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Neuronal subserving of behavior before and after chronic ethanol treatment

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Abstract

We have previously shown that an acute ethanol dose (1 g/kg), sufficient to impair the performance of a healthy rabbit, also reversibly depresses the activity of those limbic-cortex neurons that are specifically activated during recently learned behavioral acts. Our new morphological and neurophysiological data suggest a death of such neurons after 9-month chronic ethanol treatment. The effect of acute ethanol administration on neurons and performance speed in alcoholic rabbits was opposite to that found in healthy animals. Our results help to understand why neurocognition of alcoholics changes and why acute low-level alcohol ingestion influences them differently than healthy individuals. © 2000 Elsevier Science Inc. All rights reserved.

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1. Introduction

Ethanol has selective influence on various brain structures and even neighboring neurons (Klemm & Stevens, 1974; Klemm et al., 1976; Zornetzer et al., 1982). This selectivity has been suggested to be due to various functional and structural factors each of which can predict the effect of ethanol on a particular brain site but fail to do it on another (Alexandrov et al., 1990b). The crucial determinants underlying this selectivity have thus remained unclear (Zornetzer et al., 1982). We have suggested that the critical factor is the behavioral specialization of neurons, i.e., their belonging to a particular functional system (Anokhin, 1973; Shvyrkov, 1986; Alexandrov et al., 1997) involved in behavior. In our previous studies, different types of neuronal specializations were identified in various brain areas of freely moving rabbits performing instrumental food-acquisition behavior in a cage equipped with two pedals and two feeders (Alexandrov et al., 1990a,b, 1993).

The diverse types of specializations can be classified in two main categories. "M-neurons" are activated in relation to different *movement* systems, established early in an individual's development. Their activation is specifically related to a certain movement. They are activated during the same movement that can be performed in different behavioral contexts. "L-neurons" are activated in relation to systems of comparatively new behavioral acts formed during an animal's *learning* in the experimental cage (e.g., approaching the feeder, approaching the pedal, pressing the pedal). Their activation is specifically related to a certain behavioral act but is independent of its detailed motor execution (see Experimental Procedures and Figs. 1 and 2). *Unidentified* "U-neurons" do not show consistent activation during the given task.

Our classification accords with the idea of functional specialization, replacing the "cerebral organ concept of functional localization" (Mountcastle, 1995). The classification is compatible with other conventional classifications (see Alexandrov et al., 1993) as well as experimental

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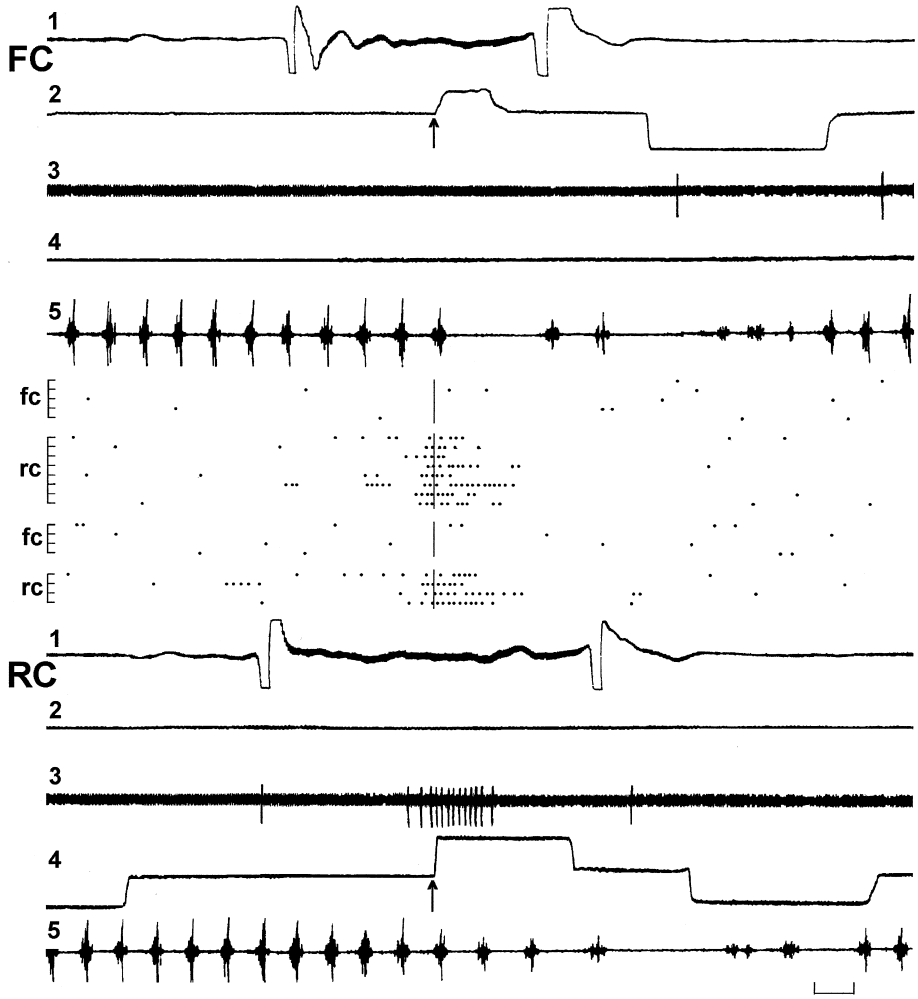

