defensive responses. Carbachol has been found to be the most effective and specific pharmacological compound to facilitate the natural killing response (3, 4) and to induce predatory aggression (6, 11, 25, 36). Another kind of defensive reaction, the escape response, is induced by injections of tubocurarine (9, 36).

Our investigations (30, 31) of the functional organization of the systems regulating defensive behavior at the hypothalamic level have proved that a direct tubocurarine injection into the hypothalamus of cats elicits specific changes in the defensive response with very distinct vegetative, somatic and behavioral symptoms, typical of increased anxiety (terrific mewing, urination, defecation, pupillary dilatation, increased respiratory rate, and attempts to escape from the chamber) (Table I). Carbachol injected into the same hypothalamic sites produced a strong

TABLE I

Characteristic symptoms elicited by intrahypothalamic injection of d-tubocurarine

Cat	Region of injections	Doses of d-tubocurarine (in μg)	Symptoms	Control injections	
				H_2O pro injectione	NaCl 0.9%
R26	Hypothalamus anterior	30	v, u, m, l, r, e	—	—
R27		10	v, u, m, d, r	—	—
R28		10	v, d, l, m, e	—	—
R54	Medial part of hypothalamus	20	v, u, d, l, m, r	—	—
R55		20	v, u, d, l, m, r	—	—
R56		20	v, u, l, m, r	—	—
R34	Hypothalamus posterior	20	v, u, d, l, m, r	—	—
R35		20	v, u, d, l, m, r, e	—	—
R45		20	v, u, l, m, e	—	—

Abbreviations: v, vocalization (mewing); u, urination; m, mydriasis; l, locomotor reaction; r, increase in respiratory rate; d, defecation; e, escape from the cage. —, no effect.

aggressive reaction (vigorous growling, snorting, piloerection, pupillary dilatation, salivation and savage attacks against any object introduced into the chamber), and indicated that a rage drive had been induced (Table II). The recorded changes in the cats' emotional behavior after carbachol and tubocurarine injections are specific, because control injections — 0.9% NaCl, H_2O pro injectione, adrenaline, noradrenaline, re-

TABLE II

Characteristic symptoms elicited by the intrahypothalamic injection of carbachol

Cat	Region of injections	Doses of carbachol (in μg)	Symptoms	Control injections	
				H_2O pro injectione	NaCl 0.9%
R26	Hypothala-	8	g, h, p, m	—	—
R27	mus anterior	8	g, h, m, s, l	—	—
R28		8	g, h, p, m	—	—
R54	Medial part	8	g, h, p, m, s, a	—	—
R55	of hypotha-	8	g, h, p, m	—	—
R56	lamus	8	g, m, s, r	—	—
R34	Hypothala-	8	g, h, p, r	—	—
R35	mus poste-	8	g, h, p, m	—	—
R45	rior	10	g, h, p, m, s, r	—	—

Abbreviations: g, growling; h, hissing; p, piloerection; m, mydriasis; s, salivation; l, locomotor reaction; r, increase in respiratory rate; a, attack. —, no effect.

serpine, amphetamine, serotonin, strychnine — applied by ourselves as well as others (3, 9, 36) failed to produce any changes in defensive behavior. We have also stated that aggressive and flight responses are triggered by injecting carbachol and tubocurarine into the dorsal or ventral part of the anterior, medial, or posterior hypothalamus.

According to Hess's hypothesis (18) accepted for a long time, and based on findings obtained by electrical stimulation, the anterior and posterior hypothalamus are different topographico-functional systems, viz. the trophotropic, and the ergotropic system. However, we found (31) that the aggressive response accompanied by marked sympathetic symptoms, giving evidence of stimulation predominantly of the ergotropic system, is elicited by a carbachol injection, no matter whether this is given into the anterior, medial, or posterior hypothalamus. These findings, as well as others (1, 9, 11, 25, 36) are not compatible with the generally accepted hypothesis of such topographical division of the hypothalamus.

