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EXPLORATION OF THE DEEP CEREBRAL STRUCTURES IN THE CAT BY MEANS OF BENACTYZINE * AND PERPHENAZINE **

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In pharmacologic studies on the mode of action of tranquillizing drugs and their point of attack in the central nervous system (CNS), certain nervous structures have been pointed out as those upon which the tranquillizing effect depends mainly, although not exclusively. Attention has been directed especially to the ascending, activating, reticular system of the brain stem and cortico-subcortical feedback loops [11]. In connection with the reticular system of the mesencephalon, a role has been attributed to diffuse opticocortical projection, i.e. the system of Jasper [6], as the main site of action of tranquillizers. Berger et al. [3] associate the tranquillizing effect with an influence on the relay nuclei in the thalamus. Bein [2] and Steiner et al. [13] suppose that tranquillization after phenothiazine depends on hypothalamic depression. Preston [10] was the first author to attribute importance to the limbic structures in tranquillization. Hano et al. [4], while studying the tranquillizing properties of substituted derivatives of γ-butyrolactone injected intraperitoneally and into the ventricles, demonstrated a selective influence on the limbic structures, especially on the amygdaloid nuclei system.

In view of the lack of uniform opinions on the point of attack of tranquillizing drugs administered intrasystemically or intraperitoneally, it was considered of interest to reverse the problem methodologically, i.e. to explore directly the action of tranquillizers on certain CNS structures.

In a simplified way, the behavior of animals depends on three basic nervous structures: 1) the hypothalamus, which is the seat of drive activity and, moreover, the highest control center of hormonovegetative reflexes; 2) the neocortical-reticular formation system of the brain stem, which prepares the plan of action on the basis of internal and external conditions of the body;

* β-Diethylaminoethyl benzilate hydrochloride.
** 2-Chloro-10-{3-[1-(β-hydroxyethyl)-4-piperazinyl-1]-propyl}-phenothiazine.

and 3) the limbic structures, which participate in motivation of behavior and emotional background of all reactions.

The purpose of this study was an attempt to ascertain which of the aforementioned structures, when acted upon by tranquillizers, generates the tranquillization syndrome as described in a previous paper [4].

MATERIAL AND METHODS

Chronic experiments were carried out with 32 cats of both sexes weighing 2.8—3.5 kg. Besides cortical electrodes placed supradurally in the sensory-motor region, double-walled stereotaxic cannulas of the Grossmann type were introduced into the following deep structures: hippocampus (HP), amygdaloid nuclei (AN), preoptic region (RPO), nonspecific thalamic nucleus (CL), and brain stem reticular formation (BSRF).

In the first group (16 cats) parameters according to the atlas of Jasper and Ajmone-Marsan [8] were used: HP (F = 5.0; L = 4.5; H = 8.0) — AN (F = 9.0; L = 10.0; H = 7) — RPO (F = 14.5; L = 2.0; H = − 3.5) — CL(F = 9.0; L = 3.5; H = 2.5) — BSRF)F = 2.0; L = 3.5; H = − 2.5).

In the second group, parameters according to the atlas of Snider [12] were used: HP (F = 2.5; L = 9.0; H = 6.0) AN (F = 11.0; L = 11.0; H = − 6.0) RPO (F = 14; L = 3.5; H = − 4.0) BSRF (F = 2.5; L = 4.0; H = − 1.0) CL (as in the first group). Instead of the hypothalamus, the preoptic region (RPO) was explored, which is not included into the hypothalamus by some authors [14] because of its direct connections with the remaining structures explored (HP, AN and BSRF).

