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ALCOHOL ADDICTION AS THE RESULT OF COGNITIVE ACTIVITY IN ALTERED NATURAL MAGNETIC FIELD

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ABSTRACT

The influence of information load on ethanol and water intake of altered magnetic background was studied in initially ethanol-aversive Wistar rats. A paradigm of free choice between 10% of ethanol solution and water was applied. Daily exposure to weak disturbances of ambient magnetic field (MF, up to 210 μT) caused by various ferromagnetic objects under conditions of free behavior (zero level of information load) did not alter rats' ethanol/water intake over 2 months. Cognitive activity on the background of natural MF (38 μT) caused an increase of ethanol intake in 34.8% of rats, which were mainly poor learners. This activity on the background of altered MF (up to 280 μT) modulated by magnets provoked an increase of stable ethanol addiction in all rats, independent of individual peculiarities and learning success. A possible role of the endogenous opioid system in mediating MF-induced development of alcohol addiction is discussed.

Key Words: Ethanol addiction; Free behavior; Learning; Altered magnetic field; Wistar rats

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INTRODUCTION

In spite of a long history of medicobiological research on neurobiological mechanisms of alcoholism, many questions remain open. It is common knowledge that alcoholism is a complex psychiatric disorder that is defined by certain criteria, including physical dependence, tolerance, and compulsive use of alcohol and its craving. It has long been known that genetic predisposition to alcoholism exists and numerous factors are involved in its development and symptomatic manifestation.[1] Clinical and laboratory studies have ascertained various psychological and neurobiological determinants of this disorder,[2–4] yet the underlying mechanisms are poorly understood.

Nowadays, practically all neurotransmitter systems are shown to be involved in the numerous manifestations of this disorder. Three principal pathways are arguably related to mechanisms of alcoholism: the γ-aminobutyric acid (GABA)/glutamatergic, the serotonergic, and the opioid/dopaminergic systems.[5] The role of GABAergic mechanisms is supported by a number of ethanol drinking, withdrawal, and behavioral studies.[6] It is believed that ethanol might have some mechanisms of action common with GABAergic barbiturates and benzodiazepines.[7,8] The neurochemical basis of alcohol withdrawal has been consistently characterized as an increase in neuronal excitability associated with reduced inhibition via the GABA–benzodiazepine receptor system, changes of voltage-gated Ca^{2+} channels, and increased excitation via excitatory glutamate receptors.[9] It is now well accepted that widespread excitatory *N*-methyl-D-aspartate (NMDA) receptors are cell membrane targets for ethanol that acutely inhibit NMDA receptor function.[10–12] Neuronal hyperexcitability due to the combined effect of increased excitatory and decreased inhibitory neurotransmission is suggested as a common mechanism underlying some anxiety disorders and alcohol withdrawal.[13,14] Serotonergic system down regulation has been suggested as underlying obsessive craving for alcohol and high rates of ethanol consumption, as reduced brain serotonin function has been revealed in alcohol-preferring animals, in human alcoholics, and in humans who were at risk or have exhibited alcohol misuse.[3,4] It is suggested that the effect of selective 5-hydroxytryptamine (5-HT) reuptake inhibitors on alcohol intake is mediated through counteracting dopamine (DA) and/or serotonin deficiencies caused by alcohol intoxication.[15,16] It has also been suggested that serotonergic agents' effect is indirect and mediated by their mood-regulating (antidepressant) properties.[3] The involvement of serotonergic mechanisms in cognitive and learning processes supports a general role for this system in the regulation of motivated responses, rather than a specific role in the modulation of the reinforcing properties of alcohol.

The opioid/dopaminergic system is considered to play the most important role in the development of alcoholism, as a system dealing with reinforcing, stimulating, and enhancing effects of drugs of abuse, including ethanol.[17–19] The ''opioid-deficiency hypothesis'',[20] suggesting that individuals predisposed to alcoholism have a deficiency in the basal level of activity of the endogenous opioid system, has been demonstrated in humans and experimental animals.[21] There are data on the direct effects of ethanol on binding properties of opioid receptors, as well as on its modulation of opioid peptide synthesis and secretion.[22] Opioid antagonists are widely used in the clinic for treatment of various addictive disorders. Furthermore, the mesocorticolimbic opioid/dopaminergic system seems to have an important role in regulating emotional and motivational states.[18,19] The ''tension-reduction'' hypothesis of alcoholism, presuming that alcohol reduces stress, predicts increased ethanol consumption under stressful conditions.[23] A positive correlation has been found between the basal level of anxiety and voluntary alcohol drinking in rats.[24]

Thus, a three-pathway psychobiological model of craving for alcohol[4] was an attempt to relate certain dysregulations in neural circuitries or neurotransmitter systems with a different type of craving. The opioid/dopaminergic system is proposed to play a role in the rewarding effects of alcohol and, possibly, appetite states of craving. The GABAergic/glutamatergic system is likely to be involved in neuronal hyperexcitability underlying physiological and emotional arousal and, possibly, conditioned withdrawal or other anxious states. Finally, the role of the serotonergic system in craving may operate through the regulation of impulse-control mechanisms.

