PHARMACOLOGY AND TOXICOLOGY

Effects of Afobazole on Cognitive Behavior of the Offspring of Rats Exposed to Tobacco Smoke during Gestation Period

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Experiments on the model of foraging behavior formation under conditions of free choice (T-maze) revealed learning failure against the background of reduced motor activity in the offspring of rats exposed to tobacco smoke on gestation days 1-20. Afobazole administered to pregnant rats orally in doses of 1 or 10 mg/kg daily during the whole gestation and/or entering rat pup body with breast milk from mothers receiving 200 mg/kg to day 20 of their life normalized their learning capacity. The formation of short-term and long-term memory in animals receiving afobazole did not differ from the control. Hence, afobazole corrects cognitive disorders in rats exposed to tobacco smoke during prenatal development.

Key Words: *afobazole; tobacco smoke; rat offspring; cognitive activity; memory*

Prevention and treatment of nervous system disorders developed at the period of prenatal development is one of priority areas in perinatal medicine [1]. Its pharmacological aspect is limited due to insufficiently developed experimental approaches to the search and preclinical study of agents for the development of medicinal products [7].

Previous studies showed that anxiolytic afobazole, 2-[2-(morpholino)-ethylthio]- 5-ethoxy-benzimidazole hydrochloride), exhibits a wide range of cytoprotective effects [9]. Its antioxidant [6], antimutagenic [4,5], antiteratogenic [3], and neuroprotective [2,10] properties were also reported. Pharmacokinetic methods demonstrated afobazole ability to cross the placental barrier and to enter rat mammary glands and rat pup brain [8]. These data substantiate complex evaluation of afobazole as a product for correction of pre- and postnatal development disorders in offspring. Unfavorable effects of active and passive smoking during gestation on physical and mental development the offspring are well known and well documented [11].

The objective of this study was to analyze drug effects on cognitive disorders developed during gestation under the influence of tobacco smoke.

MATERIALS AND METHODS

Experiments were carried out on white mongrel rats weighing 200-250 g (Stolbovaya nursery, Russian Academy of Medical Sciences). Day of spermatozoid detection in vaginal smears was taken as gestation day 1. Pregnant rats were randomly divided into five groups: control (group 1), model (group 2), postnatal treatment group (group 3), two groups of prenatal and

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postnatal treatment (groups 4 and 5 depending on drug dose). Females of groups 2-5 (gestation days 1-20) were daily placed for 15 min into plastic chambers (60×40×30 cm) uniformly filled with tobacco smoke of four filtered cigarettes containing 13 mg tar and 1 mg nicotine. Control group animals were exposed to the same procedures, but without tobacco smoke in the chamber. Rats of groups 4 and 5 received 1 and 10 mg/kg afobazole, respectively, *per os* immediately before exposure to tobacco smoke during the whole gestation period and during the postpartum period (200 mg/kg *per os*) for 20 days. Females of group 3 (postnatal treatment) received afobazole (200 mg/kg *per os*) only during lactation for 20 days. The doses were selected on the basis of previous data [2-6,8]. Animals of groups 1 and 2 received distilled water *per os* from 1 to 20 day of lactation.

Cognitive activity of the offspring was evaluated on the model of foraging behavior under conditions of free choice (T-maze: polyvinyl chloride, 32 cm high walls).

Training lasted for 5 days. The animals made 5 running 5 min each (300 sec). Food deprivation before the experiment lasted 24 h. Cognitive task consisted in goal-directed achievement of food reward under conditions of free choice (training result) placed in a feeder of one maze arms within certain time (speed of goal achievement) with minimal number of mistakes (spontaneous approach to empty feeder). Short-term memory functioning during the test was assessed by memory trace stability and its retrieval during the initial stages of training (1-2 day of training). Engram transfer into the long-term memory was considered to be successful when cognitive task was purposefully reproduced on days 2 to 5. Differences in time of attaining food reward between test days were used to assess the formation of short-term and long-term memories.

