corpus striatum, coupled with the dopamine D-1 receptor and also, evidently, for other dopaminergic receptors in the brain than typical neuroleptics. Consequently it may be expected that the use of thse antiarrhythmic phenothiazine preparations will not be complicated by any significant neurotropic action or by any influence on peripheral dopaminergic receptors.

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AMPHETAMINE STEREOTYPY AS A STABLE RHYTHMIC PROCESS

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KEY WORDS: amphetamine; stereotypy; mental disturbances.

Stereotyped behavior induced by large doses of amphetamine is a well known state at the present time that is widely used as a model of psychopathology and for screening psychotropic drugs [1, 2].

Stereotypy in different species of animals is characterized by an assortment of automatized actions (head turning, sniffing, licking, and so on). Their frequency characteristics and time course have not been adequately studied. Yet such an approach could be important for our understanding of the nature of mental disturbances. With this in mind, in the investigation described below some temporal parameters of stereotyped behavior were studied in detail for the first time in experiments on cats.

EXPERIMENTAL METHOD

Nineteen cats of both sexes weighing from 2 to 3.5 kg were used. Horizontal motor activity of the animals was recorded in a special chamber by means of an electromechanical rotameter of original design. To connect the cat's head securely to the mechanical part of the rotameter, the animal was anesthetized with ether and a socket was fixed to the vault of the skull. Head movements to right and left were recorded on a 4-channel N338-4P automatic writer and monitored visually.

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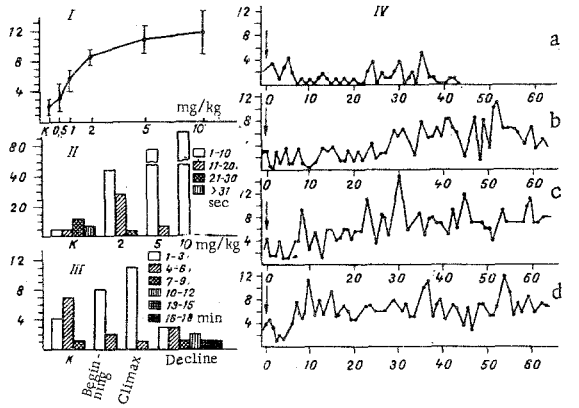

Fig. 1. Rhythmic characteristics of amphetamine stereotypy. I) Dependence of frequency of stereotyped head turning on dose of amphetamine (mean results of 12 experiments on 10 cats). Vertical axis — number of turns, K) original frequency of movements in control; II) histograms of intervals between individual head turnings after different doses of amphetamine. Vertical axis — number of intervals (duration measured in seconds on the right) during 10 min of recording. Results on different days of experiment on cat No. 35; K) control, III) histogram of periods of fluctuations in frequency of movements in different phases of amphetamine stereotypy (results of one experiment on cat No. 33). Total number of movements, irrespective of direction, estimated per minute. Vertical axis — number of periods, their duration shown on right (in min). K) control; IV) time course of fluctuations in animal's activity (cat No. 31) after injection of physiological saline (a) and a standard dose (in mg/kg) of amphetamine (b-d) on different days of experiment. Total number of movements to left and right counted. Arrow indicates time of injection.

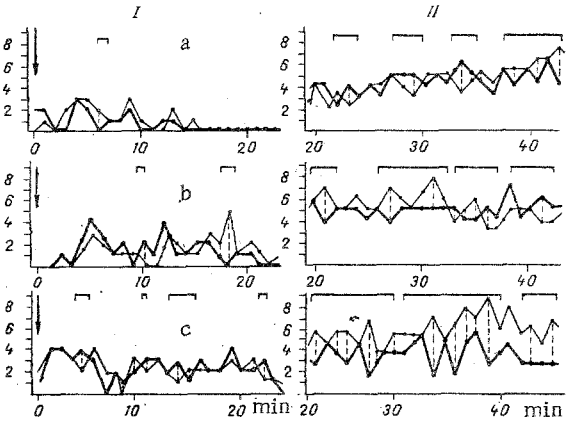

Fig. 2. Time course of correlation between number of head turns by animal (cat No. 35) to left and right after different forms of activation. Abscissa, time of recording (in min); ordinate, number of turns per minute. I) Comparison of natural activation after injection of physiological saline (a) and stimulating effects of caffeine, 20 mg/kg (b), and amphetamine in substereotyped dose, 0.25 mg/kg (c). Arrow indicates time of injection. II) time course of stereotyped movements and their increasing desynchronization after injection of increasing doses of amphetamine. Bold line shows time course of head movements to right, thin line — to left. Extrema in opposite phases joined by vertical broken lines, horizontal lines above indicate total duration of desynchronized episodes.

Amphetamine was injected intraperitoneally in one of the following doses: 0.25, 1, 2, 5, and 10 mg/kg. The animals' behavior was recorded immediately after injection of the psychostimulant and for the next 3-4 h. Experiments in which physiological saline and caffeine in a dose of 20 mg/kg were injected on the same schedule served as the control. All experiments were conducted under standard conditions (intensity of illumination, noise background, feeding, time of day) and with an interval of 2-3 days. The results were analyzed by Student's test ($P < 0.05$).