In magnetobiology—a parallel field of research, there is accumulating experimental evidence that the central nervous system (CNS) is extremely sensitive to magnetic factors. It has been shown that various magnetic (MF) and electromagnetic (EMF) fields alter many behavioral and physiological functions, including motor activity, motivation, emotional component, memory consolidation, and learning.[25–28] The possible role of the endogenous opioid system in mediating some MF effects is widely discussed in the literature.[29–33] The question comes up naturally: does the magnetic factor affect opioid reward mechanisms? The lack of data about MF or EMF influence on the development of drug addiction has given impetus to the present study.

Earlier, we reported that weak disturbances of natural MF (up to 300 μT) affected rat learning and habitual performance.[34] It was shown that rats failed to form goal-directed operant behavior by themselves due to MF-induced suppression of exploratory activity. Additional stimulation made learning possible, but the habitual performance was unstable and accompanied by numerous stress reactions. The behavioral model of complex learning used in this study presumes a high level of cognitive activity and psychoemotional stress. According to clinical data, those two factors often play a causal role in the development of alcohol addiction in humans.[2,13–15,24] It was previously found that only 40% of Wistar rats succeeded in problem solving in a maze. The development of ethanol preference did not depend on the results of learning, but was usually observed in rats with unstable psychophysiological status, inclined to displaying active stress and neurotic-like reactions during learning.[35]

MATERIALS AND METHODS

Animals and Housing Conditions

Male Wistar rats (breeding company ''Stolbovaya,'' Moscow area), weighing 250–300 g at the beginning of the experiment, were housed in groups of 10 on a 12-hr light/dark cycle (lights on at 8:00 a.m.). The temperature was controlled between 21°C and 23°C. Standard food and tap water were available *ad libitum.*

Behavioral Situations and Learning Procedure

Animal behavior was studied in two experimental situations: free behavior in groups of 10 in a ''living room'' (variant of open field), and solving of a problem food-getting task individually in a complex maze. The ''living room'' was a part of the laboratory's interior (300×160×120 cm), which consisted of tables, chairs, bookshelves, boxes, sink, etc., located so that rats could move easily. This experimental situation was considered as an analog of a zero level of intellectual activity. The learning procedure was conducted in another room, where an experimental Plexiglas © box (90×70×40cm) was placed on a wooden platform (90×70×40cm). The experimental room was evenly darkened and lacked any visual or sound references. The experimental box and learning procedure have been described earlier in detail.[34] The box consisted of two areas with different information content: a free area, unconnected with food reinforcements, and a maze in which an animal may receive food, given certain rules of behavior. A one-way entry into the maze was located in the center of the free area; two one-way exits from the maze were located symmetrically to the entry. An animal was required to form a cyclic four-link habit by itself without any kind of conditioned stimuli. It had to enter the maze (1st link), and locate and get a piece of food from one or two supported feeders out of four in the maze (2nd and 3rd links). The other two feeders never contained food (false). In order to obtain more food, the animal was required to leave the maze (4th link) and reenter it again (1st link). The latter requirement was of the most cognitive difficulty, since no external stimuli pointed out the necessity to leave the food area (maze) to the area free of food in order to get more food. This experimental situation was considered a high level of information load.

All groups of animals were exposed to the living room daily for 3 hr between 10:00 a.m. and 1:00 p.m. The rats from the learning groups were removed from the living room individually and in random order, transferred to the second room and placed into the maze. Each learning session lasted for 13 min. After a learning session, a rat was placed in a feeding cage for 45 min and then transferred back to the living room. After all rats underwent this procedure, they were placed in the home cage and transferred to the vivarium. There was no food available before the next learning session. The rats from the living room group were not food-deprived during the experiment. The experimenter recording the animal behavior had a permanent location in both experimental situations and did not influence the animals' activities. Rat behavior in the living room was recorded for 15 min.

The following series of experiments were carried out. In Condition 1 ($n = 50$), rats were exposed to the living room (zero level of information load) for 3 hr daily for 2 months. Two other groups of rats were exposed to both the living room and the problem-solving situations for 30 days. In Condition 2 ($n = 50$), MF background in the learning situation was uniform and close to natural MF. In Condition 3 ($n = 50$), MF background in the learning situation was heightened and inhomogeneous. Magnets were removed after 12–15 learning sessions.

MF Background in the Experimental Situations

The MF background in the vivarium varied from 16 to 118 μT due to surrounding ferromagnetic construction members and grid cage covers. The MF background in the living room (Condition 1) was comparable to that in the vivarium, and its magnitude varied from 9 to 210 μT, again due to various ferromagnetic objects, which were present in abundance. The MF background in the maze (Condition 2) was a nearly uniform 38.6 ± 2.4 μT, as the maze was located 1 m distant from any laboratory equipment or other ferromagnetic objects. In Condition 3, three magnets placed 5 cm beneath the maze caused MF perturbation of from 55 to 280 μT. A TM75-41 magnetometer (IZMIRAN) was used for measurements. A more detailed description of the MF parameters in the experimental environment was given in our previous paper.[34]

Ethanol Self-Administration Procedure

The rats were tested for ethanol preference before learning started (test ''Before''), during the task acquisition period (test ''Learn 1,'' 6th–11th sessions), after restoration of the natural MF background in the maze (test ''Learn 2,'' 16th–21st sessions), 2 weeks later (test ''After 1''), and 4 weeks (test ''After 2'') after learning was finished. During the ethanol preference test, animals were housed in individual cages (30×30×30 cm) in the living room. Each test lasted for 5 days; the interval between tests was 7–10 days. Rats were offered a bottle filled with 10% ethanol solution (prepared from absolute ethanol with tap water). Ethanol was presented as a free choice with tap water and was available for 21 hr per day. The position of ethanol and water bottles was alternated. Consumption was measured daily at 10 a.m.