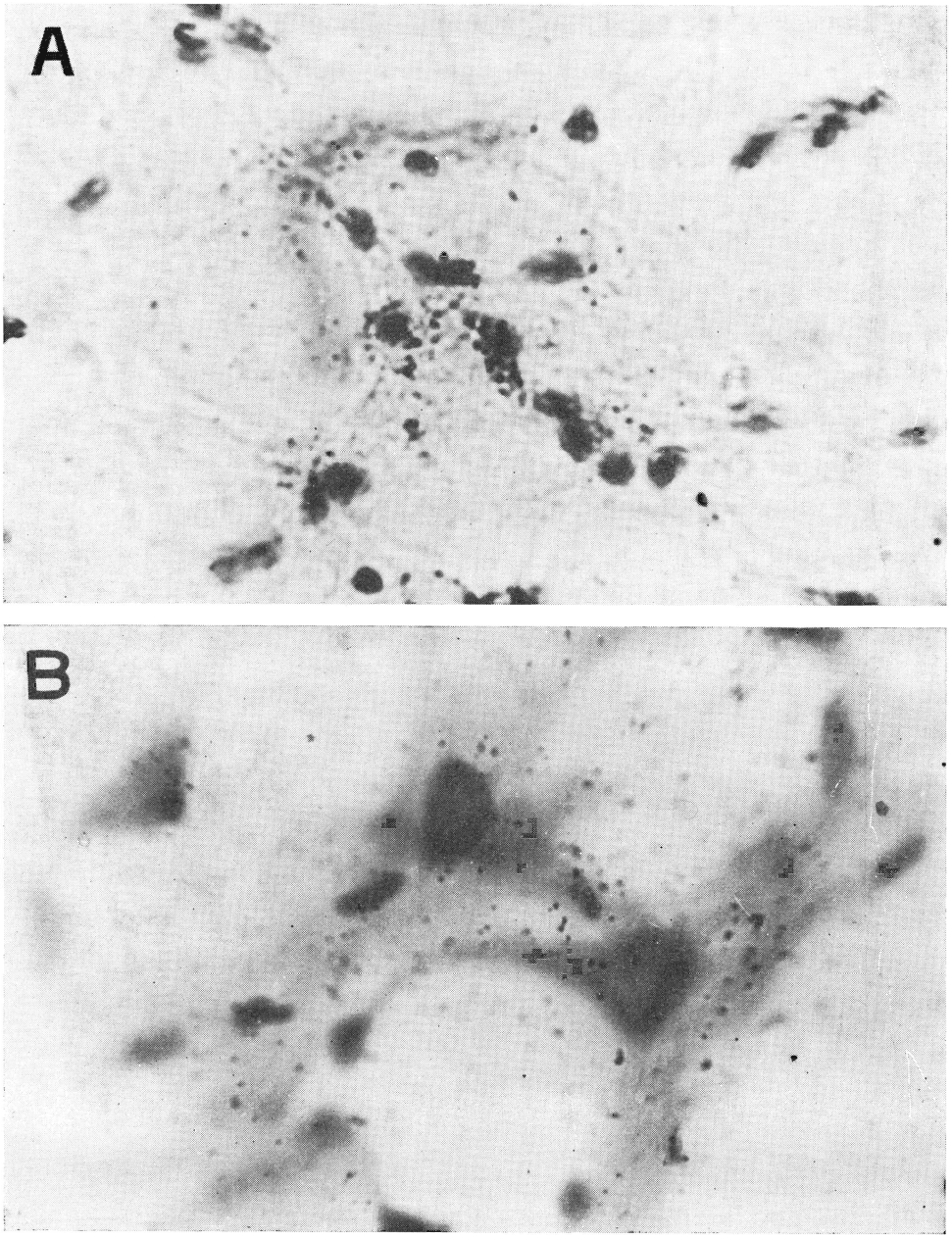

Fig. 1. Autoradiogram of representative field in the hypothalamus of the cat labelled with ^{14}C-d-tubocurarine (11.5 μCi). Exposure time 53 days. Magnification $\times$ 1400. *A*, shows one type of hypothalamic cell labelled with ^{14}C-d-tubocurarine, *B*, another type of hypothalamic cell in which no radioactivity could be discovered.

In connection with the results obtained by chemical stimulation of the brain the classical conception of hypothalamic "centers" controlling defensive responses needs modifications. Our previous investigations in which we applied electrical stimulation (28, 29), as well as the present results obtained by chemical stimulation (30, 31) allow us to put forward the following hypothesis, which attempts to elucidate the degree to which the hypothalamus participates in the mechanisms regulating defensive behavior: all hypothalamic regions are part of a functional "defensive system" consisting of two neuronal systems, which may be interrelated, but are probably differentiated in their chemical aspect in two functional systems, "the fear circuit", and "the rage circuit". Under "biologically indifferent" conditions these systems maintain some dynamic balance, but if changes threatening to the organism occur either in the inner or in the outer environment, the defensive system is aroused and the balance upset. Probably some specific chemical agents are liberated and they selectively activate either the "fear circuit", eliciting flight, or the "rage circuit", triggering aggression. And so it may be assumed that each of these systems has different and specific chemoreceptors, which are selectively sensitive to some neurohumoral substances, or other chemical compounds.

