ACTA BIOL. SZEGED. 38. pp. 57–66 (1992)

BEHAVIOUR OF AN ALCOHOL-PREFERRING STRAIN OF WISTAR RATS

M. WILHELM[1]*, H. SCHULZ[1] and R. GÁBRIEL[2]

[1]*Department of Comparative Physiology and* [2]*Zoology, Attila József University, Szeged, Hungary.*

* *Present address: Department of Pathophysiology, Albert Szentgyörgyi University School of Medicine, H–6722 Szeged, Semmelweis u. 1. Hungary.*

(Received: December 10, 1991)

Abstract

The 16th generation of alcohol preferring (P) and non-preferring (NP) rats was used for behavioural experiments and synaptological studies. In the open-field test, the male P animals showed more inner ambulations, groomings and wall rearing with shorter latencies and a lower defecation rate with longer latency. The P females did more ambulations and their motility was higher in the inner part of the open field. They showed more wall rearing and grooming than the NP females with shorter latencies. The defecation rate in the P group was lower, although its latency was longer on the first day. In the time-to-emerge test the latency of emergence of the P males was longer than that of the NPs, but not that of the P females. In the plus-maze test the latency of leaving the centre was short for all the P animals and the P males more often entered into the open arm. Both the female and male P animals spent less time there than the NPs. The defecation rate of the P rats was higher. The EM studies revealed a significant decrease in the number of synapses on pyramidal cell apical dendrites in layer 4 of the cerebral cortex and this parameter also differed – although not significantly – in the molecular layer of the hippocampus in the P rats, while an increase in the synapse density was seen in the molecular layer of the cerebellar cortex in the P strain. The results showed that the behavioural pattern of the P animals is not fear-motivated but rather non-adaptive in the stress situations. Since the parameteres observed in this study can be correlated with the behavioural elements of the human alcoholics, this animal model seems to be useful in studies of alcoholism.

Key words: rats, alcohol preference, open-field test, time to emerge test, plus maze test, synapse counting.

Introduction

Several laboratories have described the effects of acute and chronic ethanol administration both in animal experiments and in clinical investigations (e. g. MILLER, 1986; WARTBURG, 1979; WOOD et al., 1987). Most of these investigations are concerned with the ethanol metabolism in the liver (e. g. ROVINSKY et al., 1987) and dysfunction of the central nervous system(CNS) (e. g. CHAMACHO–NASI and TREISTMANN, 1986; MILLER, 1986; SIGGINS et al., 1986; WOOD and SCHROEDER, 1988). The other line of the experimental research has tried to elucidate the long term events, that might affect the structural organization (BAUER–MAFFETT and ALTMAN, 1977; BERACOCHEA et al., 1987; HOFF, 1988),

ongoing physiological processes in the CNS (GORDON et al., 1986; MORZORATI et al., 1988) and even the inherited genetic information (GOLDMAN et al., 1985; GOLDMAN et al., 1987; GOODWIN et al., 1974; OLIVERIO and ELEFTHERIOU, 1976). LI et al. (1986) were the first to report, that generations of an alcohol-preferring (P) line of rats were bred out. The behavioural properties of this inbred strain were examined and found to be different when compared with the nonpreferring (NP) rats.

On the basis of these experiments, an inbred P strain of Wistar rats was also derived in our laboratory (SCHULZ, 1987). The aim of the present work was to investigate the behavioural parameters of these animals with open field, time-to-emerge and plus maze methods. Furthermore, we aimed at to examine the synaptic density in three regions of the CNS (the cortical pyramidal cell apical dendrites in layer 4; the molecular layer of the cerebellum and the main dendrites of the pyramidal cells of the hippocampal CA1 region) that are known to play a major role in motor and behavioural activity.