Parameters of conditioning were recorded and processed using RealTimer software (procedure timer).

Two approaches were used for the analysis: 1) conditioned reactions of all animals of the group were analyzed; 2) the animals were divided to active and passive animals by their behavioral characteristics (learning capacity, rate of attaining the goal, and character of fear response) and the results were analyzed separately in each subgroup.

The animals were typed using cluster analysis (MacQueen's κ-means). Normalcy of data distribution in samples of the animals was tested using Shapiro–Wilks test. Statistical significance was determined by methods of nonparametric statistics. Multiple comparison approach based on Wilcoxon sign rank test and Mann–Whitney test with corrections for multiple comparisons was used.

RESULTS

The time of attaining food reward for males and females from control group at 1 day of testing was 13.2 and 8.3 sec, respectively. These values significantly decreased during subsequent days, which attests to rapid adaptation and high learning capacity of control animals (Fig. 1, *a*).

In animals antenatally exposed to tobacco smoke (group 2), the time of attaining food reward in most cases was 300 sec (Fig. 1, *a*). They demonstrated passive and stressful reaction to novelty and refused to learn.

In all animals receiving afobazole (groups 3 to 5), increased exploratory activity in males and females, accelerated adaptation under conditions of unknown environment, and accelerated formation of foraging behavior were noted in comparison with group 2 (model). Training dynamics in these groups was virtually the same as in control animals.

Table 1 presents the results characterizing the effects of afobazole on memory trace stability and engram transfer into long-term memory in rats. In control animals, the maximum difference between the time of attaining food reward (characteristic of short-term memory formation) was noted between training days 1 and 2 (10.1 and 5.5 sec for males and females, respectively). Reduction in time to reach food reward and goal-directed reproduction of cognitive task were also noted on days 2 to 5, which indicates memory trace transfer into long-term memory starting from testing day 2.

In group 2 females (model), no differences were revealed in the time of attaining food reward, while in males changes in this parameter were observed only between days 4 and 5, which can be explained by increased food motivation.

In animals receiving afobazole, the formation of short-term and long-term memory was similar to that in control animals.

Thus, in offspring from rats exposed to tobacco smoke (group 2), passive-stressful behavior reactions, reduced exploratory activity, and prolonged latency during training were detected against the background of reduced motor activity. These effects disappeared in animals receiving afobazole.

In addition to intergroup differences, within-group differences in learning capacity, rate of goal achievement, and character of fear reaction were established in rat offspring from each study group (Fig. 1, *b*, *c*). This prompted us to evaluate cognitive ability in active and passive rats separately.

Active control males and females performed short acquaintance with maze environment without demonstration of stress reactions and took food reward from the feeders at the first opportunity (Fig. 2). The time of

