2. A. I. Arditi and V. A. Kropachev, Inventor's Certificate USSR No. 516702, Otkyrtiya, No. 21 (1976).
3. Yu. D. Ignatov and Yu. N. Vasil'ev, Farmakol. Toksikol., No. 6, 676 (1976).
4. Z. V. Pavlova, Prolonged Peridural Anesthesia in Oncology [in Russian], Moscow (1976), p. 76.
5. U.S. Patent No. 3928562 (1975).
6. K. P. Khomyakov, A. D. Virnik, S. N. Ushakov, et al., Vysokomol. Soedin., 7, No. 6, 1035 (1965).
7. J. Schuster and V. D. Mikashan, Farmakol. Toskikol., No. 5, 535 (1977).
8. J. Schuster and V. D. Mikashan, Khim.-farm. Zh., No. 4, 138 (1978).
9. F. E. D'Amour and D. L. Smith, J. Pharmacol. Exp. Ther., 72, 74 (1941).

SELF-STIMULATION CHARACTERISTICS AND ENDOGENOUS ETHANOL IN RATS OF BOTH SEXES

L. M. Andronova, R. V. Kudryavtsev, M. A. Konstantinopol'skii, and A. V. Stanishevskaya

UDC 616.89-008.441.13-092.9-092 : 616.831.41-008.932.62-074

KEY WORDS: sex, hypothalamic self-stimulation, endogenous ethanol.

Recent investigations have demonstrated the great importance of functional changes in the hypothalamic positive reinforcement system in the pathogenesis of alcoholic intoxication [3, 5, 7, 15]. However, the importance of sex differences in this matter has been neglected. Meanwhile the development of the metabolic concept of the genesis of alcoholism [2, 6] has led to widening of our ideas on the role of endogenous ethanol in the etiology of alcohol dependence in animals of both sexes.

The aim of this investigation was to study sensitivity of the positive reinforcement system in the lateral hypothalamus of rats of both sexes and the effect of prolonged self-stimulation of this system on the endogenous ethanol level in these animals.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats (11 females and 11 males). To obtain the self-stimulation reaction electrodes were implanted in accordance with coordinates from the atlas [11] at the level of the lateral hypothalamus. The animals were anesthetized with hexobarbital (150 mg/kg, intraperitoneally) for the operation. The rats were taught a self-stimulation reaction 5-7 days after the operation in a chamber with a pedal until a stable threshold and a stable frequency of self-stimulation had been reached for a period of 6 min, using currents of different strengths. Pressing the pedal was accompanied by stimulation of the brain with square pulses (Alvar stimulator, duration 0.1 msec, frequency 100 Hz, duration of volley 0.25 sec). The strength of the pulsed current was monitored on the scale of an S1-10 oscilloscope and with a M-195 microammeter. The sessions of self-stimulation lasted 30 min daily. Every week mean values of the threshold current were calculated for animals of each sex. Self-stimulation was studied in six females in two stages of the es-

TABLE 1. Self-Stimulation Parameters in Females Depending on Stage of Estrous Cycle

Stage of cycle	Threshold current strength, μA	Frequency of self-stimulations	
		threshold current	maximal current (350-400 μA)
Diestrus	205 (172—236)	176 (148—204)	406 (377—439)
Estrus	191 (158—224)	277 (254—300)	426 (388—464)

Laboratory of Pharmacology of Narcotics and Laboratory of Psychopharmacology, V. P. Serbskii All-Union Research Institute of General and Forensic Psychiatry, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 6, pp. 688-690, June, 1984. Original article submitted July 12, 1983.

 0007-4888/84/9706-0756$08.50

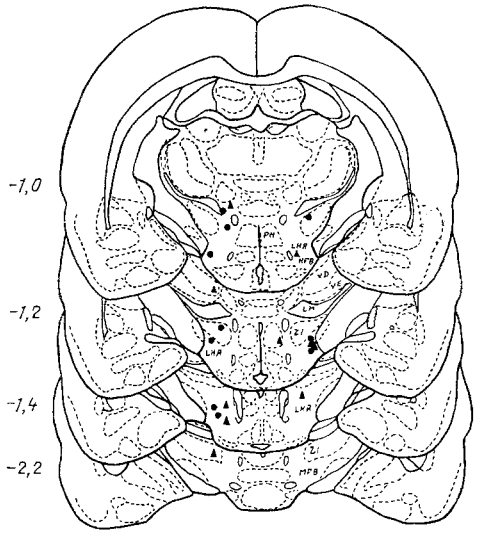

Fig. 1

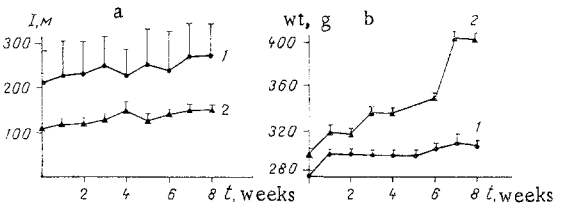

Fig. 2

Fig. 1. Location of tips of stimulating electrodes in rat brain corresponding to coordinates from atlas [11]. On left – coordinates of transverse brain sections in millimeters relative to the bregma. LHA) lateral hypothalamus, PH) posterior hypothalamic nucleus, MFB) medial forebrain bundle, VD-VE) ventrodorsal thalamic nuclei, LM) medial lemniscus, ZI) zona incerta. Circles denote females, triangles – males.