The experimental situation was unequivocal, and the alimentary reaction of the animals, fasted two days, to meat or mouse placed in the cage was easy to evaluate. Direct chemical stimulation was performed with benactyzine (B) a tranquillizer with parasympathicolytic activity, and perphenazine (P), which has sympathicolytic activity. Both compounds were injected intracerebrally with an „Agla" syringe in doses of 1—3 mg contained in 0.01—0.03 ml of physiologic saline solution. Infusions were performed once in the course of 1—2 minutes. Experiments with the same cat were performed at intervals of 1—2 weeks. Before injecting the compounds into the tissues, the animals were adapted to the experimental situation for one hour and the strength of the alimentary reaction was checked. The behavior of the animals was observed while EEG tracings were being made. The apparatus used and other details of the method have been described in a previous paper [15].

RESULTS

Since similar results were obtained with B and P, they will be discussed jointly and summarized in Tables 1 and 2. Minor differences between the action of the two compounds after infusion into the BSRF will be commented in the discussion.

The most pronounced changes in the bioelectric activity of the brain of cats and in the behavior of the animals were observed when the compounds were injected into the limbic structures, especially into the amygdaloid nuclei. After 28 out of 32 infusions the alimentary reaction was abolished, i.e. the animals exhibited no interest or aversion to meat or mice placed in the cage. In 3 cases the alimentary reaction was only weakened, manifested by turning

Table 1. Changes of bioelectric activity

Site of registration / Site of infusion		Total number of infusions	Neocortex			Reticular formation of the mesencephalon			Preoptic region			System of amygdaloid nuclei			Hippocampus		
			S	D	O	S	D	O	S	D	O	S	D	O	S	D	O
Limbic structures	Amygdaloid system	32	7	23	2	8	23	1	—	28	4	31	—	1	22	—	10
	Hippocampus	11	—	11	—	2	9	—	—	11	—	8	—	3	10	—	1
Preoptic region		28	2	7	19	—	24	4	—	26	2	14	2	12	4	10	14
Reticular formation of the mesencephalon		26	—	26	—	—	26	—	—	19	7	10	12	4	12	8	6

Designation: S — synchronization, D — desynchronization, O — out of effect.

Table 2. Changes of somatovegetative activity

Effect registered / Site of infusion		Total number of infusions	Alimentary reaction			Motor system			Vegetative system	
			abolished	weakened	unaffected	motor disorders	abolished spontaneous motility	unaffected	stimulated	unaffected
Limbic structures	Amygdaloid system	32	28	3	1	12	16	4	30	2
	Hippocampus	11	2	5	4	—	8	3	11	—
Preoptic region		28	2	4	22	2	10	16	27	1
Reticular formation of the mesencephalon		26	1	5	20	11	4	11	10	16

away from proferred meat, smelling it only, or uncoordinated attempts to eat. In one case infusion into the AN had no effect on the alimentary reaction, suppression of which is a fundamental symptom in the tranquillization syndrome. The effect of infusion into the AN on the motor system in 16 cases consisted in loss of spontaneous motility, and in 12 cases disorders such as loss of righting reflex, and incoordinated gait (swaying, striking the head against walls) were observed. In one case catatonic symptoms were noted (the cat lay on its back with extended, rigid paws). In four cases motor function was unaffected. In 30 cases the motivational and motor disorders were accom-

panied by vegetative stimulation manifested by dilated pupils, accelerated breathing, slight exophthalmos, salivation and urination. Some of the somatovegetative disorders, which will be discussed separately, were named the tranquillization syndrome, observed in 31 cases after infusion into the AN.

Typical EEG changes after infusion of P into the AN are illustrated in Fig. 1. Immediately after the infusion desynchronization appeared in all the leads. After 10 minutes the bioelectric activity of the AN underwent gradual synchronization; in place of the flat runs with fast rhythm, high-voltage sharp waves with a few free elements appeared. After 35 minutes, at the peak of the action of P, hypersynchronization included the HP and neocortex (Fig. 1 D). In 20 cases, however, the neocortex and BSRF remained desynchronized, displaying a typical pattern of dissociation between the bioelectric activity of the phylogenetically younger system and older limbic structures. After 2 hours the tracing returned to normal (Fig. 1 F). Hypersynchronization was observed in AN leads in all cases with the tranquillization syndrome.