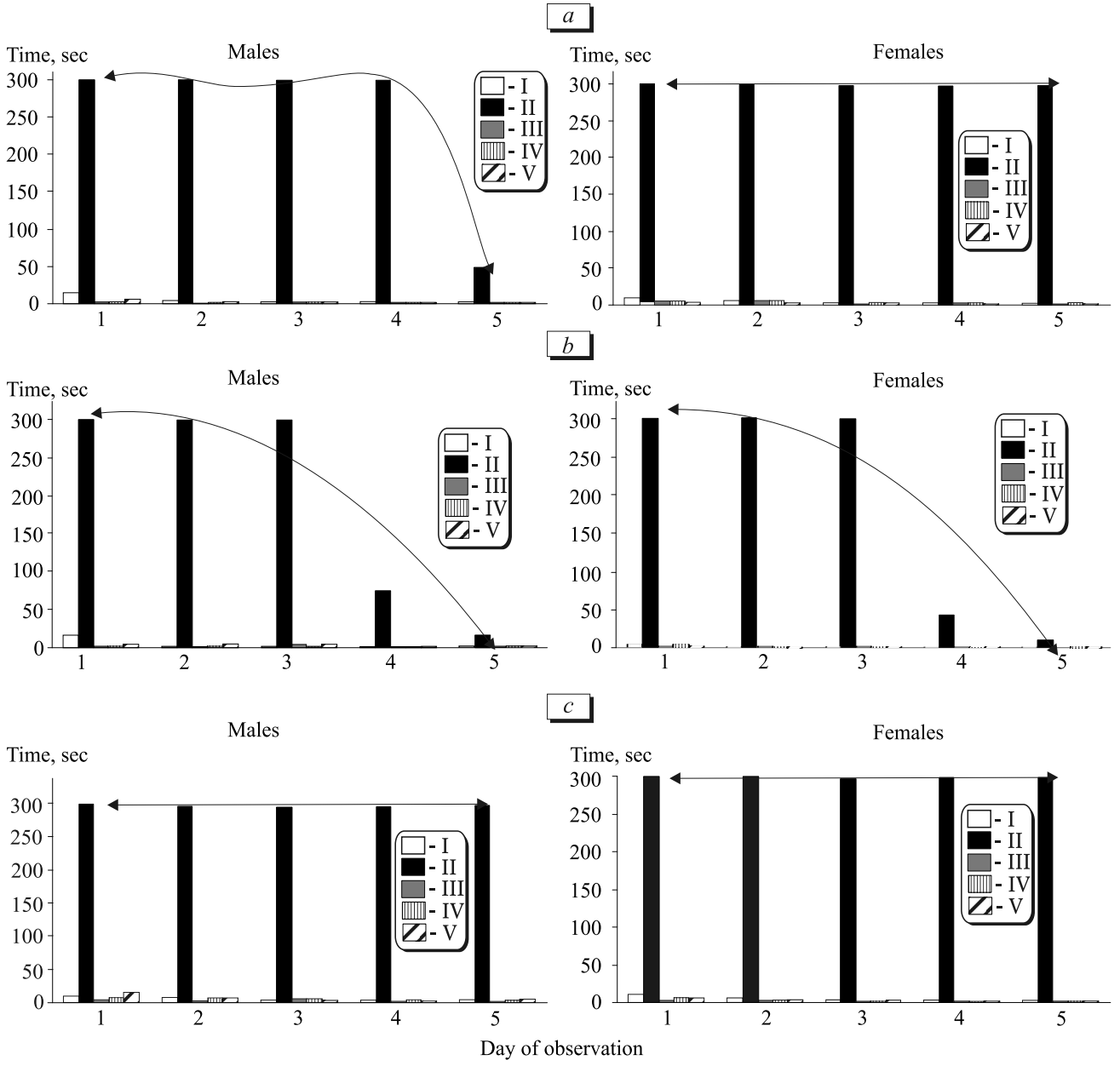

Fig. 1. Dynamics of formation of foraging behavior in rats without typing and after typing according to behavioral characteristics. *a* – without typing; *b* – active animals; *c* – passive animals. Here and in Figs. 2 and 3: I: control, II: model, III: model+postnatal treatment, IV: model+afobazole, 1 mg/kg, V: model+afobazole, 10 mg/kg.

attaining food reward significantly decreased starting from test day 2 in both males and females. By the end of training, the time of getting food reward in males was 1.1-1.5 sec and in females 1.2-2.1 sec. Goal-directed performance of cognitive task was observed from the test day 1. Maximal number of food rewards was noted in males starting from day 1 and in females starting from day 2. Females also demonstrated significantly lower number of mistakes on day 5.

Active males and females of group 2 (model) demonstrated pronounced passive-stressful reactions to novelty resulting in motor inhibition and refusal to learn on days 1-3. Against the background of increasing food motivation, motor retardation was changed by active behavior in terms of random running in the maze. Dynamics of foraging behavior formation in this group differed from that in the control group by the character of reduction of the time to reach food reward. Significant reduction of this parameter in males and females was noted only on days 4 and 5. Minimal time of attaining the food was observed on day 5 (11.7-31.7 sec in males and 8.2-31.8 sec in females). The maximum number of food rewards was observed only on day 5.