Fig. 2. Dependence of threshold current strength (a) and body weight of rats (b) on sex of animals during prolonged self-stimulation. 1) Females, 2) males. Abscissa, time (in weeks); ordinate: in a) current (in μA), in b) body weight (in g).

trous cyele: estrus and diestrus, diagnosed with the aid of vaginal smears. The mean number of self-stimulations at threshold and maximal current strengths was then calculated. All rats were weighed weekly.

The location of the electrode tips at the end of the experiments was verified histologically. The animals were killed with chloral hydrate and the brain immersed in 10% formalin solution for 10-14 days. Serial brain sections 50 μ thick were cut on the PMS-2 microtome with TOS-II freezing stage. Sections containing areas of injured tissue in the region of the electrode tip were selected. By means of a photographic enlarger these sections were projected on high-contrast photographic paper to obtain photographs. The position of the electrodes in the rat brain was then determined, using coordinates from the stereotaxic atlas [11].

To measure the endogenous ethanol level 0.1 ml of blood was taken with a capillary tube after amputation of the tip of the tail. Samples were taken 5 min after a routine self-stimulation session once or twice a week for 2-14 weeks depending on the time of fixation of the electrodes in the animals' brain. Ethanol in the blood was determined quantitatively by gas chromatography.

The experimental data were subjected to statistical analysis by the Student and Wilcoxon–Mann–Whitney tests.

EXPERIMENTAL RESULTS

Data showing the position of the electrodes in the brain of individual animals are given in Fig. 1. They show that most self-stimulation points were either actually in the lateral hypothalamus (six females and six males) or in adjacent structures (four females and four males). Consequently, there was no appreciable difference in the position of the stimulating electrodes in animals of the two sexes. However the thresholds of hypothalamic self-stimulation depended on the animals' sex. It will be clear from Fig. 2 that during 8 weeks of self-stimulation the magnitude of the threshold current in females was 1.5-2 times higher than in males ($P < 0.05$). It can be tentatively suggested that the higher threshold of self-stimulation in females is due to the fact that the positive reinforcement system of their brain is more resistant to external influences of different kinds. If activation of the positive reinforcement system of the brain by certain substances (ethanol in particular) is regarded as one of the main causes of mental dependence on them [4, 14], it can be postulated that such activation is more difficult to induce in females than in males. The slower formation of preference for ethanol to

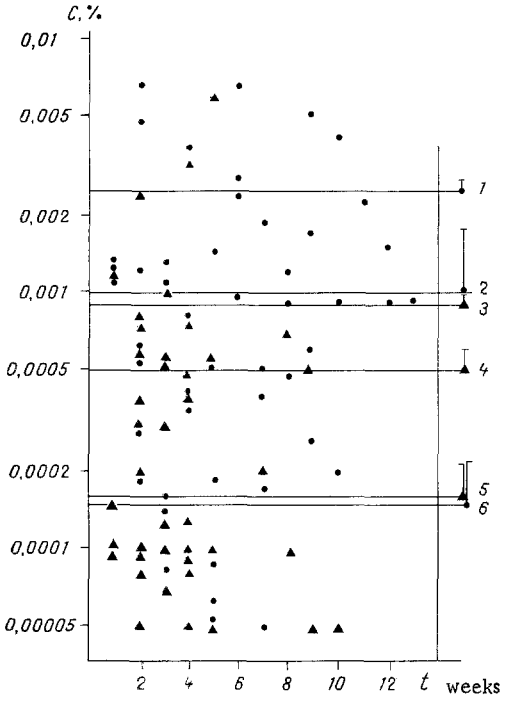

Fig. 3. Endogenous ethanol level in individual rats of both sexes during prolonged self-stimulation. Mean endogenous ethanol levels obtained in previous experiments shown for comparison: 1) intact females; 2) alcoholized females; 3) intact males; 4) alcoholized males; 5 and 6) alcoholized males and females respectively during ethanol withdrawal. Legend: differences in times of experiments for individual animals due to different times of fixation of stimulating electrodes in animals' brain (for females 2-11 weeks, for males 1-5 weeks). Abscissa, time (in weeks); ordinate, blood ethanol concentration (in %, logarithmic scale). Circles indicate females, triangles – males.

water and the associated ability to consume large doses of ethanol [1, 12] are probably due not only to a slower rate of metabolism in females [2], but also to the greater resistance of their CNS to the action of general anesthetics.