Materials and methods

Inbred strain of Wistar rats (16th generation) were used in these experiments. Animals (n=22 in each group) were selected for their voluntary ethanol consumption with a preference index (see SHULZ, 1987) larger than 0.7, and tested in the behavioural experiments described below (P animals). Control groups (n=25) were chosen from NP rats of the same inbred strain (preference index<0.20). All groups of animals were caged individually and kept under a 12–12h light-dark cycle. Food and water was available ad libitum. Temperature of the breeding room was 22–25 °C, the relative humidity about 50%.

a. Behavioural tests

1. *Open field test* (OFT; HALL, 1934)

An 8×8 square white painted 100×100×40 cm wooden test box was illuminated by a 150 W electric bulb from 150 cm above. A background noise was constantly applied which was about 20 dB strong. The experimental animals were placed individually in the middle of the box. The length of a session was 5 min and the experiments were repeated on 3 consecutive days. The parameters examined were the number of ambulations in outer and inner squares, the total ambulation activity in the first minute, the ambulation in the inner squares, rearing activity and latency of rearing, grooming activity and latency of grooming, the defecation rate and latency of defecation.

2. *Time-to-emerge test* (TTE; CRAWLEY and GOODWIN, 1980)

The testing equipment consisted of two communicating parts: a black painted dark chamber and a white painted indirectly illuminated one. The size of both chambers was 30×20×30 cm and they were connected with a guillotine door. On the first day of the experiments, each animal was placed 4 times into the dark chamber for 5 min to allow habituation to occur. On the second day, animals were placed into the dark compartment for 5 min, then the quillotine door was opened. TTE latencies were recorded when the animals entered the light compartment with all 4 paws.

3. *Plus maze test* (PMT; HANDLEY and MITHANI, 1984)

The test apparatus consisted of 4 arms (45×10 cm each); two were open while the other two were closed with a 9 cm high wall. The maze was elevated 80 cm from ground level. Each animal was tested in a 5 min session and the following parameters were recorded: latency to leave the centre of the maze, first choice of the open or closed arm, the number of entries onto and time spent on open arms.

b. Electron microscopic processing

Brain tissues from animals used in behavioural tests were processed for electron microscopy. Both the P and NP groups (6—6 specimens: 3—3 males and females) were perfused transcardially under Nembutal anaesthesia. First with 0.12 M phosphate buffer (PB) followed by a fixative solution (4% paraformaldehyde, 2.5% glutaraldehyde in 0.12 M PB at pH 7.4) for 20 min. After removing the brains from the skull, small tissue blocks were cut from the parietal cerebral cortex close to the midline, the hippocampal grey matter in the CA1 region and the lower vermis region of the cerebellar cortex. The tissue pieces were postfixed in the same fixative for 3 h, then washed in 0.12 M PB containing 7.5% sucrose. Buffered 1% OsO_4 was applied for 1 h, followed by dehydration through an ethanol series and propylene oxide. Block contrasting took place in 70% ethanol saturated with uranyl acetate. Tissue pieces were then embedded in Durcupan ACM resin. Semithin and ultrathin sections were cut with a Reichert Om U2 microtome. The ultrathin sections were counterstained with lead citrate, viewed and photographed in a Jeol 100B or Tesla BS 540 electron microscope. In the case of the cerebral cortex, the number of synapses/100 μm of pyramidal cell apical dendrite membrane in layer 4 was recorded and the same parameter was determined in the molecular layer of the hippocampus. In the cerebellum, the synapse density in the molecular layer was calculated.

c. Statistical evaluation

The results of both the behavioural tests and also the EM observations were processed by using the information statistics (KULBACK, 1978). For establishing significances, the F-test was used.

Results

The alcohol preference index was determined for all the groups as described by SCHULZ (1987). The preference index of the female P rats was 0.83 ± 0.11, while that of the NP rats was 0.15 ± 0.13. It is interesting to note, that this index was smaller among the male P animals (0.6 ± 0.12), while in case of the NP animals it was 0.16 ± 0.07.

In the OFT, the males (Fig. 1) of the P strain had significantly higher inner ambulation rate; more wall rearings and groomings with shorter latency to these parameters were observed on the first day. The defecation rate of the P males was lower, than that of the NPs on all the days, and the latency of defecation was higher in P male animals. The female P rats (Fig. 2) showed much higher motility than the members of the NP group. There was a higher rate of both the total ambulation and the inner ambulation on the first day. The P females showed more rearing and grooming, and the latency of these parameters was mostly shorter than that of the NP females. The defecation rate of the P animals was also lower, while its latency was significantly longer on the first day only.