Fig. 1. Example of the activity of the L-neuron that was activated during pressing the pedal in the rear-wall cycle (RC) but not front-wall cycle (FC) of food-acquisition behaviour. Recording is from an ethanol-treated rabbit after acute ethanol administration (E+). (1) Actogram showing the position of the head in relation to the wall of the cage between the corresponding pedal and feeder (downward deflection = head is near middle of the wall; see Experimental Procedures). (2) Actogram of behaviour in the FC (upward deflection = pedal pressing, downward deflection = lowering head into feeder). (3) Neuron activity. (4) Actogram of behavior in the RC (upward deflection = pedal pressing, downward deflection = lowering head into feeder). (5) EMG of m. masseter. The rasters in the middle of the figure indicate unit activity (each point represents one impulse, each row — one act) plotted at the instant of the beginning of displacement of the pedal during its pressing (arrow). An example of the neuronal activity in the RC (numbers (1)–(5) are the same as above) is shown under the rasters. This neuron was activated during pressing of the rear — but not front-wall pedal. The rabbit was tested repeatedly in each cycle during recording of the given neuron in the following order: fc, rc, fc, rc. Rasters are arranged according to the order of realization of corresponding acts during recording. Horizontal bar on the right indicates 200 ms.

findings of cortical and subcortical unit activity measured from various species. Neurons compatible with the present classification to M- and L-neurons have been described in several previous studies (Luschei et al., 1971; Ranck, 1973; Wong et al., 1982; Winer, 1996; Winer et al., 1989; Shima et al., 1991; Chang et al., 1994; Martin et al., 1997; Stackman & Taube, 1998; Hampson et al., 1999). Our classification has been used successfully in studies of neuronal mechanisms of formation and realization of behavior (Gorkin & Shevchenko, 1996; Alexandrov et al., 1997) including alcohol-acquisition behavior (Alexandrov et al., 1998a), for comparative investigations of neuronal subserving of instrumental behavior in different species (Gavrilov et al., 1998), for studying effects of local brain damages (Alexandrov et al., 1990a), and acute ethanol administration (Alexandrov & Alexandrov, 1993; Alexandrov et al., 1990b, 1993, 1998b). We demonstrate here that this classification helps also to understand the selective effect of chronic ethanol administration: belonging to M- or L-group determines a neuron's susceptibility.

We recently analyzed the activity of almost 700 neurons in the limbic (cingulate) cortex and hippocampus of freely moving rabbits, here after called "healthy" animals (Alexandrov et al., 1990b, 1993). In the limbic cortex acute ethanol administration (1 g/kg, ip) decreased the number of active units due to selective depression of activity of L-