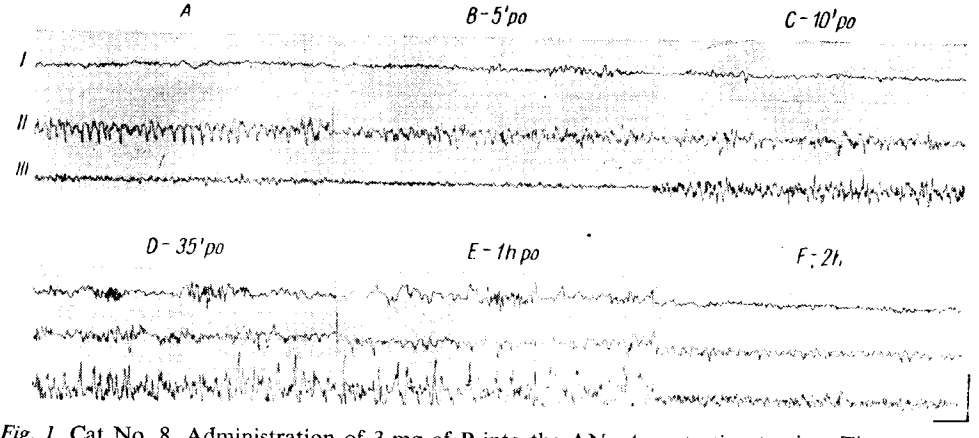

Fig. 1. Cat No. 8. Administration of 3 mg of P into the AN. *A* — starting tracing. Time counted from the beginning of infusion. *I* — neocortex, *II* — HP, *III* — AN. The horizontal line denotes 1 second, and the vertical line 100 μV. (The same designations are used in the following figures).

Infusion of the substances into the HP produced qualitatively similar results to those observed after infusion into the AN. An effect on the alimentary reaction was obtained in 7 out of 11 cases, and spontaneous motility was abolished in 8 cats. All the infusions into the HP elicited vegetative stimulation. Concurrently with symptoms of tranquillization, EEG changes, illustrated in Fig. 2, were observed. Ten minutes after injection of 3 into the HP the regular theta rhytm was replaced by high-voltage discharges of sharp waves and spikes radiating to the AN. Hypersynchronization in the HP occurred on the background of generalized desynchronization. After 55 minutes the band of frequent hypersynchronic discharges moved in the direction of the δ waves. After 80 minutes the EEG returned to normal.

Because of the close anatomophysiologic relations of the AN and HP, in 30 out of 43 cases after infusion into the limbic structures it was difficult to decide which of these centers was the direct source of tranquillization.