Numerous anatomical and physiological data prove beyond doubt that the "defensive system" is not confined to the hypothalamus but is a very complicated system with many connections involving structures on various levels of the central nervous system. According to Konorski (22) the central defensive mechanisms are regulated on at least two levels: the hypothalamic, which is functionally lower, and the limbic, mostly the amygdala, which is a higher level of the defensive system. Particular links of this system regulate only certain fragments of defensive responses, whereas the full development of emotional behavior is brought in by coordinated activity of the entire functional system. Injecting into various brain structures specific chemical compounds which elicit some definite types of defensive responses will certainly help to localize with more precision, and to gain a better understanding of the complete functional system which regulates defensive behavior. Various combinations of chemicals, acting synergetically or antagonistically, and administered in concurrent or competitive injections into particular links of this system will probably allow one to establish the functional interdependence between the levels of the whole defensive system, and reveal the neurochemical basis of its activity. If the hypothesis of the chemical differentiation of the hypothalamic defensive system is accepted, it may be assumed that as carbachol and tubocurarine elicit two different responses from the same hypothalamic site, acting through different central

receptors. From previous reports (3, 9, 11, 36) it is only known that tubocurarine and carbachol injections elicit defensive responses probably due to the effects these compounds produce on the central receptors belonging in general to the cholinergic system. Carbachol was believed to produce stimulation comparable to acetylocholine, the natural mediator of the cholinergic system, as well through the muscarinic receptors as through the nicotinic ones. We have tried to clarify experimentally whether carbachol may really act through the intermediary of both of these types of receptor of the cholinergic system. The investigations recently carried out (unpublished) have proved that carbachol injected into the hypothalamus, after previous application of atropine at the same site, i.e. after blocking the muscarinic receptors, fails to elicit any defensive response, whereas carbachol injection administered after the nicotinic receptors had been blocked by betamon or hexamethonium, induces a full aggressive response (Table III). Injection of chemicals, selectively stimu-

TABLE III

Effect of muscarinic and nicotinic receptor blocking agents on the rage reaction elicited by intrahypothalamic injection of carbachol. Abbreviations as in Table II.

Cat	Symptoms elicited by intrahypothalamic chemical stimulation		
	8 μg carbachol	10 μg atropine +8 μg carbachol	50 μg betamon +8 μg carbachol
R52	g, h, s, r, d, a	—	g, h, m, r
R53	g, p, m, r	—	g, p, m
R54	g, h, p, m, s, a	—	g, h, p, m, s, a
R55	g, h, p, m	—	g, h, p, m, s, a

lating the nicotinic or muscarinic receptors, confirmed these findings. Arecoline and carbachol injected simultaneously into the hypothalamus intensified the somatic, vegetative and behavioral symptoms typical of rage, whereas the effects following simultaneous injections of nicotinic acid and carbachol did not differ from those elicited by application only of carbachol. The investigations carried out with the use of anticholinergic substances blocking selectively muscarinic and nicotinic receptors, as well as those performed with these receptors functional, prove that in eliciting rage, carbachol acts through the muscarinic receptors. The mechanism of tubocurarine action seems to be more complex, because this compound induces a fear response after either the muscarinic, or the nicotinic receptors have been blocked (Table IV). Tubocurarine is likely to act through some receptors of the cholinergic system which have not been identified as yet, or, perhaps, through receptors which

TABLE IV

Effect of muscarinic and nicotinic receptor blocking agents on the fear reaction elicited by intrahypothalamic injection of d-tubocurarine. Abbreviations as in Table I.

Cat	Symptoms elicited by intrahypothalamic chemical stimulation		
	20 μg d-tubocurarine	10 μg atropine + 20 μg d-tubocurarine	50 μg betamon + 20 μg d-tubocurarine
R52	v, u, m, l, r, d	v, u, m, l, r	u, m, l, r
R53	v, u, m, l	v, u, m, l, r	u, m, l, d
R54	v, u, m, l, d, r	v, u, m, l, d	v, u, m, l
R55	v, u, m, r	v, u, m, r	v, u, r, l, d

do not belong to this system at all. We wish to emphasize that the data presented in this paper are preliminary, as the problem of chemically specific receptive sites is a separate, complex question necessitating further, extensive research. We hope that use of autoradiography with injection of tubocurarine marked with isotopes, will allow us to identify the specific sites where this agent acts in the brain. Electron microscopic analysis may also help to reveal the specific receptive elements for this compound. The combination of two techniques: chemical stimulation of the brain with substances eliciting some determined physiological effect, and autoradiography, ought to contribute to a determination of neuronal functional circuits, and to provide some fundamental information concerning neurochemical processes which induce particular central drives (Fig. 1).