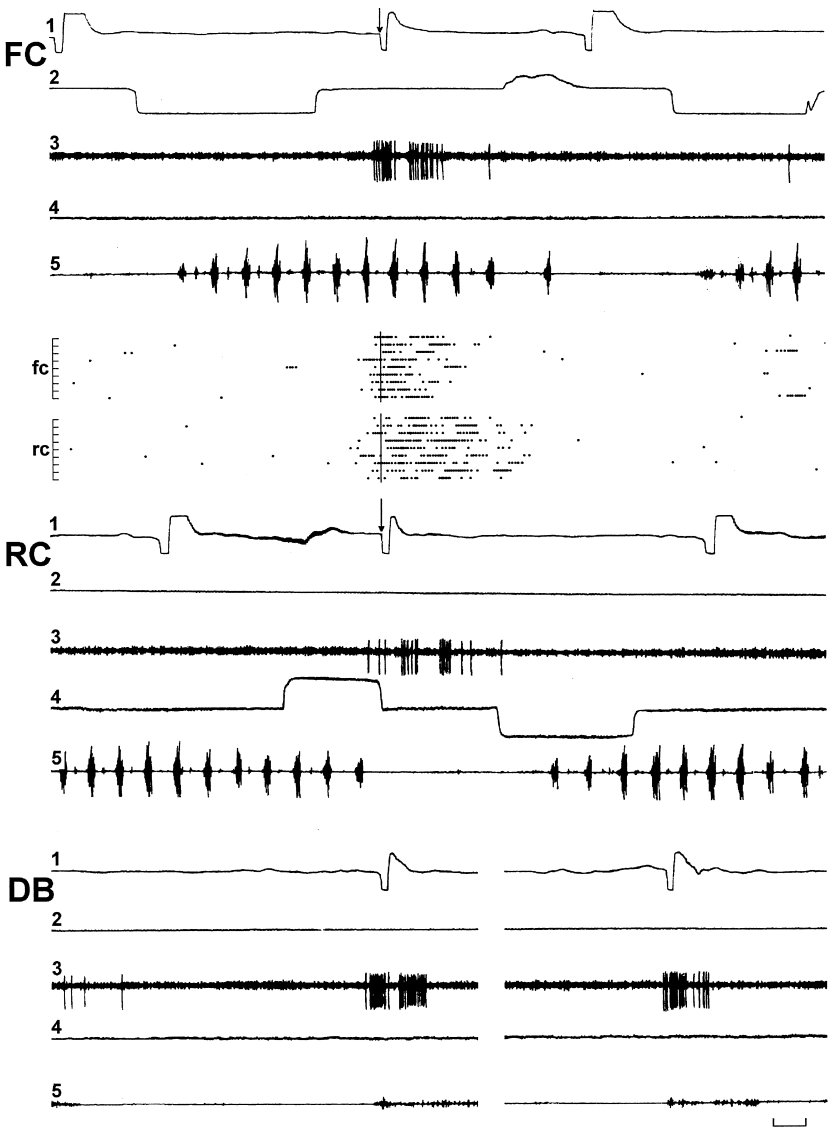

Fig. 2. Example of the activity of the M-neuron that was activated during rightward body movement in the rear-wall (RC) and front-wall cycle (FC) of food-acquisition behaviour, and in defensive behavior (DB) performed by ethanol-treated rabbit in control experiment with no ethanol (E−). An example of neuronal activity in FC is shown in the upper part of the figure. Rasters in the middle of the figure show unit activity plotted at the instant of the beginning of downward deflection of actogram showing the position of the head in relation to the either wall of the cage (arrow). An example of the neuronal activity in RC is indicated below the rasters. The bottom part of the figure shows neuronal activity in the defensive behavior (DB) during rightward movements under the experimenters' hand coercion. Numbers (1)–(5) are the same as in Fig. 1. Each deflection in (1) corresponds to the rightward turn. Activations of this neuron appear during the turning to the right when approaching the pedal on one side of the cage (FC), approaching the feeder on the opposite side of the cage (RC) or during this movement in defensive behavior (DB). Horizontal bar on the right indicates 200 ms.

neurons. As a consequence, the relative amounts of L- and M-neurons changed (see Fig. 4). Both the upper (II–IV, small cells with short axons) and the lower (V–VI, larger pyramidal cells with long axons) cortical layers contained L- as well as M-neurons. However, depression was more prominent in the upper than in the lower cortical layers. The inhibitory influence of ethanol on L-neurons was also found in the hippocampus. Similar suppressive effects were recently obtained when ethanol was applied via microdialysis on hippocampal neurons of behaving rats (Ludvig et al., 1995). We have also demonstrated increased susceptibility of relatively new brain systems to ethanol in nestlings (Alexandrov & Alexandrov, 1993) and in humans (Alexandrov et al., 1998b).

Also chronic ethanol treatment (CET) has selective effects on neuronal tissue (Walker & Hunter, 1987; Fadda & Rossetti, 1998). However, it has remained unclear "why some cells are lost, and others are not" (Walker & Hunter, 1987, p. 124). We hypothesized that if analogous sites were modified through acute and chronic drug intoxication (Koob & Bloom, 1988; Koob et al., 1989), the main target for chronic effects in the limbic cortex were L-neurons located

in the upper cortical layers. We studied limbic–cortex morphology and neuronal activity in chronically ethanol-treated rabbits performing instrumental food-acquisition behavior both in the control experiments with no ethanol (E−) and after acute ethanol administration (E+).

2. Experimental procedures

2.1. Subjects

Six male adult rabbits (*Orictolagus cuniculus*; weight about 3 kg), preferring ethanol over water, received a nutritionally adequate diet. The animals were taken care of according to institutional guidelines. All efforts were made to minimize animal suffering. Animals, used in the study of acute ethanol effects on the limbic cortex (eight male rabbits), served as weight- and age-matched healthy controls. Although healthy animals were studied in separate experiments (Alexandrov et al., 1990b), we are confident that the data obtained from them and the present results are well comparable because of the following reasons. The same experimenters carried out experiments. Age and weight of animals, duration, and number of experimental sessions, room, experimental cage, training, electrodes, recording techniques, way and routine of acute ethanol administration, and data analysis were identical for the control and experimental groups (see below).