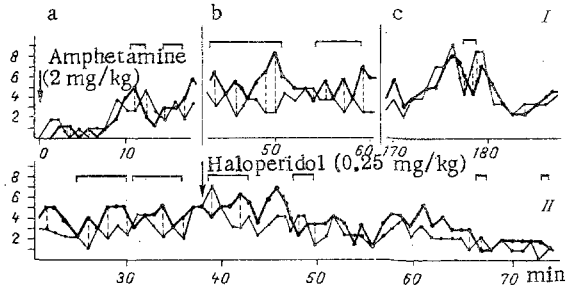

Fig. 3. Time course of head turns to left and right during natural evolution of amphetamine stereotypy (I) and under the influence of a neuroleptic (II). I): a) Beginning, b) climax, c) decline of stereotypy (cat No. 33). II) on another day of exerpiment 38 min after amphetamine at climax of stereotypy haloperidol was injected. Remainder of legend as to Fig. 2.

EXPERIMENTAL RESULTS

The special features of amphetamine stereotypy in cats were fully described previously [3]. It was emphasized then that monotonous head turning from side to side is the most constant component of abnormal behavior compared with other components of stereotypy (movements in the vertical plane, sniffing, grooming, and so on).

The results showed that the frequency of these turns (the total number of head movements to left and right per minute was counted) varied with the dose. After a substereotype dose of amphetamine (in this series of experiments 0.4 mg/kg) the small increase in frequency of horizontal movements did not exceed the control value statistically significantly. With an increase in the dose and with the appearance of definite stereotypy the number of turns gradually increased, to exceed the initial level significantly (Fig. 1: I). The maximal frequency of movement (14-16 turns/min) was obtained after injection of a dose of 10 mg/kg, after which this parameter stabilized or declined, or the actual structure of the stereotypy changed, with predominance of other components. Previous observations [5] showed that a dose of 1-2 mg/kg of amphetamine is sufficient to disturb the adequacy of cat's behavior completely.

Random testing of histograms of frequency of turns when increasing doses of the drug were used showed at first glance that the formation of stable stereotype is accompanied not only by an increase in frequency, but also by a more regular rhythm of horizontal movements. In particular, when time intervals between individual turns were determined, a regular increase in the number of short periods was found (Fig. 1: II).

Meanwhile successive evaluation of the process every minute for several hours of recording demonstrated its distinct oscillatory character. Normal activity of the animals also varied, but this activity declined with adaptation whereas stereotypy exhibited stable rhythmic fluctuations over a long period of time (Fig. 1: IV).

Waves with different period and amplitude can be detected in an oscillating trace of this kind: The regularity of their appearance, moreover, depends to some extent on the intensity of stereotypy. At its climax waves with a short period (1-3 min and low amplitude 3-4 turns/min) predominated (Fig. 1: III). Smoothing of the trace by the sliding means method [4] reveals oscillations with longer periods (12-14 min). However, superposition of the activity traces obtained in different experiments on the same animals, in response to injection of the standard dose of amphetamine, failed to reveal a rhythm with fixed frequency. The pattern of the curve varied appreciably from one experiment to another (Fig. 1: IV).

Inhibition of stereotypy took place through a stage of definite destabilization of the oscillatory process. As adequate behavior of the animals was restored, waves with long periods appeared more often (Fig. 1: III) and the amplitude of the oscillations increased (from 3-4 to 5-6 turns/min). Similar destabilization of the rhythmic pattern of stereotypy preceded complete abolition following injection of the neuroleptics haloperidol and clozapine.

Behavioral analysis thus shows that amphetamine stereotypy is a nonstationary oscillatory process, whose formation and decline obey certain rules. However, it is difficult to apply such information to the explaination of the mechanisms of stereotyped behavior. In our view, another approach, taking account of the time course of the frequency of movement depending on the direction of the turns could prove more informative.

A more careful study of the pattern of the movements themselves revealed some interesting phenomena. In particular, strict correlation was by no means always found between the intensity and number of turns to the left and right.

During natural activation animals adapted to the experimental chamber began to make a series of surveying movements with the head. Outwardly they were quite symmetrical, when a turn to one side was followed by movement in the opposite direction. This pendulum-like quality of the rotations is clearly visible on the actographic record. Behavior in the case of artificial stimulation by caffeine (20 mg/kg), which does not induce stereotypy, or by a small dose of amphetamine (0.25 mg/kg) was similar in appearance.

Meanwhile, when actograms of turns of the head to left and right were assessed separately, some significant fine differences could be detected. In some cats, for example, definite preference for one side could be discerned, in the form of a larger amplitude (angle) and duration of movement performed in that direction. In addition, at certain moments there were more turns in this direction. Averaging of the number of turns to left and right per minute gave two curves, each distinctly oscillatory in character.

For normal, adequately stimulated animals, a definite pattern could be observed in the position of these curves relative to each other (Fig. 2: I). Usually the shifts were synchronized, when the frequency of head turns to both sides either increased or decreased simultaneously, or their frequency was reduced without reaching complete numerical parity between them, and some delay in time also was possible. Moments of desynchronization occurred less frequently when the frequency shifts were in opposite directions: More frequent movements to one side coincided with less frequent turns to the other side. Under normal conditions the asynchronous periods were short and had low amplitude (the distance between the extrema in opposite phases).