In the TTE test there was a significant difference in the latency of leaving the dark box both by the males and females (Fig. 3). The female P group showed an extremely short latency, while in the male P group the same parameter was substantially higher, than that of the NP males (Fig. 3).

In the PMT, the males differed in the latency of leaving the centre, which was prolonged in the NP group; in their entries to the open arm, which was highest for the P males. The time spent there was much less than that of the NP group. The defecation rate of the P males was higher (Fig. 4).

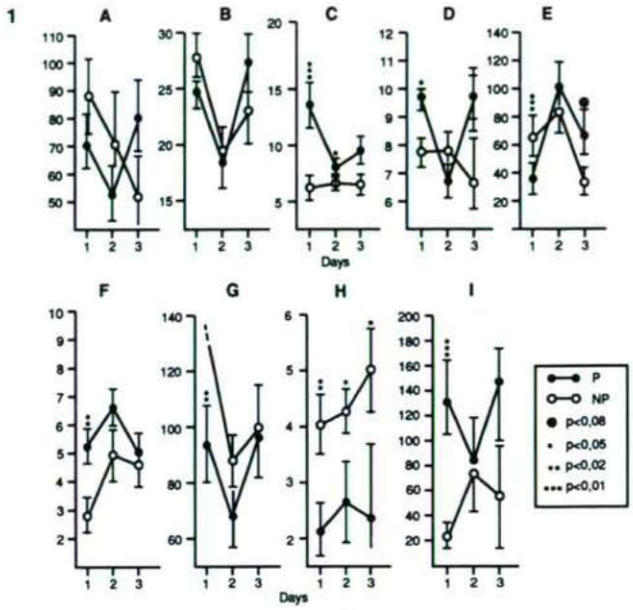
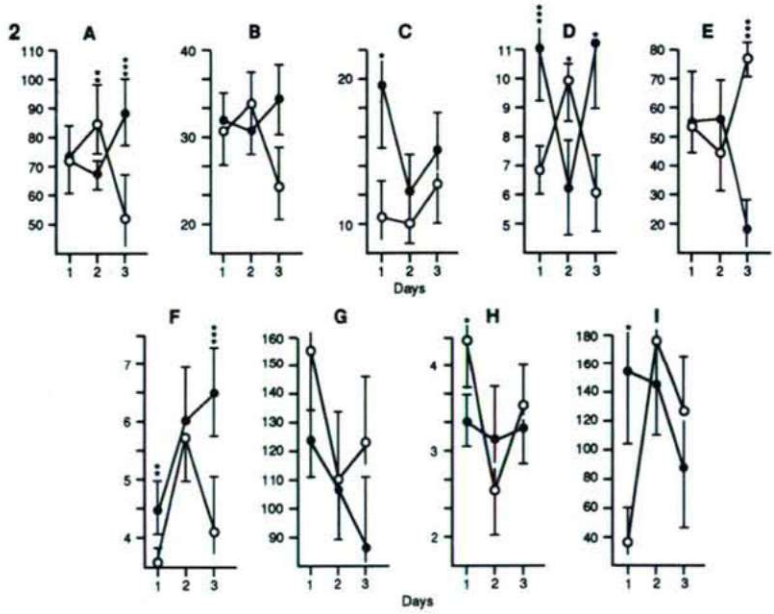


Fig. 1. The results of the open-field test. The filled circles represent the P (preferring), while the empty circles the NP (non-preferring) males. A: total ambulations; B: ambulations in the first min; C: ambulations in the inner part of the open-field; D: wall rearing; E: latency of rearing; F: grooming; G: latency of grooming; H: defecation rate; I: latency of defecation. $p<0.08$; *$p<0.05$; **$p<0.02$; ***$p<0.01$. The test days are indicated on the abscissa, while the time in secs on the ordinate. Conventions also apply to the Figures 2—4.



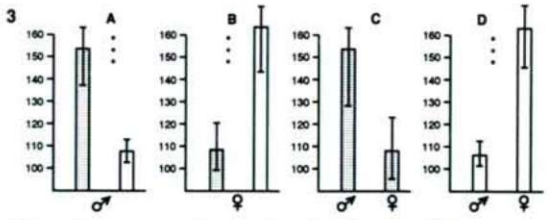


Fig 3. The TTE test latencies compared to each other in two different ways. A and B show the results of P and NP males and females, while in the C, and D graphs the two sexes of the same strain.