2.2. Chronic ethanol treatment

In the process of selecting and during CET lasting 9 months, a duration that causes permanent structural and functional alterations of the brain (Walker et al., 1981), the animals could freely choose between ethanol (7% first 2 weeks, 10% later) and water permanently present in water bottles (Cemic, Finland). The number of days when the animals drank more water than ethanol decreased during CET from 18% during the first 2 months of the treatment to 4% within 6–9 months. Alcohol consumption increased during the first 2 months from 3.8 ± 0.2 (mean ± S.E.M.) to 4.4 ± 0.2 g/kg/day ($t = 2.56$, $P < .05$). Thereafter, consumption gradually decreased and at the end of CET consumption was 2.7 ± 0.2 g/kg/day. A similar reduction at the end of 8 months of CET has been observed in rats and is related to the decreased tolerance, characteristic of late stages of alcoholism development (Burov et al., 1983). We have shown in another experiment that instrumental alcohol-acquisition behavior can be formed in rabbits after the same CET as used here (Alexandrov et al., 1998a). When ethanol is able to maintain operant action, dependence is supposed to have occurred (Li et al., 1988).

In order to test ethanol need on the eighth month of the treatment, ethanol withdrawal was induced. After removal of ethanol for 24 h, the consumption increased by 25% in comparison to the average amount per month. However, we have not noticed physical signs of abstinence that can rarely be observed in animals voluntarily administering the drug and are not necessary indicators of dependence (Koob & Bloom, 1988; Koob et al., 1989).

2.3. Acute ethanol administration

In E+ ethanol was injected intraperitoneally (12% ethanol in isotonic solution) in a dose of 1 g/kg just before recording, and thereafter every 1.5–2 h, 0.3–0.5 g/kg ethanol was added until the end of experiment, as in experiments with healthy controls. This routine allows to reach maximum of blood alcohol concentration about 0.9 g/l (defined by gas chromatography; see Alexandrov et al., 1990b) 15–20 min after the first injection and to maintain a level about 0.4 g/l during the recording session. This concentration is enough to evoke changes in behavior and brain activity in rabbits, birds, and humans (Alexandrov & Alexandrov, 1993; Alexandrov et al., 1990b, 1993, 1994, 1998b, present results). In the E−, the equivalent amount of isotonic solution was used. Same animals participated both in E+ and E− experiments.

To make the data obtained in E+ and E− experiments comparable, we had to make animal states similar before the experiments. Previous results from other laboratories (Chapin & Woodward, 1989) and our present data indicate that neither alcohol withdrawal nor acute alcohol injection brings ethanol-treated animals to a "normal" healthy state. Therefore, our task could only be to equalize animals' states before E+ and E−. For this aim, the dose of ethanol consumed by an animal in a home cage during the night before the experiment depended on a previous experimental session (E+ or E−). Ethanol doses were about 1 g/kg after E− and 0.5 g/kg after E+. Blood alcohol concentration in the night just before recording did not differ significantly after E+ and E−, being 0.022 ± 0.039 g/l. This concentration slightly exceeds the upper limit of endogenous ethanol level in rabbits (0.020 g/l).

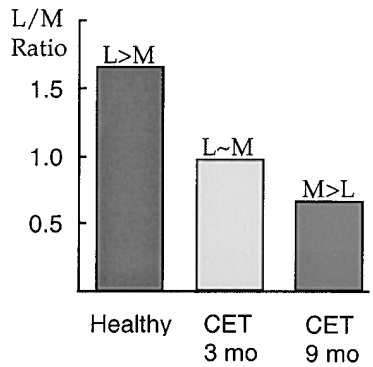

Fig. 3. Ratio of L- and M-neurons in healthy rabbits and after CET for 3 and 9 months (control conditions with no ethanol).

2.4. Food-acquisition training

Recording techniques, experimental cage, training, and data analysis were the same for ethanol-treated and healthy animals. They have been thoroughly described earlier (Alexandrov et al., 1990a,b, 1993).

Before CET all rabbits were taught to acquire food by pressing one of the two pedals in the experimental cage (described in detail in Alexandrov et al., 1990a). Pressing of the pedal activated an automatic feeder on the same side of the cage. Each rabbit repeatedly carried out the food-acquisition task involving a constant series of acts (behavioral cycle: pressing the pedal, turning to the feeder, taking food from the feeder, turning to the pedal) at both sides of the cage (front and rear walls in relation to the video camera; see Recording Techniques). During training and recording periods in the home cage animals received ca. 25% of the normal daily amount of food.

2.5. Recording techniques

Single-unit activity was recorded with glass microelectrodes from the limbic (cingulate) cortex (area 29d; P 10.0–11.5, L 2.5–3.5, according to Vogt et al., 1986). Glass microelectrodes with 2.5 m KCl, tips of 1–3 mm diameter, and impedance of 1–5 MΩ at 1.5 kHz were used and driven by a micromanipulator.