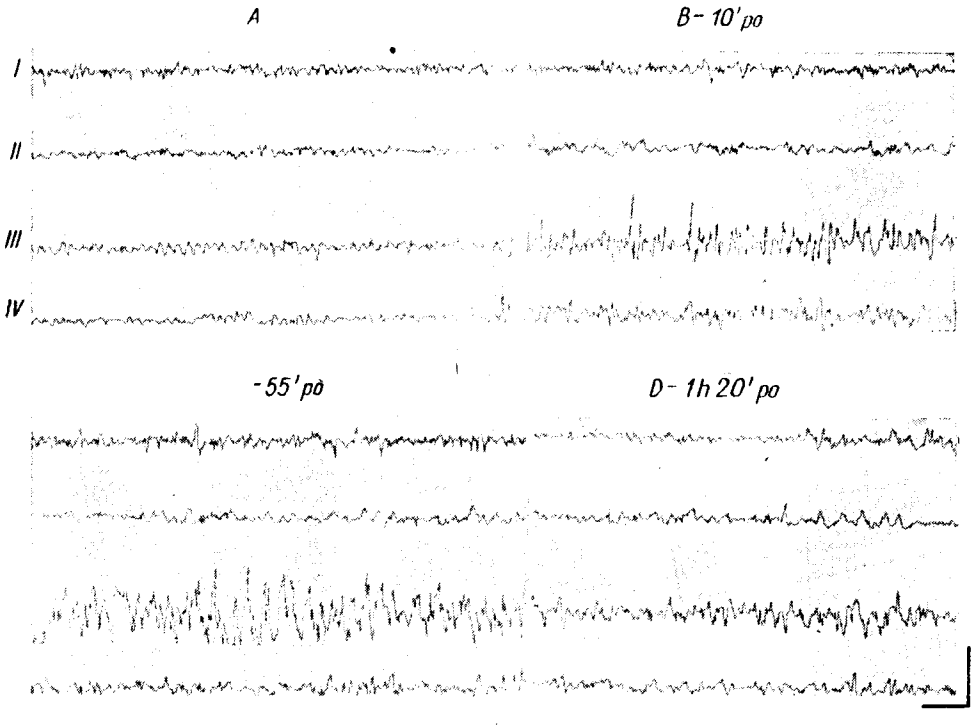

Fig. 2 Cat No. IV. Administration of 2.5 mg of B into the HP. *A* — starting tracing, time counted from the beginning of infusion. *I* — neocortex, *II* — BSRF, *III* - HP, *IV* — AN.

Nevertheless, it was demonstrated that the EEG and behavioral changes caused by chemical stimulation of the AN had the shortest latent period — 2—5 min.

Injection of the compounds into the RPO abolished or weakened the alimentary reaction in only 21.5% of cases; motor disorders manifested by unrest as well as loss of spontaneous motility occurred in 43%, and vegetative stimulation in all cases. Infusion of the tranquillizers into the RPO produced a more differentiated pattern of bioelectric activity. Whereas infusion into the RPO always caused desynchronization of BSRF, the influence on the cortex was less uniform; signs of synchronization (in two cases) on a background of BSRF desynchronization were observed exceptionally. The full tranquillization syndrome, observed in only 6 cases, was usually associated with hypersynchronization in the AN. The EEG changes after infusion of B into the RPO are illustrated in Fig. 3. For 32 minutes after the infusion (Fig. 3B

and C) all the leads showed desynchronization, especially distinct in the flat leads from the neocortex. After 32 minutes the EEG pattern began to change, signs of synchronization appearing first in the HP. After 50 minutes synchronization spread to all the leads, but least to those from the neocortex (Fig. 3D).

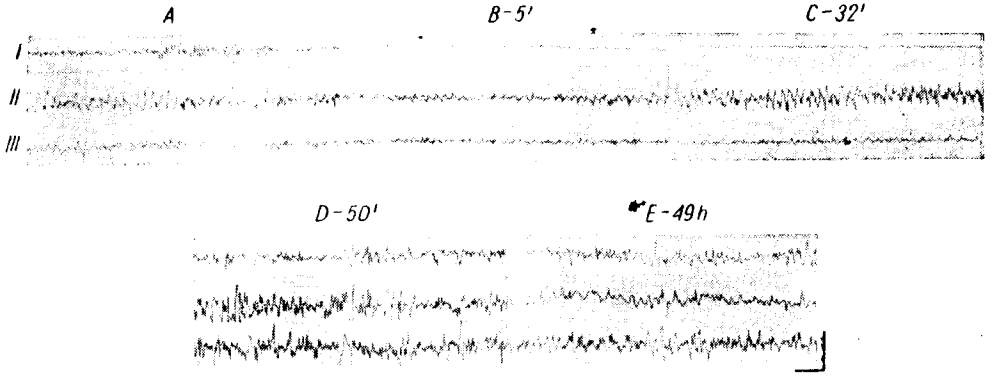

Fig. 3. Cat No. VII. Administration of 3 mg of B into the RPO. *A* — starting tracing, time counted from the beginning of infusion. *I* — neocortex, *II* — HP, *III* — AN.