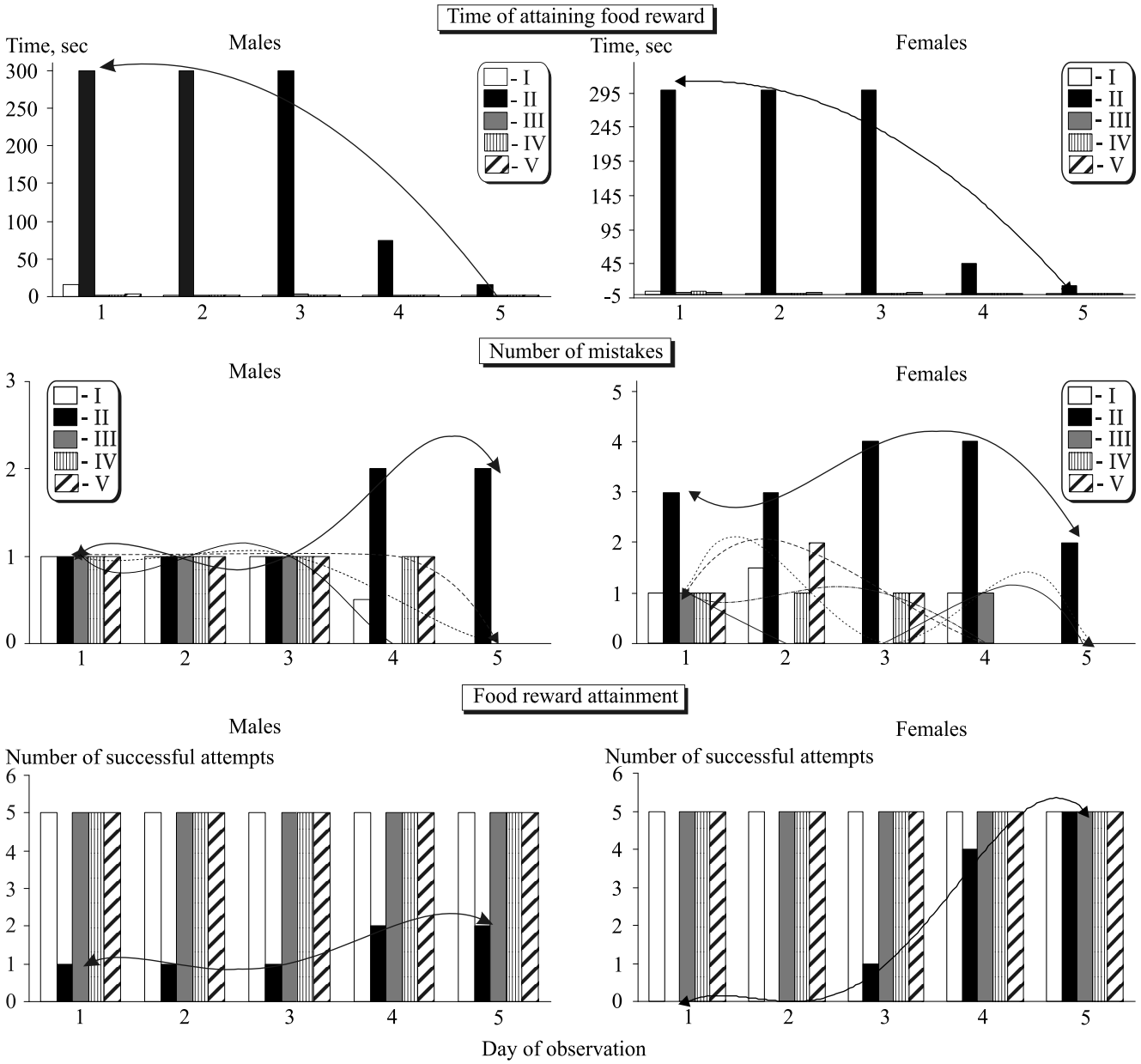

Fig. 2. Effects of afobazole on learning capacity in active animals.