Unit activity, EMG (m. masseter pars profundus) and actographic marks of the behavior were tape-recorded. Animals' movement from the pedal to the feeder, or vice versa, was recorded by a photocell, fixed to the head of the animal, which responded to photodiodes located in the middle of the front and rear walls of the cage (front and rear behavioral cycles, correspondingly) between the pedal and the feeder. The rabbit's behavior was video-recorded with the unit activity (audio-channel), the light indicators of the pedal pressing and head lowering, the counters of the cumulative number of spikes, and of time (timer).

The depth of each active unit's location, encountered during microelectrode penetration, was measured by means of a micromanipulator with a scale showing the vertical location of the recording tip.

2.6. Data analysis

Unit activity was analyzed by conventional means. Rasters and histograms were plotted with reference to different phases of behavioral phases determined by the actographic marks and EMG of m. masseter, as well as to other behavioral events determined by an analysis of video-recording. In the latter case we calculated, by means of readings of the timer and impulse counter in the stop-frame mode of the video recorder, the number of impulses during each of the successive 20-ms intervals with reference to different phases of behavior. The data obtained from successive realizations of behavioral cycles were then summed, yielding histograms plotted in relation to different behavioral events. The average action potential frequency during the entire recording was calculated for each neuron (''background frequency''). Activations were estimated during different phases of behavior by relating increases of firing frequency to the background activity. Activation was considered to be present if a discharge appeared (in a unit without background activity) or there was a marked increase in firing frequency (in a unit with background activity) during a behavioral phase in all realizations of the behavior. As others (Yamamoto et al., 1989), we consider a change in the firing frequency to be an activation when it deviates by more than two standard deviations from the mean background frequency.

Classification of the units was based on a constant appearance of their activation in relation to certain phases of repeated behavior. The neurons were divided into two groups: unidentified neurons (no consistent activation during the behavioral cycles of instrumental behavior, U-neurons) and neurons involved in subserving of behavior (activated in constant relation to a certain stage of the repeated behavioral cycle). The latter group was further divided into two groups with different behavioral specialization: L- and M-neurons (see Introduction).

L-neurons showed activation in relation to novel behavioral acts established late in individual development, such as during animal's learning in the experimental cage (e.g., approaching the feeder, approaching the pedal, pressing the pedal). Whether their activation appeared or not was specifically related to a certain behavioral act but independent of its motor characteristics. Similar activity was elicited when the animal pressed the pedal with the left paw, right paw, or both. Many of the L-neurons became active only when the animal pressed a certain pedal, say in the rear-wall but not in the front-wall behavioral cycle (Fig. 1). Neurons that showed activation in relation to a particular movement of the body, head or lower jaw, were considered to be specialized relative to the systems formed earlier in ontogeny (Shvyrkov, 1986; Alexandrov et al., 1997). Whether their activation appeared or not was related specifically to a certain movement but independent of its behavioral context. Activation appeared during the same movement in different behaviors, e.g., turning to the right when approaching the feeder on one side of the cage or approaching the pedal on the opposite side of the cage. It was also activated during such a movement in defensive behavior provoked by experimenter's hand intrusion and jogging an animal (M-neurons; Fig. 2).

The duration of the behavioral cycle (running from the pedal to the feeder and back) and the number of mistakes in the performance (missing a pedal, checking feeders without pressing a pedal) were determined.

2.7. Morphological analysis

After the experiments the rabbits were sacrificed with an overdose of Nembutal and their brains were fixed in 10%

formalin. Serial frontal slides, 10–20 μm thick, were cut and stained by the Nissl method. In the contralateral hemisphere (symmetrical to the site of the recording), neural structure was analyzed by light microscopy ("Cytopan," Austria). The total thickness of the cortex, the thickness of cortical layers (compared with the locations of the units encountered during the recordings), and the numerical density of neurons (in a grid with a square side of 80 μm) were determined. The calculations were taken in each animal in 20 vision fields in the upper (II–IV) layers and in 30–40 vision fields in the lower (V–VI) layers. Then, the volumetric number was calculated. This is an essential parameter since, despite the absence of significant differences in cell packing densities after CET, a reduction of their total number can be present (Tavares et al., 1987).

In data analyses, χ^2 tests, t tests, ANOVAs, and log-linear analysis were used (significance limit $P < .05$).

3. Results

3.1. Effect of chronic ethanol treatment on the relative number of L- and M-neurons

The activity of 484 limbic cortex neurons in ethanol-treated rabbits was analyzed (249 in E− and 235 in E+) and compared with the activity of 360 neurons of the healthy animals (173 in E− and 187 in E+). Same types of neuronal specializations (L and M) were found in ethanol-treated animals as in healthy ones. Representative examples of L- and M-neurons' activity recorded in ethanol-treated animals are exhibited in Figs. 1 and 2, respectively.