Results of the investigation of self-stimulation in females in two stages of the estrous cycle are given below. It will be clear from Table 1 that the intensity of the reaction in females in the estrus stage when currents of threshold strength were used was 57% greater than in the stage of diestrus ($P < 0.05$). The threshold strength of the current and the number of self-stimulations at maximal current strength showed no significant change. This observation suggests increased sensitivity of the positive reinforcement system of the brain in females to the action of general anesthetics in estrus.

The endogenous ethanol level in individual animals (11 females and 11 males) during prolonged self-stimulation is illustrated in Fig. 3. Incidentally the endogenous ethanol level was higher on the whole in females (0.002-0.0005%) than in males (0.0008-0.00005%). These differences were statistically significant in the second (females and males 0.0033 and 0.00005% respectively; $P = 0.01$) and third weeks of self-stimulation (females and males 0.0012 and 0.0004% respectively; $P = 0.05$). Compared with the data on the endogenous ethanol level obtained previously [2], it is clear that during self-stimulation the endogenous ethanol concentration was lower in both females and males than in intact animals (females 0.0025%, males 0.0009%). Moreover, its values moved closer to the endogenous ethanol levels obtained in animals with long-lasting preference for ethanol to water (females 0.001%, males 0.0005%). In a high proportion of males with self-stimulation the endogenous ethanol level reached values characteristic of alcoholized animals in the period of ethanol withdrawal (females 0.00015%), males 0.00016%).

As Fig. 2b shows, during daily self-stimulation for 7 weeks, no increase in body weight was observed in the females whereas the males increased in weight by 33% ($P < 0.05$).

The study of the endogenous ethanol level and body weight of the animals during prolonged self-stimulation provides a parallel with the analogous values obtained in rats of different sexes with long preference for ethanol to water. In both cases lower values were obtained than in intact animals. However, differences in the concentrations of endogenous ethanol depending on sex still remained: They were higher in females than in males. In addition, an increase in body weight was observed only in males. This latter circumstance, just as during chronic alcoholization, suggests that the action of prolonged self-stimulation is more injurious to females. On the basis of the catecholamine hypothesis of brain self-stimulation [8, 9, 13], the argument runs as follows. One possible way of formation of endogenous ethanol in the body is by reduction of acetaldehyde. During activation of the catecholaminergic system and increased catecholamine release, acetaldehyde may react with catecholamines [10]. As a result, probably, the quantity of acetaldehyde usually converted into ethanol is reduced, and this may lead to the observed fall in the endogenous ethanol level.

LITERATURE CITED

1. L. M. Andronova and N. K. Barkov, Farmakol. Toksikol., No. 1, 71 (1979).
2. L. M. Andronova, M. M. Ushakova, R. V. Kudryavtsev, et al., Farmakol. Toksikol., No. 5, 101 (1982).
3. Yu. V. Burov and S. A. Borisenko, Farmakol. Toksikol., No. 3, 291 (1979).
4. É. É. Zvartau, in: Neuropharmacological Aspects of Emotional Stress and Drug Dependence [in Russian], Leningrad (1978), pp. 143-158.
5. É. É. Zvartau, Zh. Vyssh. Nerv. Deyat., No. 4, 651 (1982).
6. Yu. M. Ostrovskii, in: Ethanol and Metabolism [in Russian], Minsk (1983), pp. 6-14.
7. A. V. Stanishevskaya, in: Experimental and Clinical Psychopharmacology [in Russian], Moscow (1980), pp. 102-106.
8. R. J. Barrett and D. K. White, Pharmacol. Biochem. Behav., 13, 555 (1980).
9. P. Cazala, Psychopharmacology, 71, 143 (1980).
10. V. E. Davis and N. Y. Walsh, J. Pharmacol. Exp. Ther., 174, 401 (1970).
11. L. J. Pellegrino, A. S. Pellegrino, and A. J. Cushman, A Stereotaxic Atlas of the Rat Brain, New York (1979).
12. A. Shimon, Psychopharmacology, 57, 97 (1978).
13. A. Wauquier, Acta Neurobiol. Exp., 40, 665 (1980).
14. R. A. Wise and M. A. Bozarth, Prog. Neuro-Psychopharmacol., 5, 467 (1981).
15. P. Witte, L. Dekens, and M. F. Bada, Neurosci. Lett., 19, Suppl. No. 5, 324 (1980).