Thus, active animals from model group were characterized by increased anxiety and longer latent period for training.

In active rats of groups 3 to 5 received afobazole, the character of behavioral reactions to unknown environment and formation of cognitive activity were similar to those in control animals. Reduction in time to food reward and significant differences in comparison to corresponding parameter in group 2 were noted on days 1-5 in both males and females. In males of groups 4 and 5 and group 5 females, the number of mistakes significantly decreased compared to that in group 2 (model) starting from day 4. The task was performed with maximal number of food rewards during the entire testing period. Significant increase in the number of food rewards was noted in males and females receiving afobazole in comparison with active rats of group 2.

Analysis of cognitive activity of control passive rats is presented in Fig. 3. At the end of training, the time of attaining food reward was 1.3-4.4 sec in males and 1.5-5.6 sec in females. Both males and females got maximal number of food rewards starting from day 1.

The behavior of passive control animals was characterized by high level of passive stressful reactions throughout the training period. In these animals, fear reduced motor activity and led to refusal of learning.

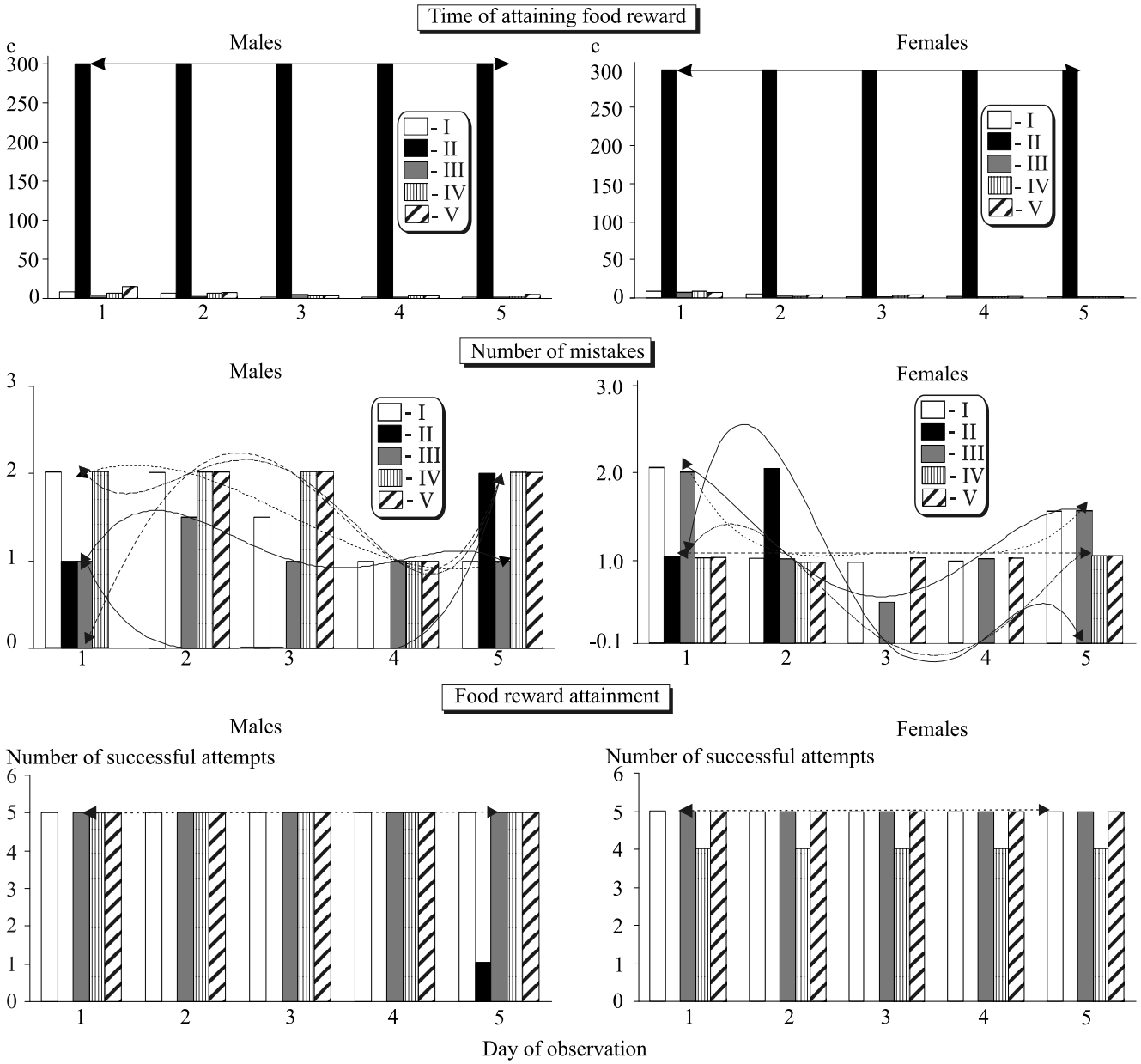

Fig. 3. Effects of afobazole on learning capacity in passive animals.

By day 5 (with increasing food motivation), partial task performance with getting food reward was observed in males.

In passive males of group 3, the time of attaining food reward significantly decreased starting from day 2 and reached 1.2-4.3 sec by the end of the experiment. In females, this parameter significantly decreased on days 3-5 and by the end of training it was 1.2-6.1 sec. Maximal number of food rewards in this group was recorded from day 1 for both males and females.

In passive animals of groups 4 and 5, the time of attaining food reward also decreased. Administration of 1 mg/kg afobazole during the whole period of prenatal development significantly reduced this parameter in males starting from day 4 and in females on days 1-5. Maximal number of food rewards in males was detected starting from day 1. Females got 4 food rewards on day 1. Afobazole in a dose of 10 mg/kg significantly reduced the time of getting food reward from day 2 to day 5 in both males and females. Both male and females of these groups got maximum number of food rewards starting from day 1.