In the healthy animals in E−, the relative number of L-neurons was significantly higher than the number of M-neurons (L/M ratio = 1.7, $\chi^2 = 4.68$, $P < .05$). The reverse was true in ethanol-treated animals (L/M ratio = 0.7, $\chi^2 = 7.31$, $P < .01$). A decrease in the number of U-neurons in ethanol-treated animals was also significant ($\chi^2 = 5.20$, $P < .05$). The dynamics of the L/M ratio changes become even more evident if we add to the present data the L/M ratios observed in our earlier experiment after 3 months of CET (Alexandrov et al., 1994). The latter value (0.96) is intermediate between that found in healthy animals and after 9-month CET (Fig. 3).

The effect of ethanol was most evident in the upper cortical layers where the relative number of L units was 15% less than in healthy animals (difference significant, $\chi^2 = 4.87$, $P < .05$). In the lower layers, the number was only 3% less (ns).

3.2. Effect of chronic ethanol treatment on the number of active neurons

In E−, the number of active neurons in the upper layers per penetration was 21% smaller in ethanol-treated rabbits than in healthy animals (5.7 ± 0.4 and 7.2 ± 0.4, respectively; $t = 2.48$, $P < .05$). In the lower layers, the number was 6% smaller (ns). We predicted the number of L-neurons assuming that this number remained constant when the total number of active units in the upper layers decreased. This value significantly exceeded the experimental one ($\chi^2 = 8.49$, $P < .01$). Thus, the decreases in the relative number of L-neurons and in the total number of active neurons in the upper cortical layers are likely due to the same phenomenon: a decrease in the absolute number of active L-neurons.

3.3. Effect of chronic ethanol treatment on the volumetric number of neurons

Morphological analysis revealed a significant 35% decrease in the volumetric number of neurons in the upper cortical layers of ethanol-treated rabbits in comparison to the healthy animals (65.2 ± 3.9 and 99.8 ± 15.7, respectively; $F = 6.4$, $df = 1$, $P < .05$). The number was 2% smaller in the lower layers (ns).

3.4. Effect of acute ethanol administration on the performance

Ethanol-treated rabbits started performing faster in E+ (by 7%: 4.8 ± 0.07 s/cycle in E− and 4.5 ± 0.06 s/cycle in E+; $t = 3.22$, $P < .01$), and in that sense their behavior was improved. However, the number of mistakes increased by 44% (0.16 ± 0.008 mistakes/cycle in E− and 0.23 ± 0.01 mistakes/cycle in E+; $t = 4.93$, $P < .001$).

3.5. Effect of acute ethanol administration on the number of active neurons and on the relative number of L- and M-neurons

In healthy animals, acute ethanol induced a decrease in the number of active neurons in the track (from 17.1 ± 0.8

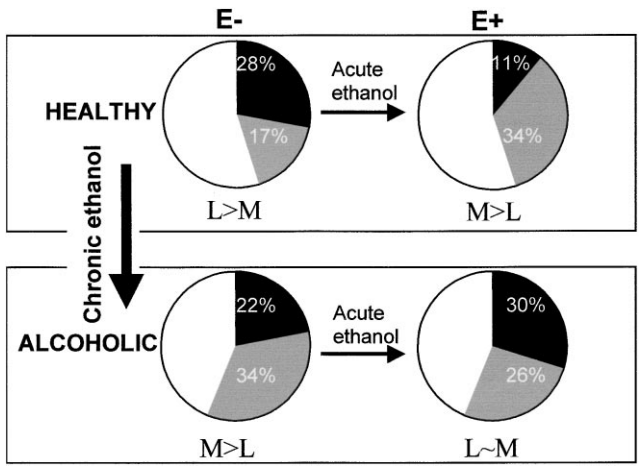

Fig. 4. Relative number of limbic–cortex L- (black), M- (grey), and U- (white) neurons in healthy and ethanol-treated (alcoholic) rabbits in control experiment with no ethanol (E−) and after acute ethanol administration (E+).

to 10.8 ± 0.5) and a concordant twofold decrease in the relative number of active L-neurons (from 28% to 11%). This resulted in a dramatic abnormality of the L/M ratio if compared with the sober state (Fig. 4, above). On the contrary, in the ethanol-treated animals (except one whose alcohol consumption during CET was significantly lower than in other animals; $F = 8.24$, $df = 1$, $P < .01$), the number of active neurons increased in the upper cortical layers under acute ethanol administration. The mean increase in these animals was significant (from 4.7 ± 0.4 to 6.2 ± 0.5, $F = 4.72$, $df = 1$, $P < .05$). Acute ethanol concomitantly *increased* the relative number of L-neurons, resulting in a *normalization* of the L/M ratio compared with the initial (E−) state (bottom part of Fig. 4). However, the L/M/U ratio in ethanol-treated animals even in E+ was significantly different from that in healthy animals ($\chi^2 = 6.79$, $df = 2$, $P < .05$).