BSRF activity after local administration of tranquillizers had little influence on motivated behavior of the animal. Absence of an influence on the alimentary reaction after infusion into the BSRF was recorded in 20 out of 26 cases. In 15 cases motor disorders were observed (atactic gait, loss of righting reflex, abolishment of spontaneous motility). Of all the types of administration,

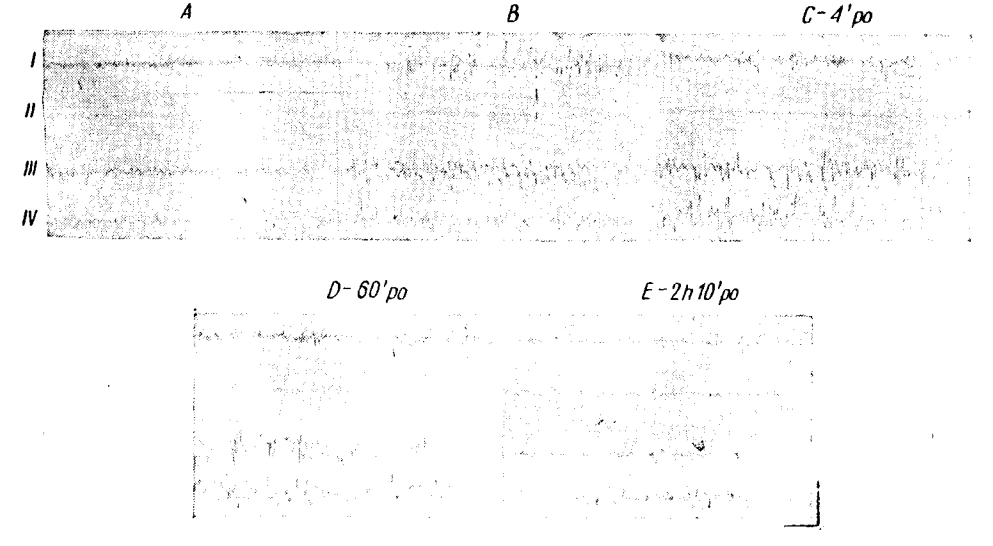

Fig. 4. Cat No. XIII. Administration of 3 mg of B into the BSRF. *A* — starting tracing, resting; time counted from the beginning of infusion. *I* — neocortex, *II* — BSRF, *III* — HP, *IV* — AN.

infusions into the BSRF exhibited the weakest influence on vegetative functions, which were noted in only 10 out of 26 cases. Chemical stimulation of the BSRF in 14 cases produced uniformly generalized desynchronization. In 10 cases, as can be seen in Fig. 4, results were differentiated. Four minutes after stopping the infusion (Fig. 4C) EEG activity in the limbic structures showed sudden synchronization; after 35 minutes synchronization passed into hypersynchronization lasting 130 minutes. In 6 cases a typical tranquillization syndrome appeared simultaneously with synchronization of the limbic structures.

Differences between the effect of infusions of P and B were observed only in the BSRF. Somatovegetative disorders occurred after 11 out of 13 infusions of B, but only in 3 out of 13 after P. Doses of 1 mg and 3 mg produced identical changes in the EEG pattern.

DISCUSSION

The structural model of external manifestations of emotional behavior consists of three fundamental subsystems: from the limbic system, hypothalamic centers, and from structures in the brain stem.

The limbic system is an integrating center, where the demands of the body are ranked and the emotional character of behavioral reactions is determined. Among the limbic structures, the AN plays a key role, concentrating all the impulses from other parts of the limbic lobe. The hypothalamus, which contains, among others, neurodetectors sensitive to changes in blood composition in hunger and after feeding, is regarded as a triggering link and source of impulses for all types of behavior.