Thus, significant differences were noted in males and females receiving afobazole in comparison with group 2 (model) in the parameters characterizing learning capacity. Significant differences from the control

TABLE 1. Effects of Afobazole on Memory in Rats Prenatally Exposed to Tobacco Smoke (Time, sec)

Group	Males					Females				
	I	II	III	IV	V	I	II	III	IV	V
Control	10.1	1.4	0	0.3	11.8	5.5	1.3	-0.2	0.1	6.7
Tobacco smoke+ physiological solution	0	0	0	251.3	251.3	0	0	0	0	0
Tobacco smoke+ postnatal treatment	0.5	-1.4	1.88	0.22	1.2	1.5	0.4	-0.2	0.5	2.2
Tobacco smoke+ afobazole, 1 mg/kg+ postnatal treatment	0.8	0	0.4	0.3	1.5	3.2	0.3	0.5	0.2	4.2
Tobacco smoke+ afobazole, 10 mg/kg+ postnatal treatment	3.3	0.2	0.8	0.3	4.6	2.1	0	0.8	0.2	3.1

Note. Roman numerals indicate differences in time of attaining food reward at different stages of training: between days 1 and 2 (I), between days 2 and 3 (II), between days 3 and 4 (III), between days 4 and 5 (IV), between days 1 and 5 (V).

group were noted from day 1 to day 3. By the end of the experiment, the rats receiving afobazole during prenatal or postnatal period did not differ from controls by parameters of learning capacity, except passive females of group 4. Fragmentary formation of foraging behavior was observed in group 2 (model) on days 4-5 only in active animals. Passive males and females of this group demonstrating passive defensive behavior during the whole testing period were considered to be unable to learn.

Afobazole equally effectively improved adaptation and learning capacity in active and passive rats. This conclusion agrees with the results obtained in all animals in each group.

Thus, afobazole corrects cognitive disorders in rats exposed to tobacco smoke during prenatal development. It makes further investigations promising in terms of creation of a product for correction of unfavorable effects of active and passive smoking during gestation on the development of the fetus.

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