Although the technique of chemical stimulation of the brain contributed a lot to broadening the knowledge of neurochemical mechanisms regulating particular kinds of animal behavior, this method has many shortcomings. The brain consists of complicated, interlaced entities, and functional systems spread on different levels. This is why the behavioral effects induced by injections of some chemical substance into a defined site should not be viewed as evidence of a direct activity of the injected compound at this locus. These effects may result from disturbance of the balance between particular links of the same functional system, or between different neuronal systems. The chemoinjection of the brain may also induce some non-specific effects connected with changes of pH, osmotic pressure, or with mechanical action on the central nervous system. This is why experiments permitting the elimination with great accuracy of all kinds of artefacts and non-specific symptoms are extremely important in neurochemical investigations. It must be born in mind that all knowledge of the activity of particular neurotransmitters, neurohormones, and other biologically active substances has been acquired

mostly form investigating their influence on peripheral synapses. In this connection there are many instances which raise doubts whether the mechanism of action of some particular substance is comparable in the central nervous system.

Synaptic transmission in the brain is performed within complicated neuronal systems through various neurotransmitters acetylocholine, adrenaline, noradrenaline, or serotonin. Chemical agents injected directly into the central nervous system may bring on behavioral alterations via complicated, indirect ways, for instance by synergistic stimulation or antagonistic blockade of synaptic, chemosensitive structures, activated by some definite neurotransmitter. It is also likely that a biologically active substance injected into brain takes part in the synthesis, or disintegration of a given neurotransmitter, and it may bring on its inactivation, or protect it from de-activating enzymes.

The various effects produced by pharmacological substances introduced into the brain have been reviewed to show how vast are the possibilities and perspectives offered by neurochemical investigations, and, on the other hand, how many difficulties and complications must be overcome to understand the central mechanisms that regulate particular behavioral responses.

REFERENCES

1. ALLIKMETS, L. H. 1970. Problems of studying neurochemical mechanisms of behavior control (in Russian). Zh. Vyssh. Nerv. Deyat. 20: 386–393.
2. AMIN, A. N., CRAWFORD, T. B. B. and GADDUM, J. H. 1954. The distribution of substance P and 5-hydroxytryptamine in the central nervous system of the dog. J. Physiol. 126: 596–618.
3. BANDLER, R. J. 1970. Cholinergic synapses in the lateral hypothalamus for the control of predatory aggression in the rat. Brain Res. 20: 409–424.
4. BANDLER, R. J. 1971. Direct chemical stimulation of the thalamus: effects on aggressive behavior in the rat. Brain Res. 26: 81–93.
5. BARD, P. 1928. A diencephalic mechanism for the expression of rage with special reference to the symphatetic nervous system. Amer. J. Physiol. 84: 490–515.
6. BAXTER, B. L. 1966. Chemical and electrical stimulation of hypothalamic sites mediating "emotional" behavior. Pharmacologist 8: 205.
7. BERTLER, A. and ROSENGREN, A. 1959. Occurrence and distribution of catecholamine in brain. Acta Physiol. Scand. 47: 350–361.
8. BROWN, J. L., HUNSPERGER, R. W. and ROSVOLD, H. E. 1969. Defence, attack, and flight elicited by electrical stimulation of the hypothalamus of the cat. Exp. Brain Res. 8: 113–129.
9. DESCI, L. and KARMOS-VÁRSZEGI, M. 1969. Fear and escape reaction evoked by intraphypothalamic injection of d-tubocurarine in unrestrained cats. Acta Physiol. Acad. Sci. Hung. 36: 95–104.