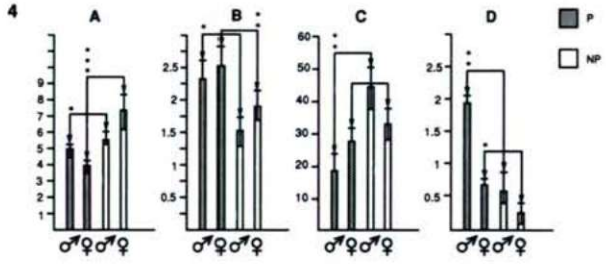


Fig. 4. The results of the plus maze test in bargraphs. A: Latency of leaving the centre; B: first choice; C: the number of the entries to the open arm; D: time spent in the open arm.

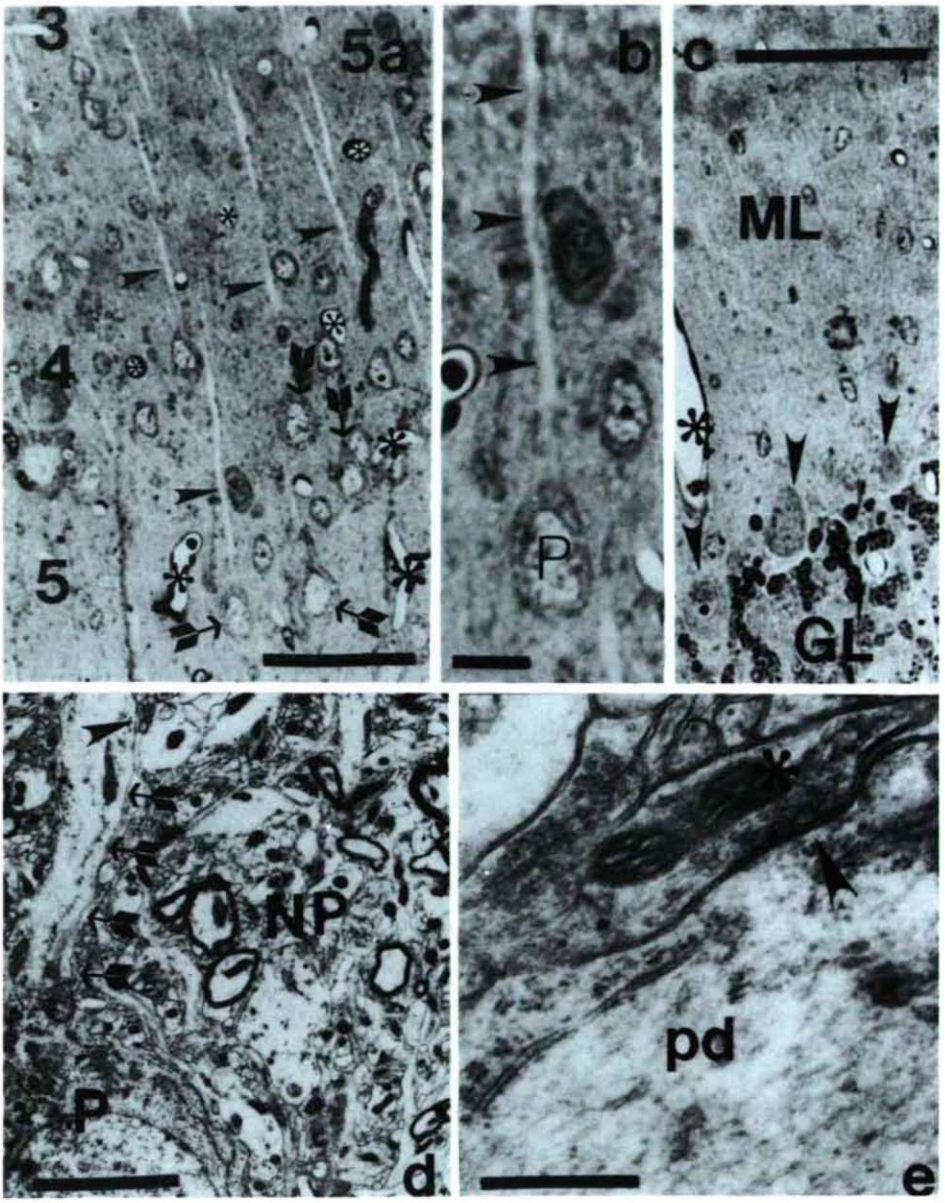
There was no difference in the entries to the open arm between the P and NP females, but all of the other parameters were similar to that of the males (Fig. 4).