Data Analysis

Clear visible and/or audible reactions were chosen for analysis.[32] Compartments of the experimental environment and animal behaviors were assigned their own symbols, which allowed each behavioral session to be recorded as a text. The animal trajectory, number of different reactions, and contacts between animals were recorded. Rat behavior during learning was classified as unconditioned and conditioned. Unconditioned behaviors were not related directly to the task performance. They included various inborn reactions, like grooming, rearing, tics, jumps, manges, freezing, etc.

The following conditioned behavioral parameters were used: 1) number of trials (N_T); 2) rate of unconditioned reactions in all actions per sessions (P_{UC}); 3) level of locomotor activity—number of maze compartments traveled per sessions (LA); 4) number of pieces of food taken (N_F) per session; and 5) probability of mistake—number rechecking of empty feeders after food taking per trial ($P_M = N_M/N_T$).

According to results of the drinking test, rats were classified as *ethanol aversive* (W_{AV})—ratio of consumed 10% ethanol solution was less then 0.1 in a daily total liquid volume; *ethanol preferring* (W_{PR})—ratio of consumed 10% ethanol solution was more then 0.5 of total fluid intake; and *ethanol consuming but non-preferring* (W_{NPR})—ratio of consumed 10% ethanol solution was in between 0.1 and 0.5 in daily total liquid volume.

The authors' computer program ''Labyrinth'' (K. Nikolskaya and A. Osipov) was used for real time recording of the behavioral data and its primary analysis. Liquid intake data and behavioral parameters were statistically analyzed using Kolmogorov–Smirnov and Student's tests, Wilcoxon's matched-pairs test, and analysis of variance (ANOVA).

RESULTS

Learning on the Natural MF Background (Condition 2)

Forty percent of rats were able to solve the food-getting problem and to form a four-link cyclic habit within 9.3 ± 2.7 sessions. The habitual performance in these rats was characterized by 28.6 ± 8.4 cycles per session, a high speed of locomotion ($V_{LA} = 0.76 \pm 0.03$ zones/sec), and a minimal rate of unconditioned reactions ($P_{UC} = .24 \pm .05$). Detailed analysis of conditioned parameters of rat behavior and unconditioned reactions accompanying learning has revealed that the speed of learning correlates well with the level of fear and with anxiety and the level of locomotor activity (Tables 1 and 2). According to the above-noted behavioral parameters, rats were subdivided into two groups (learning Types I and II). The Type I rats displayed a low level of fear and anxiety and a high locomotor activity in the first session. They learned quickly the location of feeding points (P_{Rf}, $P_{En-F1-F2}$; Table 1), but extinction of mistakes was very slow (V_M; Table 1). The much slower speed of learning in Type II rats was due to a high level of fear and anxiety and low locomotor activity in a novel environment. The quick inhibition of mistakes (V_M; Table 1), high efficiency of learning, and high stability of habit were specific features of learning Type II. Thus, the quicker the learning, the less efficient it was, and the lower the level of habit organization (Table 1).

Sixty percent of rats (learning Type III) failed to solve the problem task and did not form the four-link food-getting cyclic habit within 28–30 sessions. Basically, these rats formed two- or three-link habit (entering the maze and getting food from one or two feeders) and performed 2.7±1.3 trials per session at most. These rats displayed the highest level of fear and anxiety and the lowest level of locomotor activity in the first learning sessions (Tables 1 and 2).

Learning on the Altered MF Background (Condition 3)

All rats tested failed problem solving and were not able to form the food-getting habit by themselves. The failure of learning was due to a deep and prolonged locomotor depression, which developed very quickly after well-expressed orienting to novelty, and lasted up to the end of the learning session. The same behavioral scenario was observed in the following four sessions. Orienting activity was not spontaneously followed by exploratory behavior. Learning was possible only after additional external stimulation (pushing an animal into the maze by hand at the beginning of the 6th session). This procedure resulted in the elimination of locomotor depression, and 50% rats formed the four-link food-getting cyclic habit within 5.8 ± 2.2 sessions. There were no clearly detectable learning types (I or II). This group of rats displayed a nonstandard combination of low level of fear and anxiety and low locomotor activity, but the speed and efficiency of learning were higher than in Condition 2 (Tables 1 and 2). Habit was unstable in all rats after learning was finished. The remaining 50% of rats resembled learning Type III, as they formed two- or three-link habits only (entering the maze and getting food from one or two feeders), and performed 3.4 ± 0.8 cycles per session. Despite displaying lower levels of fear and anxiety, and higher locomotor activity in comparison to learning Type III rats in Condition 2 (Tables 1 and 2), it was not sufficient to complete the learning task successfully.

