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Acute effects of alcohol on unit activity in the motor cortex of freely moving rabbits: comparison with the limbic cortex

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Unit activity was recorded from the motor cortex of eight freely moving rabbits in order to examine the acute effect of ethanol (1 g kg^{-1}) on organization of unit activity and to compare it with our earlier results from the limbic cortex. The rabbits performed a food-acquisition task in the experimental cage. Unit activity was recorded during behaviour in the control experiment followed by the alcohol experiment on the next day. After ethanol, behavioural mistakes and the duration of the behavioural cycle significantly increased. In the control experiments activation of 58% of the units had no constant relation to the phases of the behavioural cycle (non-involved units), whereas 42% of the units were constantly activated during certain phases (involved units). Two per cent of the latter units were activated in relation to newly learned behavioural acts (e.g. pedal pressing; L units), 28% in relation to food seizure and/or grinding (S units) and 12% in relation to certain movements during different behavioural acts (M units). Ethanol had no effect on the number of active units and the same relation between the number of non-involved and involved units or between the number of different types of involved units was found. However, the number of involved units decreased in the upper and increased in the lower cortical layers. Also the number of units with low background frequency increased, although the frequency within activations did not change. In our earlier study the number of active units in the limbic cortex decreased after ethanol by one third and the relation between the number of L and M units was reversed. Thus, acute effects of ethanol on unit activity in the motor and limbic cortex differ in the number of active units and in their behavioural specialization pattern, both of which change in the limbic, but not in the motor cortex. However, also in the latter the set of involved units changed and manifested as changes in the number of these units in the upper and lower cortical layers. Consequently, the differences between the effects of ethanol on the motor and limbic cortex are not simply quantitative, but ethanol changes the behavioural role of these two cortical areas in qualitatively different ways.

Key words: alcohol, behaviour, limbic cortex, motor cortex, neural unit activity, rabbit

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The cerebral cortex belongs to the 'target site' structures of the ethanol influence on the nervous system (Klemm *et al.* 1976). In this part of the

brain the effects of ethanol on neural activity are found with the minimal dose and delay. On the other hand, limbic structures are known to be important in the development of the alcohol dependence (Kriganovsky & Evseev 1988).

In our previous paper (Alexandrov *et al.* 1990b) we described in freely moving rabbits the acute effect of ethanol on units with different behavioural specialization in the limbic cortex: M units related to the movements of the rabbit and L units related to the newly learned behavioural acts. After ethanol injection (1 g kg^{-1}) the number of active units markedly decreased (by one third) due to a selective depression of the activity of L units; the number of M units did not change. The pronounced change in the activity of the limbic cortex after ethanol may result from the predominance of L units in this area. In contrast, in the anterolateral part of the motor cortex L units form a minority (Alexandrov *et al.* 1990a). Therefore, we recorded unit activity also from this area in the same experimental situation and partly with the same rabbits as above (Alexandrov *et al.* 1990b).

In the present paper we report the data on the acute effect of alcohol on unit activity in the motor cortex and compare the results with those found in the limbic cortex.

MATERIALS AND METHODS

Subjects. The experimental animals were eight experimentally naive male adult rabbits (*Orictolagus cuniculus*; weight 2–3 kg) which were kept in separate cages in the vivarium with a 12-h light-dark cycle. In seven of the rabbits recordings were made also from the limbic cortex (Alexandrov *et al.* 1990b).

Experimental procedure. Freely moving animals were taught to acquire food by pressing one of two pedals in the experimental cage (described in detail by Alexandrov *et al.* 1990a).

After the training, experiments with unit recording (see later) were started. Each rabbit carried out the food acquisition task (behavioural cycle: pressing the pedal and receiving food from the automatic feeder at the same side of the cage) repeatedly at the both sides of the cage, during the first day in the control experiment and during the next day in the alcohol experiment. Five rabbits participated in both experiments twice. In these cases the interval between the alcohol experiments was longer than 65–70 h.