The results of the synapse counting are summarised in Table I. Some of the sample areas are shown in Fig. 5. There was a significantly lower synapse number in the P strain on the pyramidal cell apical dendrites in layer 4 of the cerebral cortex, while in the molecular layer of the cerebellar cortex the density of synapses was higher in the P group. The two strains did not differ significantly from each other in number of synapses converging to the apical dendrites of the hippocampal pyramidal cells in the molecular layer.

Discussion

The breeding of our P strain of rats has now reached the 16th generation. During this time, it was possible to follow the diverging and common elements in the behaviour of P rats compared to the control group, with continuous screening. The changes clearly reflect the differences in the emotional state of P and NP rats, which may be related to alterations within the CNS. Thus, in parallel to

Fig. 2. The open-field results of the female groups. The individual graphs A—I present the same parameters as in Fig. 1.



the behavioural experiments, we hoped to find ultrastructural alterations in important brain centres such as cerebral cortex, hippocampus and cerebellar cortex.

It is necessary to make a clear distinction between alcohol dependence and preference. Physical alcohol dependence could not be developed during our

Fig. 5. The sample areas for electron microscopy from the P animals. A: low power photograph of the cerebral cortex showing layers 3, 4 and 5, with pyramidal neurons (arrows) in layer 5 and their apical dendrites in layer 4 (arrowheads). Asterisks: capillaries. Scale bar: 15 μm. B: The initial part of a pyramidal cell (P) dendrite (asterisks). Scale bar: 15 μm. C: Cerebellar cortex. ML: molecular layer, GL: Granule cell layer, arrowheads: Purkinje cell, asterisks: capillary. Scale bar: 10 μm. D: Low power electron micrograph of a hippocampal pyramidal neuron (P). NP: neuropile, arrows: pyramidal cell dendrites, arrowhead: synaptic connection shown with higher magnification in E. Scale bar: 5 μm. E: Symmetric synaptic contact (arrowhead) to a pyramidal cell dendrite (d) from a clear-vesicle containing profile (asterisks). Scale bar: 100 nm.

breeding procedure, since the experimental animals received ethanol ad libitum 4 times only. The volume of the consumed ethanol and the low number of drinking trials is not enough for the emergence of an alcohol dependence (see SHULZ, 1987).

Among the OFT parameters the ambulations and the rearing are fear-motivated (HALL, 1934; SANTACANA et al., 1972; WALSH and CUMMINS, 1976). As presented in the Results, these parameters were at higher values in P rats in both male and female populations. The higher intensity of movements shows a higher level of fear, against the lower defecation levels with longer latency. At the same time, the high grooming activity of the P animals allows the speculation, that unusual environmental cues play important roles in the activation of the attention system of these animals.

In the TTE test, a conflict situation (i. e. dark preference versus curiosity) is employed, to examine the decision making and fear-overcoming process, when the animals have to leave the dark well known chamber to enter the light, not known part of the test box. Interestingly, only the female P rats left the dark box with a significantly shorter latency, while the results of the P and NP males were opposite. These results are important when considering the human analogy where the female alcohol—driven behaviour is even more uncontrolled than that of the males (MILLER et al., 1989; SCHMIDT et al., 1990).

A higher threshold of fear and a more uncontrolled behaviour of female rats can also be observed in the PMT test. Both the short latency of leaving the

Table 1. The number of synapses in the three brain areas, mean and standard deviation

	CORTEX No/100 μm membrane	CEREBELLUM No/100 μm^2	HIPPOCAMPUS No/100 μm membrane
P strain	9±4.1*	51±10.2*	14±3.6
NP strain	16±2.7	36±8.8	17±4.2

* — $p<0.025$ significant difference between the elements of the same column.

centre of the maze and the number of the entries to the open arms support this view.