10. EGGER, M. D. and FLYNN, J. P. 1967. Further studies on the effects of amygdaloid stimulation and ablation on hypothalamically elicited attack behavior in cats. Progr. Brain Res. 27: 165–182.
11. ENDRÖCZI, E., SCHREIBERG, G. and LISSÁK, K. 1964. The role of the activating and inhibitory structures in the control of the pituitary-adreno-cortical function. Effects of intracerebral cholinergic and adrenergic stimulation. Acta Physiol. Acad. Sci. Hung. 24: 211–221.
12. FELDBERG, W. and MYERS, R. D. 1964. Effects on temperature of amines injected into the cerebral ventricles. A new concept of temperature regulation. J. Physiol. 173: 226–237.
13. FERNANDEZ de MOLINA, A. and HUNSPERGER, R. W. 1959. Central representation of affective reactions in forebrain and brainstem: Electrical stimulation of amygdala, stria terminalis, and adjacent structures. J. Physiol. 145: 251–269.
14. FISHER, A. E. and COURY, J. N. 1962. Cholinergic tracing of a central neural circuit underlying the thirst drive. Science 138: 691–693.
15. FONBERG, E. 1965. Effect of partial destruction of the amygdaloid complex on the emotional-defensive behavior of dogs. Bull. Acad. Pol. Sci. Ser. Sci. Biol. 13: 429–432.
16. GROSSMAN, S. P. 1960. Eating or drinking elicited by direct adrenergic or cholinergic stimulation of hypothalamus. Science 132: 301–302.
17. HERNANDEZ-PEON, R., CHAVEZ-IBARRA, G., MORGANE, P. J. and TIMOIARIA, C. 1963. Limbic cholinergic pathways involved in sleep and emotional behavior. Exp. Neurol. 8: 93–111.
18. HESS, W. R. 1949. Das Zwischenhirn. Syndrome, Lokalisationen, Funktionen. Benno Schwabe, Basel. 187 p.
19. HILTON, S. M. and ZBROŻYNA, A. W. 1963. Amygdaloid region for defence reactions and its efferent pathway to the brainstem. J. Physiol. 165: 160–173.
20. HUNSPERGER, R. W. 1956. Affektreaktionen auf elektrische Reizung im Hirnstamm der Katze. Helv. Physiol. Pharmacol. Acta 14: 70–92.
21. KAADA, B. R. 1951. Somato-motor, autonomic and electrocorticographic responses to electrical stimulation of "rhinencephalic" and other structures in primates, cat and dog. Acta Physiol. Scand. 24, Suppl. 83: 1–285.
22. KONORSKI, J. 1967. The integrative activity of the brain. An interdisciplinary approach. Chicago Univ. Press, Chicago 531 p.
23. MAC LEAN, P. D. 1955. The limbic system ("visceral brain") and emotional behavior. Arch. Neurol. Psychiat. 73: 130–134.
24. MILLER, N. E. 1965. Chemical coding of behavior in the brain. Science 148: 328–338.
25. MYERS, R. D. 1964. Emotional and autonomic responses following hypothalamic chemical stimulation. Canadian J. Psychol. 18: 6–14.
26. NAKAO, H. 1958. Emotional behavior produced by hypothalamic stimulation. Amer. J. Physiol. 194: 411–418.
27. PAPEZ, J. W. 1937. A proposed mechanism of emotion. Arch. Neurol. Psychiat. 38: 725–744.
28. ROMANIUK, A. 1963. Emotional responses evoked by hypothalamic stimulation in cats. Bull. Acad. Pol. Sci. Ser. Sci. Biol. 11: 437–440.
29. ROMANIUK, A. 1965. Representation of aggression and flight reaction in the hypothalamus of the cat. Acta Biol. Exp. 25: 177–186.
30. ROMANIUK, A., BRUDZYŃSKI, S. and GROŃSKA, J. 1973. Specific changes

in the defensive behavior of cats following injection of d-tubocurarine into the hypothalamic areas. Acta Physiol. Pol. 24: 305–313.
31. ROMANIUK, A., BRUDZYŃSKI, S. and GROŃSKA, J. 1973. Rage reaction evoked by intrahypothalamic injection of carbachol in cats. Acta Physiol. Pol. 24: 623–630.
32. SCHILDKRAUT, J. J. and KETY, S. S. 1967. Biogenic amines and emotions. Science 156: 21–30.
33. SEPINWALL, J. 1966. Cholineric stimulation of the brain and avoidance behavior. Psychonom. Sci. 5: 93–94.
34. URSIN, H. 1965. Effect of amygdaloid lesions on avoidance behavior and visual discrimination in cats. Exp. Neurol. 11: 298–317.
35. URSIN, H. 1969. The cingulate gyrus — a fear zone? J. Comp. Physiol. Psychol. 68: 235–238.
36. VÁRSZEGI, M. K. and DECSI, L. 1967. Some characteristics of the rage reaction evoked by chemical stimulation of the hypothalamus. Acta Physiol. Acad. Sci. Hung. 32: 61–68.
37. VOGT, M. 1954. The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. J. Physiol. 123: 451–481.
38. WASMAN, M. and FLYNN, J. P. 1962. Directed attack elicited from hypothalamus. Arch. Neurol. 6: 220–227.
39. WHEATLEY, M. D. 1944. The hypothalamus and affective behavior in cats. A study of the effects of experimental lesions with autonomic correlations. Arch. Neurol. Psychiat. 52: 296–316.

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