In the alcohol experiments, ethanol was injected intraperitoneally (12% ethanol in isotonic solution) using a dose of 1 g kg^{-1} and thereafter every 1.5–2 h 0.3–0.5 g kg^{-1} ethanol was added until the end of the experiment. Blood alcohol level was determined by gas chromatography. In the control experiments the same amount of isotonic solution was used.

Recording techniques. Electrophysiological and behavioural recording techniques, analysis of unit activity, the criteria for activation of a unit as well as for the classification of the behavioural specialization of the units have been described in detail elsewhere (Alexandrov *et al.* 1990a).

Unit activity was recorded in the control and alcohol experiments from the anterolateral part of the motor cortex (A3.6 $\pm$ 0.7, L3.9. $\pm$ 0.6; part of the jaw motor area). Electrical stimulation of this region resulted in marked lower jaw movements (Sumi 1969, Lund *et al.* 1984). Microelectrodes were driven by a micromanipulator with a scale showing the vertical location of the recording tip with a resolution of 50 μm. The number of units encountered during each penetration was counted.

Unit activity, EMG and actographic marks of the behaviour (see Alexandrov *et al.* 1990a) were tape-recorded. The rabbit's behaviour was simultaneously video-recorded with the unit activity (audio-channel), the light indicators of the pedal pressing and head lowering, and the counters for the cumulative number of spikes and for time.

Behavioural and neural analysis. Both the duration of each behavioural cycle and the number of mistakes in the performance during the control and alcohol experiments were determined and compared (t and X^2 -tests, respectively). As mistakes were regarded: movements from the effective pedal to the uneffective pedal, cessation of the behavioural cycle, checking an empty feeder without an approach to the pedal, approach to the pedal without pressing it, and failure to achieve the aimed pedal pressure.

The units were first divided into two groups: units non-involved (not activated) and involved (activated in constant relation to a certain phase of the repeated behavioural cycle) in the food-acquisition behaviour. The latter group was further divided into three groups with different behavioural specialization (M, S, and L units, Alexandrov *et al.* 1990a, also see Results). The statistical significance of differences between the number of units belonging to the different groups and between the number of units belonging to the same groups in the control and alcohol experiments was estimated by X^2-test (significance limit $P < 0.05$).

Morphological analysis. After the experiments the rabbits were killed with an overdose of nembutal, the brains were fixed in 10% formalin and dehydrated by increasing concentrations of alcohol. Serial frontal slides were cut (thickness 10–20 μm) and every 10th section was stained by Nissl method. In the contralateral hemisphere (symmetrical to the site of the recording) neural structure was analysed with light microscope; also the total thickness of the cortex and the thickness of the II-IV cortical layers (containing