Considering the results of the 3 behavioural tests we concluded that the P rats showed a modified behavioural pattern. The fear-motivated elements in the behaviour were far less dominant in test situations than in the NP rats. Also, they showed a substantially higher level of motor activity, which has already been shown in P rats in response to low dose ethanol treatment by others (WALLER et al., 1984).

The results obtained in the behavioural tests made it possible to consider, that both major limbic and motor structures might have been genetically affected during the forced drinking experience followed by the selection procedure. Previous findings have shown that chronic ethanol consumption reduces the number of dendritic spines on the pyramidal cells in the cerebral cortex of humans (FERRER et al., 1986) and in the hippocampal CA1 pyramidal cells in experimental animals (McMULLEN et al., 1984). Reduction of the Purkinje cell dendritic tree after chronic ethanol treatment has also been observed (PENTNEY, 1982). Our results cannot be directly compared to those of the above cited studies, since our experimental animals did not get enough alcohol to be considered as a chronic treatment. Furthermore, the tendency of the changes revealed by our experiments, suggests a kind of compensatory mechanism in different brain centres. While the hippocampal CA1 region remained relatively unchanged, the number of synapses on the cerebral pyramidal neurons dramatically decreased. At the same time, the cerebellar molecular layer seemed to be enriched in synapses. A possible explanation for this fact is, that in parallel with the lower density of synapses in certain associative centres, a higher density of them may be formed in certain motor regions like cerebellum. This may be partly in connection with the higher motoric activity of P rats (WALLER et al., 1984). However these speculations need further experimental corroboration.

References

BAUER-MAFFETT, C. and ALTMAN, J. (1977): The effects of ethanol chronically administered to prewealing rats on cerebellar development: A morphological study. — Brain Res. *119*, 249—268.

BERACOCHEA, D. B., LESCAUDRON, L., VERNA, A. and JAFFARD, R. (1987): Neuroanatomical effects of chronic ethanol consumption on dorsomedial and anterior thalamic nuclei and on substantia innominata in mice. — Neurosci. Lett. *73*, 81—84.

CHAMACO-NASI, P. and TRIESTMAN, S. N. (1986): Ethanol effects on voltage-dependent membrane conductances: comparative sensitivity of channel populations in *Aplysia* neurons. — Cell. Mol. Neurobiol. *3*, 263—278.

CRAWLEY, J. N. and GOODWIN, F. K. (1980): Preliminary report of a simple animal behavioural model for the anxiolytic effects of benzodiazepines. — Pharmac. Biochem. Behav. *13*, 167—170.

FERRER, I., FABREGUES, I. RAIRIZ, j. and GALOFRE, E. (1986): Decreased numbers of dendritic spines on cortical pyramidal neurons in human chronic alcoholism. — Neurosci. Letters *69*, 115—119.

GOLDMAN, D., NELSON, R., DEITRICH, R. A., BAKER, R. C., SPUHLER, K., MARKLEY, H., EBERT, M. and MERRIL, C. R. (1985): Genetic brain polypeptide variants in mouse strains with high and low sensitivity to alcohol. — Brain Res. *341*, 130—138.

GOLDMAN, D., LISTER, R. G. and CRABBE, J. C. (1987): Mapping of a putative genetic locus determining ethanol intake in the mouse. — Brain Res. *420*, 220—226.

GOODWIN, D. W., SCHULSINGER, S., MOLLER, N., HERMANSEN, L., WINOKUR, G. and GUZE, S. B. (1974): Drinking problems in adopted and nonadopted sons of alcoholics. — Arch. Gen. Psychiatry *31*, 164—169.

GORDON, S. A., COLLIER, K. and DIAMOND, J. (1986): Ethanol regulation of adenosine receptor stimulated cAMP levels in a clonal neural cell line: In vitro model of cellular tolerance to ethanol. — Proc. Natl. Acad. Sci. USA *83*, 2105—2108.