The relations between the two curves after injection of a stereotyped dose of amphetamine presented at different appearance. During marked stereotypy a significant increase in the duration of the desynchronized and, conversely, shortening of the synchronized episodes were typical. With an increase in dose of the drug the total duration and amplitude of the first rose progressively, corresponding to stabilization of the period of observations when the total number of movements per minute was counted (Fig. 2: II). The same time course was found during analysis of different phases (beginning, climax, decline) of stereotypy following a standard dose of amphetamine (Fig. 3: I). Extinction of the process was accompanied by shortening of the desynchronized episodes and approximation of the curves. If a neuroleptic was injected at the climax of stereotypy, as in the case shown in Fig. 3: II, disparity between the direction of turns to the left and right quickly disappeared, culminating in complete abolition of the behavioral disorders.

Consequently, amphetamine and stereotypy is a complex oscillatory process in the course of time. To judge from the observations described above, besides a regular rhythm, it also is accompanied by defects in harmonious interhemispheric interaction, for turning the head to the side can be taken as an indicator of excitation of the contralateral half of the brain.

Among brain systems involved in turning movements a special place is occupied by nigrostriatal mechanisms [2]. Amphetamine increases the release of nigral dopamine and can thus readily induce asymmetry in the working of the two nigrostriatal systems [6, 7]. As the facts show, disturbance of this equilibrium is rhythmic in nature. The possibility likewise cannot be ruled out that the stable character of stereotypy and the degree of behavioral disorders (and, consequently, the severity of the psychopathology) depend not only on enhancement of rhythmic processes in the nigrostriatal systems of the opposite hemispheres, but also on defects in their synchronized, harmonious working.

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EFFECT OF BUTACLAMOL ENANTIOMERS ON TYROSINE HYDROXYLASE IN THE RAT HYPOTHALAMUS

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In view of its important regulatory role in catecholaminergic processes, the tyrosine hydroxylase reaction is interesting not only as an indicator of the velocity of these processes but also as a possible object for pharmacologic intervention [1]. Experiments *in vitro* showed previously that neuroleptics can exert a direct influence on tyrosine hydroxylase (TH), which is expressed as abolition of substrate inhibition of the enzyme [4]. The specificity [11] and stereospecificity of this effect have been established in relation to geometric isomers of flupenthixol [3]. At the present time butaclamol enantiomers, which differ in their clinical efficacy [10], their effect on animal behavior [11], and also in the specific competitive with spiroperidol binding test with membrane preparations from brain structures [13], are used as reference substances for the study of the mechanism of action of neuroleptics. Being equally soluble in lipids, the (+)- and (−)-enantiomers of butaclamol are virtually indistinguishable in their nonspecific membranotropic effects, and for that reason differences in their biological activity can be ascribed to their specific and stereoselective action [13].

The aim of this investigation was to study effects of butaclamol enantiomers, both direct and mediated through the synaptosomal membrane, on TH in the rat hypothalamus.

EXPERIMENTAL METHOD

Rats were decapitated and the hypothalamus removed in the cold. To obtain fractions, tissue from 10 rats was pooled, and the fractions for these experiments were isolated three times. A 10% homogenate was obtained in 0.32 M sucrose, large cell fragments were sedimented at 1000 g for 10 min, and the residue was washed with half the original volume of 0.32 M sucrose, after which unpurified synaptosomes and microsomes were obtained (100,000g, 60 min). The residue of unpurified synaptosomes was homogenized in 0.001 M K-phosphate buffer, pH 7.0, containing 0.002 M $CaCl_2$ to disintegrate the synaptosomes and interacellular organelles. The homogenate was centrifuged (100,000g, 60 min), the residue was suspended in 15 ml of 0.32 M sucrose, layered on a 0.6-0.8-1.2 M sucrose gradient, and centrifuged for 120 min at 80,000g in a bucket rotor. The fraction at the boundary between 0.8 and 1.2 M sucrose was collected. This membrane fraction is rich in TH [8] and does not contain myelin or mitochondrial material. The fraction was diluted with cold distilled water to a sucrose concentration 0.32 M, centrifuged at 100,000g for 60 min, and homogenized in 0.001 M K-phosphate buffer, pH 7.0, at the rate of 0.8 ml buffer per gram of obtained tissue. This homogenate was used as membrane-bound TH. Experiments on synaptosomes were carried out by the method described previously [5]. Synaptosomes isolated by the method in [7] were incubated in Krebs' phosphate buffer, pH 7.4, at 37°C for 15 min. After incubation the synaptosomes were separated from incubation medium by centrifugation at 25,000g (10 min). Membrane-bound TH was isolated from the residue thus obtained by the method in [8]. To obtain synaptosomes in these experiments, hypothalamic tissue from 40 rats was pooled each time (each sample during incubation contained material from six or seven rats). Altogether three experiments were carried out with incubation of synaptosomes. When the reaction velocity was measured, three

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