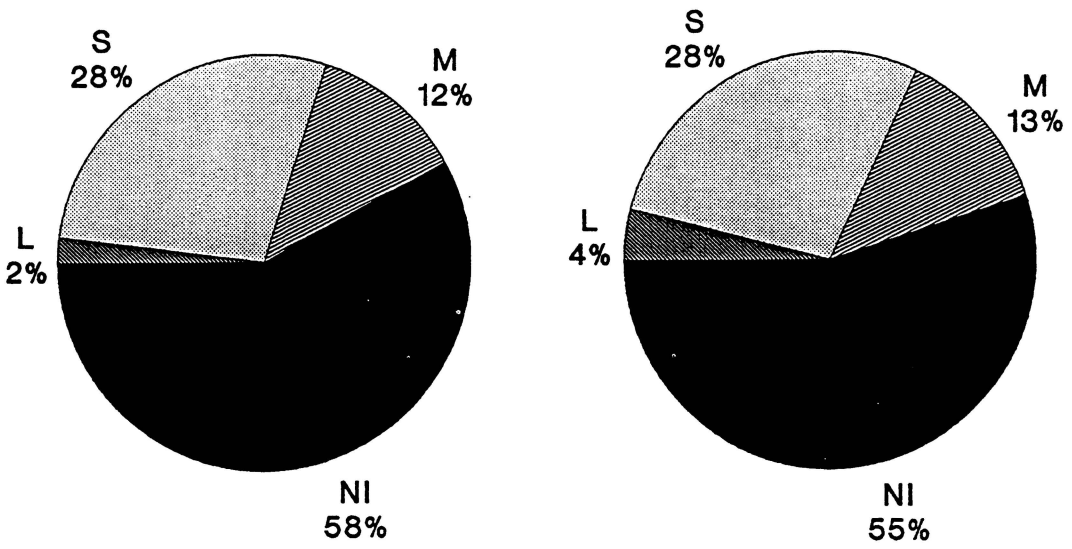

Fig. 1. Relative number of different units (L, S, and M; NI = noninvolved) in Control and Alcohol experiments.

small, densely packed cells; the IV layer was found in our recording site, also cf. Morimoto *et al.* 1985) and V–VI layers (containing large and medium size pyramidal cells) was determined. The locations of the units in the different cortical layers was determined on the basis of micromanipulator readings and this analysis.

RESULTS

The average blood alcohol level of all rabbits reached its maximum (about 0.9 l^{-1}) 15–20 min after the first injection of ethanol and decreased thereafter to a level of approximately 0.4 g l^{-1} in 40–60 min. This level was maintained by additional injections during the alcohol experiments.

The behavioural disorders after ethanol injection did not significantly differ from those found during recordings from the limbic cortex, consisting of an increase in the duration of the behavioural cycle and of an increasing number of mistakes (for details see Alexandrov *et al.* 1989).

Unit recordings were obtained from seven rabbits during food acquisition performance both in the control and alcohol experiments; for one rabbit only the number of active units could be counted during microelectrode penetrations in both experiments.

The average number of units encountered in the control experiments (16.8 ± 4.6/microelectrode penetration) did not statistically differ from that found in the alcohol experiments (16.4 ± 6.2/penetration).

In the control experiments 42% of all units ($n = 291$), recorded during performance of the food-acquisition task, had constant activations related to certain phases of the behavioural cycle (involved units). The rest of the units (58%) were non-involved.

We have previously presented the types of behavioural specialization of the units in the anterolateral motor cortex with examples on their different kinds of activation in relation to the phases of the behavioural cycle (Alexandrov *et al.* 1990a). In the control experiment of the present study the types of behavioural specialization and the quantitative relation between the number of units belonging to these types (pattern of behavioural specialization) did not differ from these earlier findings (see Fig. 1). Only 2% of all recorded units were activated in relation to acts which were formed during learning of the food-

Table 1. Relative number of units in the subgroups of L, S, M and NI (noninvolved) units in the Control and Alcohol experiments. Percentage calculated within each group

Group	Subgroup (description)	Control (%)	Alcohol (%)
L	Pedal/approaching and/or pressing	57	50
	Feeder/approaching	43	50
S	Seizure and grinding, only grinding	66	57
	Seizure of food	34	43
M	Head/body movements:		
	Vertical	44	48
	Horizontal	32	23
	Jaw movement	24	29
NI	Type of discharge during behavior:		
	Single spikes ($\leqslant 1$ imp s^{-1})	41	44
	Irregular bursts	38	38
	Regular continuous discharge	21	18

acquisition task (L-units): approaching and/or pressing the pedal or approaching to the feeder. The activation of the majority of the involved units was related to food seizure and/or grinding of the food (S units; 28% of all units) and to certain movements (e.g. vertical or horizontal movement of the body and/or head, jaw movement) in different behavioural acts (M units; 12% of all units).

In the alcohol experiments 45% of all units ($n = 264$) were involved units and 55% non-involved ones. A similar pattern of behavioural specialization of the units was found as in the control experiments: 4% L, 28% S, and 13% M units (Fig. 1). The number of units belonging to these groups or their subgroups (see also Table 1) did not differ statistically from the control experiments.