In the organization of the motor aspect, the selfreliant acting system of Hunsperger [7], elaborated by Adey [1] and Nauta [9], plays a decisive role. Laterally to the self-reliant-acting system lies the ascending activating system of Moruzzi and Magoun, connected with the neocortex by loops in two directions, and anatomophysiologically being the substrate of arousal reactions and other components of behavior. In this study, all three subsystems have been explored pharmacologically with the purpose of establishing the link on which symptoms of tranquillization depend most.

Evaluation of the behavior of the animals was based on the following elements: changes in the alimentary impulse in fasted cats and motor and vegetative disorders. Some of the elements of this behavior, taken in different proportions, constituted the tranquillization syndrome consisting of loss or weakening of the alimentary reaction, loss of spontaneous motility, maintained state of arousal, and vegetative stimulation. Not all the symptoms of tranquillization had equal diagnostic value. Greatest importance was attached to suppression or weakening of the alimentary drive as the outstanding sign of

tranquillization. The role of the somatovegetative component was secondary and complementary.

In accordance with the principle of representation of Hunsperger [7], all the explored links of the model cooperate in emotional reactions, although in different degrees. Each link may have a different level of representation (a concept that is analogous to the threshold value) for the full emotional reaction, or only for some of its components. It follows from the reported experiments that the AN has the lowest level of representation for the tranquillizing syndrome. Tranquillization symptoms were accompanied by significant EEG changes consisting in generalized desynchronization arousal, except in AN leads, where self-maintaining selective hypersynchronization manifested inhibition.

The HP also showed a low level of representation for the tranquillization syndrome. In 38 out of 43 cases infusions into the limbic structures elicited symptoms of tranquillization, although in 30 cases it was not easy to establish on which limbic center, AN or HP, the tranquillizing effect depended. It may be concluded that both structures, although in unequal degree, determine the appearance of the tranquillization syndrome.

In contrast to the centers of the limbic lobe, infusions into the RPO and BSRF had little influence on appearance of tranquillization, notwithstanding that both are important links of the structural model of emotion. The high level of representation of the RPO for tranquillization may be explained as a result of absence of direct communications between the preoptic region and hypothalamic alimentary centers. Some investigators [14] exclude the RPO from the hypothalamus. The choice of the weakly intervening RPO for neuropharmacologic exploration was dictated by the assumptions of this study taking into account the anatomic connections of this area with the remaining structures that were stimulated chemically (from AN via the stria terminalis, from HP through the fornix and from BSRF through the medial of the forebrain bundle).

The low percentage of positive results obtained after administration into the BSRF suggests that the ascending activating system of the mesencephalon stimulated chemically does not participate directly in organizing the symptoms of tranquillization. On the other hand, the fact that tranquillization was obtained in 6 cases after infusion into the BSRF can be explained either by diffusion („leaking") of the tranquillizing drugs into the self-reliant-acting system of the mesencephalon, or by an indirect influence of the ascending activating system which is connected with the system of Hunsperger [7] through the gray radiation of the tegmentum. The short latent period — less than 5 minutes — preceding the appearance of tranquillization after infusion into the BSRF is an argument against the mechanism of diffusion, while supporting the existence of anatomofunctional connections between the limbic lobe and

the system of Hunsperger, which for this reason was included into the mesencephalic limbic circuit of Nauta.

The smaller effectiveness of infusions of P, compared with B, which is a tranquillizer with parasympathicolytic activity, confirms the opinion according to which acetylcholine plays the role of a transmitter in the upper part of the brain stem [5].

It may be concluded from these experiments that, of the many structures to which a role has been attributed in the mechanism of the action of tranquillizing drugs, the limbic system, especially the AN with hypersynchronic biolectric activity, seems to be the most essential element in the production of symptoms of tranquillization.