The change in the L/M ratio in alcoholic rabbits was due to a more than twofold increase in the relative number of active L-neurons in the upper layers: from 12% in E− to 33% in E+ ($\chi^2 = 11.02$, $P < .001$). No significant changes were observed in lower layers. The increase in the relative number of L-neurons and in the total number of active neurons per penetration in the upper layers are again likely due to the same phenomenon: an increase in the absolute number of active L-neurons ($\chi^2 = 16.7$, $P < .0001$).

3.6. The source of additional L-neurons found in ethanol-treated animals after acute ethanol administration

To discover the source of additional L-neurons found in E+ in ethanol-treated animals, we applied log-linear analysis to two-way tabulation of neurons (experimental situation vs. neurons type). Table 1 presents counts of neurons of the upper cortical layers in ethanol-treated rabbits. The neurons were equally distributed across experimental situations (row totals). Table 1 also demonstrates substantial dependence of count distribution on the experimental state.

Table 2
Parameters of log-linear model of the relationship between experimental state (E− /E+) and neuron type (L, M, U) in the upper cortical layers (saturated model)

	Experimental state		
Main effects	E−	E+	
Parameter estimate	− 0.081	0.081	
Standard error	0.079	0.079	
Z value	− 1.040	1.040	
	Neuron type		
	L-neurons	M-neurons	U-neurons
Parameter estimate	− 0.392*	− 0.050	0.442*
Standard error	0.124	0.110	0.100
Z value	− 3.163	− 0.460	4.480
Interaction			
Neuron type/E−			
Parameter estimate	− 0.411*	0.203	0.208*
Standard error	0.124	0.109	0.099
Z value	− 3.321	1.850	2.112
Neuron type/E+			
Parameter estimate	0.411*	− 0.203	− 0.208*
Standard error	0.124	0.109	0.099
Z value	3.321	− 1.850	− 2.112

* Significant estimate.

Different models were applied to the frequency distribution. Only a saturated model including both main effects and interaction between two variables was found to fit the data (Table 2). Parameter estimates of experimental state were not significant, suggesting that the main effect of this variable may be neglected. The main effect of neuron type was highly significant for L- and U-neurons. The interaction between L- and U-neurons was highly significant. The observed number of L-neurons in E+ significantly exceeded the expected count. The observed number of U-neurons was much lower than the expected

Table 1
Two-way tabulation (E− /E+ vs. neuron type) for the upper layers cortical neurons in ethanol-treated rabbits. E− and E+ refer to the control and acute ethanol administration conditions, respectively

	L-neurons	M-neurons	U-neurons	Row total
E−				
Observed	12	32	53	97 (49.5%)
Expected	22.3	28.2	46.5	
Residuals	− 10.3	3.8	6.5	
E+				
Observed	33	25	41	99 (50.5%)
Expected	22.7	28.8	47.5	
Residuals	10.3	− 3.8	− 6.5	
Column total	45 (23.0%)	57 (29.0%)	94 (48.0%)	196 (100.0%)

χ^2 (Pearson) = 12.172, $df = 2$, $P = .0023$.
Cells contain observed counts, expected counts, and residuals.

Table 3
Two-way tabulation (E− /E+ vs. neuron type) for the lower layers cortical neurons in ethanol-treated rabbits. E− and E+ refer to the control and acute ethanol administration conditions, respectively

	L-neurons	M-neurons	U-neurons	Row total
E−				
Observed	42	54	56	152 (52.8%)
Expected	41.2	48.0	62.8	
Residuals	0.8	6.0	− 6.8	
E+				
Observed	36	37	63	136 (47.2%)
Expected	36.8	43.0	56.2	
Residuals	− 0.8	− 6.0	6.8	
Column total	78 (27.1%)	91 (31.6%)	119 (41.3%)	288 (100.0%)

χ^2 (Pearson) = 3.179, $df = 2$, $P = .2049$.
Cells contain observed counts, expected counts, and residuals.

number in E+. The reciprocal pattern of frequencies was detected in E− .

In the lower cortical layers (Table 3) the model with only main effect of neuron type explained well the observed frequency distribution. Interaction between neuronal type and experimental situation was not significant.