There were no differences between the control and alcohol experiments in the 'activations profile' of the units (number of activations at each behavioural phase in per cent of the total number of activations) for the preferred (the behavioural cycle at the side of the cage at which the rabbit made less mistakes, see Alexandrov *et al.* 1990b) or non-preferred behavioural cycle (Fig. 2).

In spite of the similar pattern of behavioural specialization of the units in the control and alcohol experiments differences were found in the frequency of their discharge. In the alcohol experiments the number of involved units with a low (less than 2.5 imp s^{-1}) firing frequency during behavioural phases in which no activations appeared (background frequency) significantly increased in comparison to the control experiments (Fig. 3a). There was no significant depressing effect of alcohol on the frequency of the discharge within the activations. When the discharge frequency within an activation was calculated in relation to the background frequency (activation/background coefficient) it was found that the number of units with a coefficient lower than 5 was significantly smaller in the alcohol experiments than in the control ones (Fig. 3b).

Comparison of the vertical localization of the recorded units and the thickness of the cortical layers revealed that the control and alcohol experiments did not differ in the absolute number of units encountered during microelectrode penetration, determined separately for the upper (II-IV) and the lower (V-VI) layers of the cortex.

In the control experiments 67% of the non-involved and 33% of the involved units were in the upper layers of the cortex. In the lower layers the respective numbers were 56% and 44%. There was no significant difference in the number of units belonging to these groups between the upper and lower layers of the cortex.

In the alcohol experiments 74% of the noninvolved and 26% of the involved units were in the upper layers and 50% of both unit types in the lower levels (Fig. 4) The difference between the upper and lower layers in the number of involved units was statistically significant ($P < 0.001$) indicating an increase in the

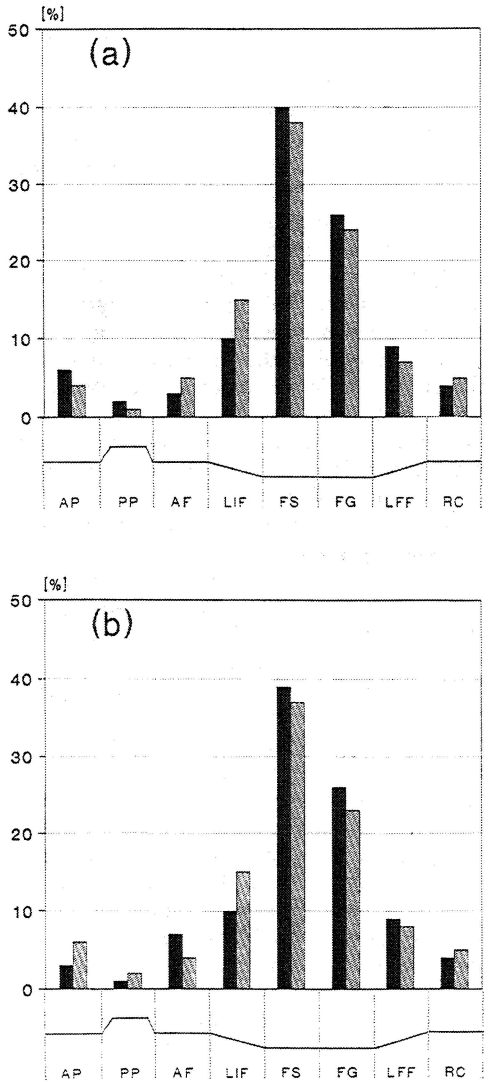

Fig. 2. 'Activation profile' of units during behaviour at the (a) 'preferred' and (b) 'non-preferred' wall. Ordinate: number of activations in per cent from the total number of activations observed in L, S, and M units in Control (black column) and Alcohol (hatched column) experiments. Total number of activations near the preferred and non-preferred wall respectively in Control = 210 and 210 and in Alcohol = 203 and 208. Abscissa shows phases of the behavioural cycle: approach to pedal (AP), pedal pressing (PP), approach to feeder (AF), lowering head into feeder (LIF), food seizure (FS), food grinding (FG), lifting head from feeder (LFF), regular chewing (RC). Actographic recording shown by the horizontal line below the histogram.