HALL, C. S. (1934): Emotional behaviour in the rat: defecation and mination as measures of individual differences in emotionality. — J. Comp. Psychol. *18*, 385—403.

HANDLEY, S. L. and MITHANI, S. (1984): Effects of alpha adrenoceptor agonists and antagonists in a maze exploration model of "fear" — motivated behaviour. — Naumyn-Schmiedersberg's Arch. Pharmacol. *327*, 1—5.

HOFF, S. F. (1988): Synaptogenesis in the hippocampal dentate gyrus: Effects of in utero ethanol exposure. — Brain Res. Bull. *21*, 47—54.

KULLBACK, S. (1978): Information theory and statistics. — Peter Smith, Boucester (Mass).

LI, T.—K., LUMENG, L., BCBRIDE, W. J., WALLER, M. B. and MURPHY, J. B. (1986): Studies on an animal model of alcoholism. Genetic and biological markers in drug abuse and alcoholism 66.

MCMULLEN, P. A., SAINT-CYR, J. A. and CARLERI, P. L. (1984): Morphological alterations in rat CA1 hippocampal pyramidal cell density resulting from chronic ethanol consumption and withdrawal. — J. Comp. Neurol. *255*, 111—118.

MILLER, B. A., DOWUS, W. R. and GONDOLI, D. M. (1989): Spousal violence among alcoholic women as compared to a random household sample of women. — J. Stud. Alcohol. *50*, 533—540.

MILLER, M. W. (1986): Effects of alcohol on the generation and migration of cerebral cortical neurons. — Science *233*, 1308—1311.

MORZORATI, S., LAMISHAW, B., LUMENG, L., LI, T.—K., BEMIS, K. and CLEMENS, J. (1988): Effect of low dose ethanol on the EEG of alcohol-preferring and non-preferring rats. — Brain Res. Bull. *21*, 101—104.

OLIVERIO, A. and ELEFTHERIOU, B. E. (1976): Motor activity and ethanol: genetic analysis in the mouse. — Physiol. Behav. *16*, 577—581.

PENTNEY, R. J. (1982): Quantitative analysis of ethanol effects on Purkinje cell dendritic tree. — Brain Res. *249*, 397—401.

ROVINSKY, B., HOSEIN, E. A., HUNG, L., LAN-YOU, H. G. and ROSTOGI, H. K. (1987): Hepatotoxicity of maternal ethanol consumption in rat offsprings an assesment with study of the ontogenetic development of ethanol oxidizing system. — Alc. Drug Res. *7*, 195—205.

SANTACANA, M. Z., ALVAREZ PELAEZ, R. and TEJEDOR, P. (1972): Effect of the mamillary bodies on the performance in the open-field. — Physiol. Behav. *9*, 501—504.

SCHMIDT, C., KLEE, L. and AMES, G. (1990): Review and analysis of literature of indicators of women's drinking problems. — Brit. J. Addict. *85*, 179—192.

SCHULZ, H. (1987): Behaviour of rats selected for their voluntary ethanol consumption. — Acta Biol. Hung. *38*, 407—422.

SIGGINS, G. R., PITTMAN, Q. J. and FRENCH, E. P. (1987): Effects of ethanol on CA1 and CA3 pyramidal cells in hippocampal slice preparation: an intracellular study. — Brain Res. *414*, 22—34.

WALLER, M. B., MURPHY, J. M., kMCBRIDE, W. J., LUMENG, L. and TING-KAI LI, M. D. (1986):

Effects of low dose ethanol on spontaneous motor activity in alcohol-preffering and -nonpreffering lines of rats. — Pharmacol. Biochem. Behaviour *24*, 617—623.

WALSH, R. N. and CUMMINS, R. A. (1976): The open-field test. — Physiol. Bull. *83*, 482—504.

WARTBURG, J. P. von (1985): Az alkoholizmus patobiokémiája. Az alkohol hatása a membránstruktúrára és működésre. — Alkohológiai kiskönyvtár, 8. Medicina, Budapest.

WOOD, W. G. and SCHROEDER, F. (1988): Membrane effects of ethanol: bulk lipid versus lipid domains. — Life Sci. *43*, 467—475.