Rat Behavior in the Living Room (Condition 1)

The observation of rat behavior in groups of 10 showed that all animals were completely habituated to the condition of the living room after 2 weeks of daily 3-hr

Table 1. Indices of Learning in Different Groups of Wistar Rats

		L_F	P_{RF}	$P_{En-F1-F2}$	V_M	V_L	E_L	O_H
Condition 2: Natural MF	Type I	0.19 ± 0.07	.44 ± .10	.34 ± .04	0.38 ± 0.06	0.16 ± 0.05	0.89 ± 0.21	0.56 ± 0.08
	Type II	0.38 ± 0.09	.34 ± .07	.08 ± .06	1.35 ± 0.09	0.10 ± 0.06	2.21 ± 0.13	0.79 ± 0.06
	Type III	0.67 ± 0.08	.08 ± .05	.001 ± .01	–	–	–	–
Condition 3: Magnets	Types I–II	0.22 ± 0.06	.58 ± .08[b]	.24 ± .08[b]	1.54 ± 0.12[a]	0.28 ± 0.06[a,b]	5.95 ± 0.45[a,b]	0.43 ± 0.12[b]
	Type III	0.34 ± 0.07[c]	.27 ± .12[c]	.06 ± .02[c]	–	–	–	–

L_F = level of fear as ratio of avoidance and freezing to all unconditioned responses; P_{RF} = probability of reinforcement in session; $P_{En-F1-F2}$ = performance probability of sequence: entrance–feeder 1–feeder 2 in session; V_M = speed of mistakes' inhibition to the criteria 10% of Nt as $\Delta_{mistakes}/\Delta_{sessions}$; V_L = speed of learning as $13/\sum$sessions; E_L = efficiency of learning as $100/\sum$trials; O_H = habit organization as ratio of minimized task solutions in session; [a,b,c] $p < .05$, Condition 3 vs. Condition 2 (*t*-test);[a] vs. type I,[b] vs. type II, and [c] vs. type III.

Table 2. Behavioral Parameters of Rats in Different Experimental Situations

			Condition 1			Condition 2	
			Type I	Type II	Type III	Types I–II	Type III
LA	1st–2nd sessions	Living room	248.3 ± 56.6	94.4 ± 22.1	33.7 ± 26.8	85.4 ± 31.8	28.2 ± 18.4
		Maze	382.4 ± 43.7	215.5 ± 36.3	136.7 ± 42.3	43.7 ± 16.4^{a,b}	33.8 ± 18.7^{c}
	15th–18th sessions	Living room	199.3 ± 44.7	42.6 ± 18.4	55.3 ± 19.5	188.2 ± 53.2^{b}	146.8 ± 31.6^{c}
		Maze	587.5 ± 63.1	674.8 ± 72.2	175.3 ± 66.8	538.4 ± 56.2	152.6 ± 77.3
Accustomed area, ratio	1st–2nd sessions	Living room	0.18 ± 0.06	0.11 ± 0.08	0.03 ± 0.02	0.13 ± 0.05	0.02 ± 0.01
		Maze	0.72 ± 0.09	0.58 ± 0.04	0.24 ± 0.06	0.31 ± 0.04^{a,b}	0.24 ± 0.05
	15th–18th sessions	Living room	0.33 ± 0.14	0.28 ± 0.02	0.11 ± 0.05	0.68 ± 0.08^{a,b}	0.56 ± 0.12^{c}
		Maze	0.99 ± 0.01	0.99 ± 0.01	0.68 ± 0.12	0.99 ± 0.01	0.58 ± 0.08
Duration of motor inhibition, sec	1st–2nd sessions	Living room	48.1 ± 18.6	83.7 ± 35.6	452.2 ± 120.3	95.8 ± 24.6^{a}	403.4 ± 90.7
		Maze	32.3 ± 16.5	128.3 ± 24.6	366.9 ± 184.2	422.5 ± 80.6^{a,b}	425.8 ± 100.5
	15th–18th sessions	Living room	33.8 ± 21.3	355.0 ± 43.1	202.5 ± 36.3	64.6 ± 22.5^{b}	86.2 ± 30.5^{c}
		Maze	28.6 ± 18.4	34.2 ± 14.6	506.4 ± 150.6	32.8 ± 24.6	306.9 ± 115.5

LA, see MATERIALS AND METHODS; total space of the Living room is 15 m^2; total number of maze zones is 32; ^{a,b,c} $p < .05$, Condition 3 vs. Condition 2 (*t*-test); ^{a} vs. Type I, ^{b} vs. Type II, and ^{c} vs. Type III.

exposure. The percent of the territory explored and preferable location differed in each individual rat and correlated with learning type. Thirty percent of rats (learning Type II) remained distant from the others, and occupied ‘‘privileged’’ places in the environment, from which they could follow the behavior of other rats. Most of the time they spent lying in a preferred location, and had extremely low locomotor activity (Table 2). Other rats displayed typical poses of subordination when colliding with them. Furthermore, these rats maintained the territorial integrity of the group, physically restraining other rats from going out of the controlled area. According to the zoo-social criteria,[36] we ranked these animals as dominant. Ten percent of rats (subdominants; learning Type I) showed the highest locomotor and exploratory activity, which was maintained continuously during 3 hr of observation period (Table 2). The remaining 60% of rats were defined as subordinates, as they expressed the highest level of fear and anxiety (Table 2), and were the least active; they preferably stayed within group in the home cage or inside the hole-like boxes on the lowest level of the Condition 1 area. They always displayed typical poses of subordination under collision with other rats of the experimental group (learning Type III).