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EKSPLORACJA GŁĘBOKICH STRUKTUR MÓZGU KOTA PRZY POMOCY BENAKTYZYNY I PERPHENAZYNY

Streszczenie

Typ zachowania zwierzęcia zależy od trzech zasadniczych struktur ośrodkowego układu nerwowego: ośrodka podwzgórzowego, układu korowo-pniowego oraz układu limbicznego.

Praca niniejsza próbuje odpowiedzieć na pytanie, który z wymienionych ośrodków warunkuje powstanie efektu trankwilizującego.

Wszystkie trzy ośrodki poddano eksploracji farmakologicznej, polegającej na bezpośrednim, domózgowym podawaniu benaktyzyny (trankwilizera o działaniu parasympatykolitycznym) i perphenazyny (trankwilizera o działaniu sympatykolitycznym). Doświadczenia chroniczne przeprowadzono na 32 kotach z elektrodami implantowanymi do kory nowej, okolicy przedwzrokowej hippokampa, zespołu jąder migdałowatych i tworu siateczkowatego pnia śródmózgowia. Sytuację doświadczalną stanowiła, reakcja pokarmowa zwierzęcia głodzonego przez dwie doby. Związki podawano w 0,01—0,03 ml roztworu soli fizjologicznej, w ilości 1—3 mg. Oprócz obserwacji zmian zachowania równolegle przeprowadzano zapis EEG.

Największe zmiany w zachowaniu, w postaci pełnego zespołu trankwilizacji (stłumienie reakcji pokarmowej, znaczne spowolnienie ruchowe, zanik spontaniczności oraz złagodzenie cech charakteru przy niezaburzonym stanie czuwania), obserwowano po podaniu związków do zespołu jąder migdałowatych i hippokampa. Niezależnie od tego, do której struktury limbicznej wprowadzano związek synchronizacja EEG, występowała równocześnie, lub naprzemiennie, zarówno w hippokampie, jak i w zespole jąder migdałowatych. Zespół objawów trankwilizacji spostrzegano w mniejszym odsetku po podaniach do okolicy przedwzrokowej i do układu siateczkowatego pnia śródmózgowia.

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ИССЛЕДОВАНИЕ ГЛУБОКИХ СТРУКТУР МОЗГА КОТА ПРИ ПОМОЩИ БЕНАКТИЗИНА И ПЕРФЕНАЗИНА

Содержание

Тип поведения животного зависит от трех основных структур центральной нервной системы: подбугровой области, кортикостволовой системы и лимбической системы.

В настоящей работе авторы стремятся ответить на вопрос — которая из трех указанных систем обусловливает появление транквилизирующего эффекта.

Все три системы подвергнуты фармакологическому исследованию, состоящему в непосредственном внутримозговом введении бенактизина (транквилизатора с парасимпатиколитическим действием) и перфеназина (транквилизатора с симпатиколитическим действием). Хронические эксперименты проведены на 32 котах с электродами, имплантированными в неокортекс, предзрительную область, гиппокамп, в комплекс миндальных ядер и ретикулярной субстанции ствола среднего мозга. Экспериментальной ситуацией явилась пищевая реакция животного, голодавшего в течение двух суток. Вводимые соединения в количестве 0,01—0,03 мл раствора физиологической соли содержали 1—3 мг препарата. Кроме наблюдений над изменениями поведения животных проведены ээг записи.

Самые большие изменения в поведении, в форме полного симптомокомплекса транквилизации (снижение пищевой реакции, значительная замедленность движений, исчезание спонтанности, а также смягчение черт характера, при ненарушенном состоянии бодрствования) наблюдались после введения соединений в комплекс миндальных ядер и гиппокамп. Независимо от того, в какую лимбическую структуру были введены соединения, синхронизация ээг появлялась одновременно, или попеременно, как в гиппокампе, так и в комплексе миндальных ядер. Симптомокомплекс транквилизации наблюдался в меньшем проценте случаев после введения препаратов в предзрительную область и в ретикулярную формацию ствола среднего мозга.

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