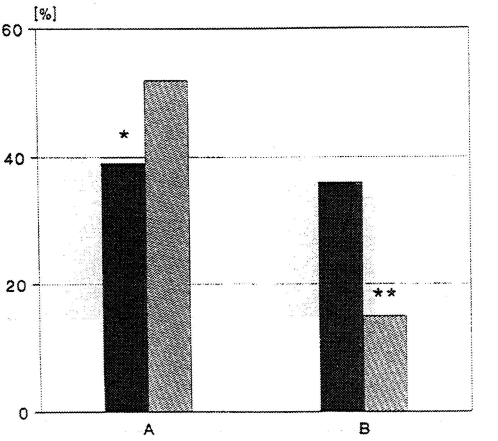

Fig. 3. Background frequency and activation/background coefficient in Control (black column) and Alcohol (hatched column) experiments. Ordinate: number of units per cent from the total number of involved units. A. Number of units with background frequency < 2.5 imp s^{-1}. B. Number of units with activation/background coefficient < 5. $^{*}P < 0.05$, $^{**}P < 0.001$.

number of units subserving food-acquisition behaviour in the lower layers of the cortex. Such a change was true for all groups (L, S, and M) of the involved units separately.

DISCUSSION

In contrast to the present results we found earlier the following acute effects of ethanol on the unit activity of limbic cortex: (1) a decrease in the number of active units by one third because of the depression of the activity of L units; (2) a change in the pattern of behavioural specialization of the units: the relative number of L units decreased to 11% from the control experiment (28%) and the number of M units increased to 34% from 17% (control) and (3) ethanol more markedly decreased the number of L units in the upper (II–IV) layers than in the phylogenetically older lower (V–VI) layers (Alexandrov *et al.* 1990b).

In the motor cortex the number of active units did not change after ethanol. Furthermore, the pattern of behavioural specialization of the units was unchanged. In the limbic cortex not only the number of L units changed, but also the relation between the number of units belonging to the

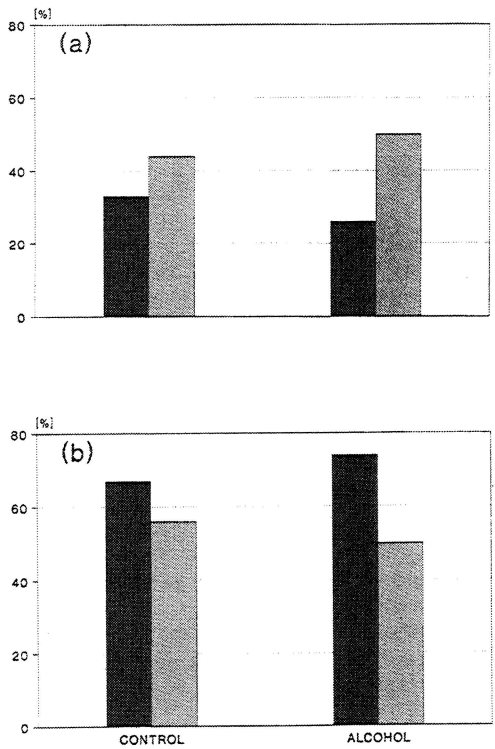

Fig. 4. Relative number of (a) involved and (b) noninvolved units in layers II–IV (black column) and V–VI (hatched column) of the motor cortex in Control and Alcohol experiments. Ordinate: number of units in per cent from the total number of localized units in each layer in corresponding experiment. Total number of localized units in layer II–IV (Control) = 101; (Alcohol) = 77; V–VI (Control) = 162; V–VI (Alcohol) = 153.

different subgroups of L units; in the motor cortex the relation between the number of units of any subgroup stayed constant. The activations profile of the units did not change in the motor cortex, whereas in the limbic cortex there were significant differences between the control and alcohol experiments.