Comparative analysis of rat behavior in two experimental situations revealed interesting relationships between rat behavior within the groups of 10 in Condition 1 and individual behavioral in the maze (Condition 2). A positive correlation of behavioral phenotype in the Living room and in the maze was observed in subdominants and subordinates. The character of behavior did not depend on the level of environmental complexity or duration of experiment (Table 2). More complicated relationships were observed in the dominants. Relatively high locomotor activity during the orientation period in the Living room combined with extremely low activity in the maze in the first learning sessions. In the later stages of the experiment, the correlation transformed to the opposite. Locomotor activity was significantly decreased in Condition 1 due to habituation, while it was dramatically increased in the maze after task learning was completed, and it was maintained at a high level in the stage of habit (Table 2).

Rat behavior in the Living room under the heightened MF background (Condition 3) differed significantly from that in Conditions 1 and 2, both in the Living room and in the maze. The specific features of rat behavior were the following: 1) the dominant–subordinate hierarchy could not be detected as all rats behaved independently from any other; 2) the territorial integrity of the group was not maintained within the Living room borders; after 10–12 sessions, most of rats were acclimated to the whole experimental space, and were continuously moving around like subdominants in Conditions 1 and 2. The accustomed space for some individuals expanded down to the laboratory’s floor; 3) rats did not display any signs of fear, readily exploring all kinds of novelty introduced into the Living room and easily interacted with the experimenter. The latter was not typical for well-handled rats in Conditions 1 and 2. Those rats remain distant from the experimenter for the whole observation period. It is important to note that an active form of behavior in the Living room was observed both before the learning session in the maze as well as immediately afterward and the rat’s activity did not depend on the level of food deprivation (Table 2).

The behavioral correlations in the two experimental situations were different: the increased locomotor and exploratory activity and low level of fear and anxiety in the Living room (simple environment) contrasted with the deep and prolonged locomotor depression in the maze (complex environment).

Table 3. Statistic Parameters of Liquid Intake in the Condition 1 (Living Room)

	Ethanol (mL/day)				Water (mL/day)			
	Test 1	Test 2	Test 3	Test 4	Test 1	Test 2	Test 3	Test 4
Mean	1.93	3.54	3.88	4.37	31.75	29.67	38.25	40.14
Standard error	0.14	0.26	0.27	0.27	1.11	1.04	1.30	1.33
Mode	0.67	0.72	0.73	0.58	26.60	26.30	27.50	25.00
Range	21	24	22	23	145	100	150	102
Number of events	298	304	288	314	298	304	288	314

Ethanol and Water Intake

After a 2-week acclimation period, all rats were tested for ethanol preference. Earlier, it was shown that the population of Wistar rats ($N = 170$) was inhomogeneous for ethanol consumption criteria.[35] Fifty-one percent of rats could be defined as ethanol aversive ($P_E < .1$) as the average volume of 10% ethanol solution consumed was 1.85 ± 0.11 mL/day or 0.6 ± 0.03 g/kg/day (mean ± SE). Twenty-four percent of

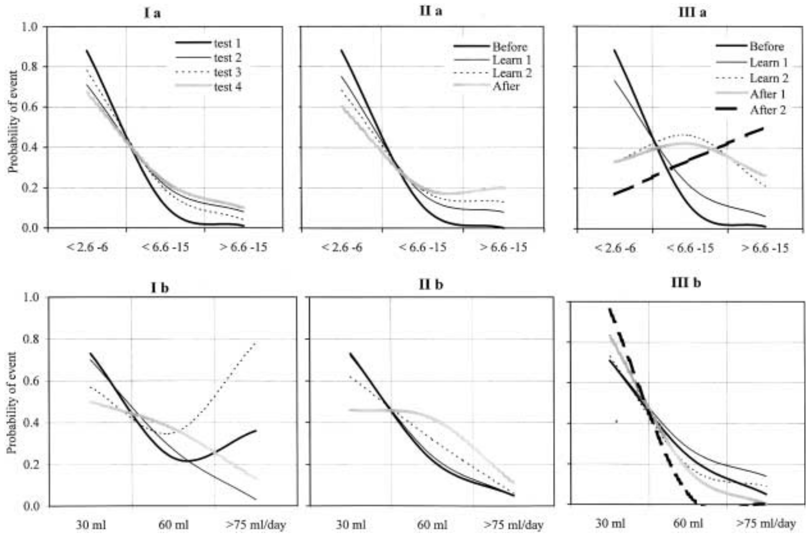

Figure 1. Ten percent (v/v) ethanol and water intake distribution in two-bottle, free-choice paradigm. (**I**) Free behavior of rats in the Living room (Condition 1) as an analog of a zero level of intellectual activity on the background of weak magnetic disturbance induced by different ferromagnetic objects, which were present in abundance (up to 210 μT). (**II**) Learning of rats in a maze on the background natural MF (Condition 2). (**III**) Learning on the altered natural MF modulated by three loudspeaker magnets placed 5 cm beneath the maze (Condtion 3). (**a**) Distribution of ethanol intake is indicated in g/kg/day (first number) and mL/day (second number); (**b**) distribution of water intake.

rats consumed 7.8±0.18 mL/day (4.1 ± 0.3 g/kg/day), and according to given criteria were defined as consuming but non-preferring ($0.1 < P_E < .5$). The final 25% of rats clearly displayed ethanol preference, as the average volume of 10% ethanol solution consumed was 17.6 ± 0.53 mL/day or 7.9 ± 0.53 g/kg/day ($P_E > .5$). Only the ethanol-aversive rats were taken for the experiment, and were randomly divided into three experimental groups.