4. Discussion

4.1. The main target of chronic ethanol administration in the limbic cortex

Present data show that the relative number of L units decreases after CET. This decrease is more prominent in the upper cortical layers. The results of morphological analysis agree well with the data on unit activity, suggesting higher probability of neuronal death of the upper-layer L-neurons. The underlying neurochemical alterations might be up-regulation of NMDA receptors and increase in voltage-operated calcium channels (Littleton & Little, 1994). Recent evidence suggests that overactivation of glutamate receptors and an increased intracellular concentration of Ca^{2+} may cause transcriptional activation of "cell-death genes," which is a part of the neuronal-death cascade (Schreiber & Baudry, 1995). We conclude that the present results confirm our suggestion that CET especially affects L-neurons in the upper cortical layers. While the reason why just L-neurons are more susceptible to above mentioned neurochemical alterations is unclear, we nevertheless propose that L-neurons are sensitive because they belong to comparatively new functional systems formed late in individual development, such as during animal's learning in the experimental cage. This explanation is in line with the Ribot–Jackson principle, stating that those mechanisms, which appear last, are most prone to disintegration. The factor determining the increased sensitivity of L-neurons located in the upper cortical layers may be "novelty" of these layers. They mature later in ontogenesis and subserve more complex neurocognitive functions (Luria, 1973; Caviness et al., 1995).

Our data showed the loss of upper-layer L-neurons. The present as well as our previous data demonstrate a relation between the animal's performance and the number of active L-neurons (Alexandrov et al., 1990b, 1993). In addition, deterioration of performance in human subjects may be related to the increased susceptibility of relatively new brain systems to ethanol (Alexandrov et al., 1998b). On the basis of the present and our previous data we suggest that the change in neural subserving of behavior and neurocognitive functions provoked by alcohol abuse is caused mainly by loss of L-neurons. However, neural subserving is deteriorated not only for this reason.

We found that acute ethanol significantly increased the number of active neurons and concomitantly increased the relative number of L-neurons in ethanol-treated rabbits. Hence, in the sober state (in E− as compared to E+) the transient decrease in the number of L-units dedicated to non-alcohol-seeking behavior (see below) may also play a role. It has been shown that in alcoholics during alcohol withdrawal the blood dopamine level changes greatly because of hampering of the transfer of dopamine to noradrenaline (Anokhina et al., 1988). In addition, the nature of the effect of dopamine (excitation vs. inhibition) is critically dependent on dopamine concentration (Williams & Millar, 1989). Catecholamine turnover is known to have a powerful influence on cognitive functions and memory performance (Goldman-Rakic, 1987; Zahrt et al., 1997). Thus, we propose that the transient metabolically dependent decrease in the number of L-units might contribute to the behavioral impairment observed when individuals are tested undrugged after a CET (File & Mabbutt, 1990).

4.2. The normalizing effect of acute ethanol administration

Acute alcohol administration satisfies the alcohol need, establishes the temporal balance in the catecholamine turnover (Airaksinen & Peura, 1987; Anokhina et al., 1988) and results, as it evident from the present data, in the involvement of additional L-neurons in subserving of behavior. Log-linear analysis utilized to find out the source of additional L-neurons showed that interaction between L- and U-neurons was highly significant, the observed number of L-neurons in E+ significantly exceeded the expected count, and the number of U-neurons was much lower than the expected number in E+. We interpret the results of this analysis, together with a significant increase in the number of active neurons in E+, to mean that additional upper layers L-neurons activated in E+ were recruited from the pool of U-neurons and of those L-neurons that were silent in E− . An alternative explanation cannot be ruled out, however.

As we argued earlier (Alexandrov et al., 1990b, 1993), U-neurons are cells specialized in relation to other (not food-acquisition) behaviors, e.g., such as alcohol seeking. During food-acquisition behavior, these neurons give inconstant, variable discharges. There is evidence of a gradual decrease in firing of some mesocorticolimbic neurons during of ethanol consumption (Woodward & Janak, 1998). Those neurons that are dedicated to alcohol-seeking behavior and appear as U-neurons in the food-acquisition behavior might become silent as a result of the satisfaction of the alcohol need (E+). If alcohol need, at least partly, inhibits other behaviors in alcoholics, its reduction may lead to disinhibition of previously silent food-acquisition L-neurons and their involvement in food-acquisition behavior.

In any case, the increase in the number of L-neurons leads to the transient and partial normalization of the L/M ratio in our ethanol-treated rabbits. Similarly, neurons in the primary somatosensory cortex of ethanol-treated rats demonstrate "normalized" (but not normal) responses to sensory stimulation after acute ethanol treatment (Chapin & Woodward, 1989). This normalizing effect upon the L/M

ratio agrees well with the data on effect of acute ethanol administration on the performance of ethanol-treated animals. While in healthy animals, acute ethanol increases the time of realization of instrumental food-acquisition cycles (Alexandrov et al., 1990b), ethanol-treated rabbits started performing faster in E+.

Our data agree with many previous experiments showing that acute ethanol administration has a normalizing effect upon test performance of alcoholics (Gibbins et al., 1971; Kissin, 1974). A couple of drinks has been shown to improve the creative output of heavy drinking writers, artists, and composers (Ludwig, 1990). On the basis of the present results, we suggest that such an improvement in performance is, at least partly, caused by transient normalization of the L/M ratio.

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