The difference in the sensitivity of the L units to ethanol between the motor and limbic cortex suggests that these units do not form a homogeneous group. At least those properties of L units are different which determine the effect of ethanol. This conclusion is supported by the fact that L units in the different layers of the limbic cortex were differently affected by ethanol.

However, the stable pattern of specialization and activations profile in the motor cortex does not mean that there had not been any change in the role of units involved in the behaviour after ethanol. The relative number of involved units in the upper layers decreased and in the lower ones increased. This tendency existed for all groups of involved units (L, S, and M). As shown by the morphological control, these changes were functional and not related to any neuronal damage. Taking into account that the absolute number of active units did not change from the control to the alcohol experiments in the upper and lower layers of the cortex, the observed change may be due at least to the following reasons: (1) part of the involved units became silent in the upper layers and at the same time part of the silent units became the involved ones in the lower layers (with the opposite direction of change in the number of non-involved units in both layers); (2) part of the involved units changed to the noninvolved ones in the upper layers and in the lower layers the opposite change took place and; (3) there was a combination of these two ways of change. In any case, the results indicate that the same pattern of behavioural specialization of units in the control and alcohol experiments is represented by different sets of units. This is true for units belonging to older systems (M and S units), because their abundance is mainly responsible for the significance of the difference in the number of the involved units between the upper and lower layers in the alcohol experiments, but probably also for L units.

It is noteworthy that a similar increase in the number of involved units in the motor cortex in the lower layers was obtained after the lesion of the visual cortex which contains a lot of L units (Alexandrov *et al.* 1990a). Thus both the damage of a cortical area containing many L units, and the depression of its L units by ethanol seem to have a similar effect of the location of the involved units in the layers of the motor cortex. On the other hand, such a possible decrease in the influence of L units located in other cortical areas upon the motor cortex may also partly explain another finding of the present study: the increase in activation/background coefficient. This conclusion is based on the assumption that activity of such L units are normally at least partly responsible for the background discharge of the units in the motor cortex (see below).

In experiments with immobilized rabbits Kogechkin (1987) observed a differential effect of ethanol on spontaneous and evoked activity: the former was depressed and the latter was not affected. Our finding about the selective depressive influence of ethanol on the background frequency of involved units (but not on activations) is in accordance with this fact. This selective depression may be explained by direct as well as by indirect influences of ethanol. Bezdeneznykh (1980) showed by using ionophoresis that the chemical sensitivity of the spontaneous activity and of the different phases of evoked discharge of cortical units differ. Such a differential chemical sensitivity of the units could form the basis for the direct selective effect of ethanol on the background frequency in the present study. Possible indirect influence may be exerted at least by the following way: Activation of a unit reflects the realization of the system to which the unit belongs. Background activity may be at least partly explained by an interrelation between the given unit and units which belong to other neural systems (Shvyrkov 1990). Thus, the decreasing number of L units in other cortical areas after ethanol may reduce these intersystemic relations and lead to slower background discharge in the motor cortex.

In conclusion, the present results show that the main differences in the influence of ethanol on unit activity in the motor and limbic cortex are in the number of active units and in the pattern of their behavioral specialization, both of which change in the limbic, but not in the motor cortex after ethanol. However, both in the limbic and in the motor cortex the set of involved units changed and in the motor cortex there were also changes in the background discharge. Thus, ethanol influences both cortical areas, but in different ways. Therefore, the behavioural disorders after ethanol could be explained by a combination of these complex influences: A change of the set of units involved in the food-acquisition behaviour, a decrease in the number of L units and a depression of the intersystemic relations.

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