Ethanol and Water Intake in the Experimental Conditions

Ninety percent of rats in Condition 1 consumed 0.67 ± 0.14 (mode ± SE) mL/day of 10% ethanol solution and 26.6 ± 1.1 mL/day of water (Table 3). The higher volumes of ethanol intake (more than 15 mL/day) were observed in some individuals with extremely low probability $P = .06$ (Figure 1-Ia). It caused the slightly higher group's mean values of ethanol intake (Figure 2a). The analysis of water intake after Test 1 revealed three areas with heightened density of the values: low (10–30 mL/day), average (30–60 mL/day), and high (more than 60mL/day) volumes of water intake with probabilities of .73, .33, and .03, respectively (Figures 1-Ib and 2b). The frequency of cases of high ethanol consumption grew gradually during two months of daily exposure to the living room, although it did not exceed $P < .23$. The cases of extremely high ethanol intake (up to 18–25 mL/day), which were episodically observed in some individuals, caused the shift of the group's mean to larger values in Test 2 (Table 3; Figure 2a). It should be emphasized that water intake was increasing in parallel with ethanol, but the shift to larger values was observed later in Test 3 (Figure 2a). At the same time, the modal values in the repeated tests (the most typical values for the group) did not change significantly for either ethanol or water intake by comparison with Test 1 (Figure 2).

Despite the discovered changes in liquid intake, 82.6% of rats in the Condition 1 remained ethanol aversive after Test 4, as the increase in ethanol consumption was in parallel with the increase of water intake (Figure 3a).

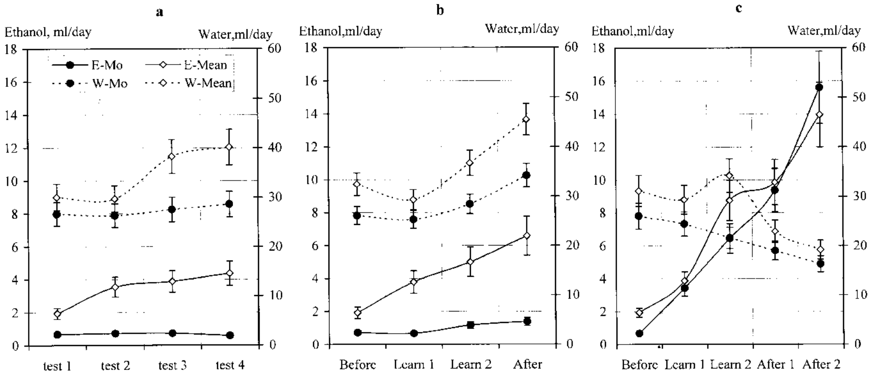

Figure 2. The mean (M) and mode (M_o) values of ethanol (E) and water (W) consumption in Wistar rats in different experimental situations. (**a**) Condition 1, (**b**) Condition 2, (**c**) Condition 3. Before, Learn 1, Learn 2, After 1 and After 2, see text. Data are presented as means and modes ± SE.

The character of liquid intake in Condition 2 (learning on the natural MF) was similar to that observed in Condition 1 (Table 4; Figures 1-II and 2b). The main difference consisted of continuous increase of mean values for both ethanol and water intake in repeated tests. Analysis of modal values showed that most of the rats did not change their ethanol intake, although the learning situation correlated with a significant increase in water intake. As is shown in Figure 1-IIb, the probability of average-drinking events (30–60 mL/day) doubled from $P = .2$ in test ''Before'' to $P = .43$ in test ''After.'' Consequently, the distribution of water consumption changed (Figure 1-IIb). According to ethanol preference criteria, 34.8% of initially ethanol-aversive rats transformed into ethanol-consuming animals, 4.8% of which became ethanol preferring (Figure 3b).

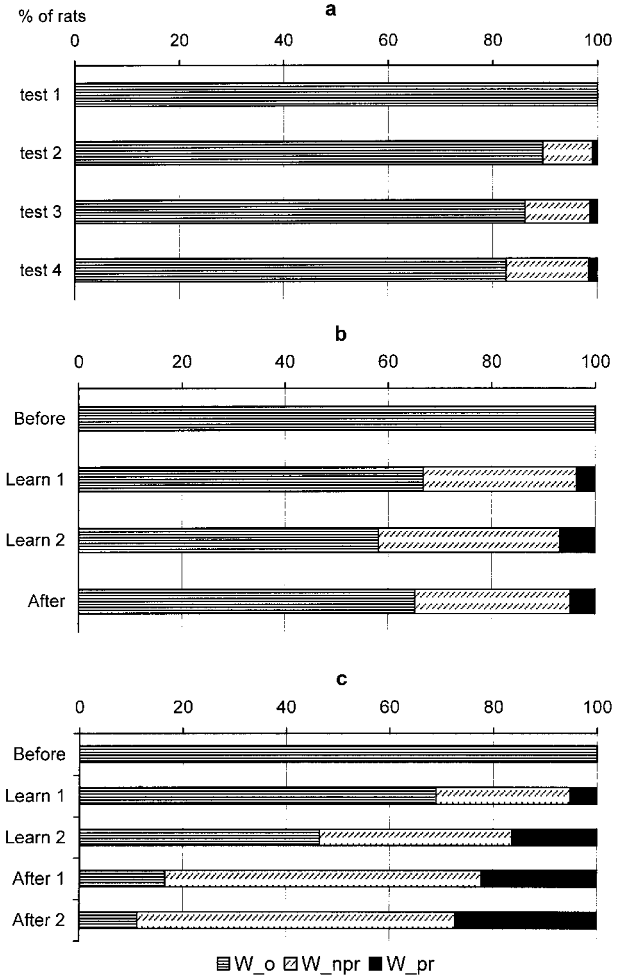

Figure 3. Ratio of ethanol-aversive (horizontal line), consuming but non-preferring (oblique stroke), and ethanol-preferring (black bar) rats in different experimental conditions. (**a**, **b**, **c**) Designations as in Figure 2; for the rest, see text.

Table 4. Statistical Parameters of Liquid Intake in Condition 2 (Natural MF)*

	Ethanol (mL/day)				Water (mL/day)			
	Before	Learn 1	Learn 2	After	Before	Learn 1	Learn 2	After
Mean	1.9	3.8	5.0	6.6	31.75	29.31	34.53	43.00
Standard error	0.1	0.3	0.4	0.4	1.03	0.98	1.12	1.09
Mode	0.72	0.67	1.16	0.86	26.60	25.30	27.20	34.2–66.1
Range	21	18	27	33	145	100	150	150
Number of events	321	302	296	300	321	302	296	300

*Before = before learning; Learn 1 = 1st–13th sessions; Learn 2 = 14th–25th sessions; After = intake testing in 15 days after finishing of learning.

The most dramatic change in the dynamic of liquid intake was observed in Condition 3 (learning on altered natural MF). The character of both ethanol and water intake differed from Conditions 1 and 2 (Figures 1-IIIa,b and 2c). Interestingly, the mean and modal values did not differ much, which pointed out the similarity of changes in all animals of this group. The opposite-directional dynamic of liquid intake was observed in MF-exposed rats during learning: continuous increase of ethanol intake and decrease of water intake. A shift to increased ethanol intake was observed after only six learning sessions: 25.8% of rats became ethanol consuming and 5.2% ethanol preferring (Figure 3c; test ''Learn 1''). More significant changes were observed after the magnets were removed in the 12th session, and the natural MF background in the maze was restored (Figure 3c; test ''Learn 2''). It is important to note that these tendencies in liquid intake remained the same even after exposure to intellectual activity was finished (Figure 2c). The analysis of distribution of rats by water consumption criteria revealed that all rats took a lower volume of water (Figure 1-IIIb). The average daily volume of water intake did not exceed 13.9 mL by the end of the experiment (Table 5). According to

Table 5. Statistic Parameters of Liquid Intake in Condition 3 (Magnets)*

	Ethanol (mL/day)					Water (mL/day)				
	Before	Learn 1	Learn 2	After 1	After 2	Before	Learn 1	Learn 2	After 1	After 2
Mean	1.93	3.87	8.75	9.44	13.30	31.75	29.35	35.25	24.49	20.04
Standard error	0.17	0.28	0.45	0.42	0.46	1.48	1.22	2.03	0.96	0.64
Mode	0.67	3.42	6.44	9.38	18.60	26.04	24.42	22.46	20.70	17.90
Range	22	23	32	27	27	145	115	125	84	58
Number of events	265	276	261	257	253	265	276	261	257	253

*Before = before learning; Learn 1= learning on the Magnets (1st–13th sessions); Learn 2 = learning without Magnets (14th–25th sessions); After = intake testing in 30 days after finishing of learning.

ethanol preference criteria, only 11.2% of rats remained ethanol aversive, although their absolute ethanol intake increased from 0.5 mL/day in test ‘‘Before’’ to 3.9 mL/day in test ‘‘After 2.’’ Four weeks after learning was finished, 88.8% of initially ethanol-aversive rats were classified as ethanol-consuming animals, and 27.3% of those rats became ethanol preferring.

DISCUSSION

The study revealed a rather interesting and unexpected phenomenon: learning, or in other words, intellectual activity, on a background of weak disturbances of the natural MF was accompanied by the development of ethanol addiction. We would like to focus on some aspects of this phenomenon. First, the development of ethanol addiction did not depend on psychophysiological peculiarities of some animals: all *initially ethanol-aversive* rats increased ethanol intake; individual differences were reflected in the level of addiction. In rats that increased ethanol intake but remained ethanol aversive by our classification ($P_E < .1$), the process of development of alcohol addiction was very slow. As a rule, those animals were low-level liquid drinkers (less then 15 mL/day); the ethanol intake increased 5.5–7.8 times during learning, despite the fact that ethanol as a part of the daily volume of liquid increased little from test to test, for example, 0.02–0.05–0.07–0.1–0.12. In average-drinking rats, the process was much faster, as the ratio of ethanol intake could be increased up to 15–18 times over the same period.

Naturally, the question comes up—what factor caused the development of such a strong ethanol addiction: intellectual activity, altered MF background, or the combined effect of two factors. It was mentioned earlier that 39% of Wistar rats increased their ethanol intake while learning was on the background of natural MF.[35] Interestingly, poor-learners, which were characterized by increased anxiety and unbalanced excitatory/inhibitory neurosis processes, usually reacted to information load by ethanol intake. The cases of decreased or unchanged ethanol intake were also observed to be independent of the success of learning.[35]

Furthermore, comparative analysis of water intake in two conditions of learning showed that the increase in consumption of 10% ethanol solution on the background of natural MF (Condition 2) usually correlated well with increase in water intake. As a result, the variation in the rat population of both ethanol and water intake parameters became larger. In contrast, in the case of MF disturbances (Condition 3), increase of ethanol intake was associated with a significant decrease of water intake. It resulted in narrowing of the initial distribution of ethanol and water intake, and the population as a whole became more homogeneous (Figure 2).

These facts suggest that acquired ethanol addiction while learning on an altered MF background was not provoked by the cognitive factor only. Weak MF daily exposure alone (zero information load, Condition 1) did not affect ethanol intake, although the magnitude and character of MF heterogeneity were comparable to Condition 3. Thus, all effects observed in Condition 3 were apparently caused by the combined influence of two factors: intellectual activity and MF disturbances.

It is important to emphasize that despite the weakness of the MF oscillations within the envelope of geomagnetic deviations, MF-induced effects were rather large. On the one hand, the MF factor provoked a profound suppression of exploratory activity or ''initiative,'' which caused the rats' failure to learn spontaneously. The depression could be easily eliminated by some external stimulation (light, sound, or movement forced by an experimenter). On the other hand, after additional activation, learning became possible and was extremely fast. Thus, taking into account the facts mentioned above, we conclude that cognitive activity is very sensitive to the MF factor, and cognitive functioning is sensitive to some MF parameters.

The psychostimulant-like effect of MF was similar to the effects of a pharmacological psychostimulant—opilong, an analogue of dermorphine.[37] Both influences resulted in a decreased level of fear and anxiety in a novel environment, increased sensory sensitivity (including MF parameters) and facilitation of associative processing.[32,34,37] In our opinion, these results could serve as experimental evidence of a hypothesis for the involvement of the endogenous opioid system in mediation of MF effects. It was shown earlier that MF effects could be blocked by pretreatment with opiate antagonists.[28,33,38] In a more detailed study, it was found that both mu- and delta-opiate receptors in the brain are involved in MF-induced decreases in cholinergic activity in the rat frontal cortex and hippocampus.[33] It also was found that a specific, pulsed-MF-induced analgesic effect in the land snail was mediated in part by delta- and, to a lesser extent, mu-opioid receptors, while the selective kappa-opioid receptor antagonist did not affect this analgesia.[38]

According to our data, psychostimulant-like effects of MF were accompanied by some negative manifestations, indicating that MF-exposed animals had trouble in realization of cognitive activity. Among those were unstable habit, a high level of stress reactions, and an hypercoagulation state of the fibrinolysis system at the latter stages of learning.[39] All of the data obtained allow us to suggest that the MF factor provoked an imbalance of brain functioning, consisting of a state where CNS substrate activity (speed of biochemical and physiological processes) did not correspond to potential cognitive capabilities (speed of the information processing). This imbalance can be argued as a possible cause of the development of ethanol addiction. The endogenous opioid system, being the most probable candidate for mediating MF effects on living organism, could play an important role in the alcohol phenomenon of our study.

There is considerable evidence that the endogenous opioid system plays a key role in alcohol addiction.[17,18,23] The mu- or delta-antagonists have been reported to reduce alcohol drinking in animals[40,41] and in humans.[20,42] The ''incentive-sensitization theory''[43] proposes that the dependence-producing properties of opioids, psychostimulants, and alcohol share an ability to enhance dopamine (DA) neurotransmission in reward pathways. It is believed that maintenance of a basal release state of DA depends on a balance between the stimulatory mu- and delta-opioid systems and the inhibitory kappa-opioid system.[44] Interestingly, it was demonstrated that the dopaminergic system was responsive to EMF in rats.[45] Undoubtedly, the hypothetical mechanisms of MF-induced development of alcohol dependence discussed in this paper need direct experimental proof.

As concluding remarks, we would like to point out that magnetobiology, in spite of being a relatively new field, has already accumulated a great number of experimental results about many different kinds of MF influences on living systems, from molecular interaction and cell metabolism to physiological functions and behavior. Interactions between MF and organisms are becoming more and more frequent nowadays because of various types of chronic exposure to man-made electromagnetic fields at places of work, increasing use of communication systems, various new medical procedures, and electrical devices at home. However, the question of how well a living system can adapt to an altered MF background in the modern human habitat still remains open, and obviously, needs further research.

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