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Effects of alcohol and aging on the cingular (area 24) and frontal (area 6) cortical areas of the mouse

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Summary. We have studied the morphometric changes of the neurons of the cingular area 24 and frontal area 6 of the mouse, produced by age and/or chronic alcohol intake. The parameters analyzed were nuclear area of these cortical neurons and cellular density (cell/neuropil coefficient). We detected a decrease in the number of neurons with age in practically all layers of the control animals. In the animals that chronically ingested the alcoholic solution, we also detected a decrease in the number of neurons with age, but only in layer V of the frontal cortex and in layer VI of the cingular area 24. The comparison between the control and the alcoholic group showed that alcohol intake caused an increase in the nuclear area of the neurons in layer II-III of the frontal cortex at 180 days, while in the cingular cortex the increase in nuclear area of its neurons was significative at 180 days in layer II-III and at 35 and 180 days in layers V and VI. We think that these changes are the expression of the neuronal plasticity in both cortical areas in response to the alcohol exposure.

Key words: Alcohol, Aging, Motor cortex, Cingular cortex, Morphometry, Cellular density, Mouse

Introduction

The cingular cortex in rats can be defined as the portion of the medial surface of the hemisphere that surrounds the callosal sulcus, and is one of the largest compounds of the limbic cortex. The six typical layers of the neocortex are not present in the majority of the cingular areas of the rat. So, in area 24 we cannot define layer IV of the small cells (Vogt and Peters, 1981). The cingular cortex has been related to a variety of functions: affections, attention, memory, object analysis (Mesulam, 1985), emotional expression (Jurgens, 1986), mediator functions of somatic and automatic motor responses, phonation, response to pain, and sexual behavior (Ward, 1948; Foltz and White, 1968; Talairach et al., 1973).

In mice, the frontal cortex is the motor area 6 of Caviness (1975), which functionally corresponds to the functional motor area of the rat (Hall and Lindholm 1974).

Several evidences confirm that alcohol produces cerebral damage; produced by alcohol itself, and by associated malnutrition, vitamin deficiency and even genetic factors (Lieber, 1988; Victor et al., 1989; Martin et al., 1993). Proof that ethanol is a toxin by itself has been obtained from animal experiments in which feeding could be better controlled. The findings suggest that alcohol is neurotoxic in several murine species, occasionally producing lesions that are similar to those seen in humans.

Qualitative and morphometric research in animals have revealed morphological alterations caused by alcohol consumption in several cerebral areas (Tavares and Paula Barbosa, 1982; Andrade et al., 1988; Cadete-Leite et al., 1990).

The alcoholism model used in the present work with experimental animals may be useful in helping to understand cerebral effects of chronic alcohol consumption, under conditions in which the amount of alcohol and food intake is controlled. Furthermore, the application of this experimental model to a nervous structure, which governs motor action and other superior functions such as memory and attention, can help us to better understand the actions of alcohol on the central nervous system. So, the aim of the present study was to study possible morphological changes in neurons of the motor and cingular areas of mice subjected to chronic alcohol intake, in relation with age.

Materials and methods

A total of 30 Swiss albino mice were used in this study. They were divided into two groups: a control group of 15 animals (5 animals for each age group studied), and an experimental group also comprising 15 mice (5 animals per age group). The animals were sacrificed at 35, 180 and 365 days in both control and alcoholic groups. Food and water were given ad libitum

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to both groups of animals, but in the alcoholic group, alcohol was added to the drinking water at a concentration of 20% from the first day of life. During the early days of life, pups received the alcohol through theirs mother's milk. After weaning, they received alcohol by direct intake from the drinking water.

Animals were weighed and their motor activity measured before sacrifice. To determine the motor activity, we used a gyroscope (Apelab) that counted the number of half-revolutions in 15 minutes. Activity was always measured at the same hour of the day.

Mice were intraperitoneally anaesthetized using chloral hydrate (200 mg/kg bw), and we perfused saline and Bouin's fluid into the heart's left ventricle to fix the animal. Brains were removed and postfixed for a further 24 hours in the same solution, dehydrated and embedded in paraffin under standard conditions. The volume and weight of the brain were calculated before and after dehydration to determine tissue shrinkage. Coronal sections, 10 micrometers thick, were performed and we studied the cingular cortical area 24 and frontal motor area 6 in their layers II-III, V and VI. We used an Image Analysis System (Magiscan Joyce Loebl) and the Genias semiautomatic Program to determine nuclear area and cellular density index in these cortical areas. The cellular density index expresses the relation of the number of cellular elements contained in the visual field with respect to the neuropil. A two-way ANOVA and the Bonferroni test were used for the statistical evaluation of the data obtained from the morphometric study and from the determination of weight and motor activity.

Results

Body weight

Body weight increased progressively and significantly with age in both groups, but the control group showed significantly higher weight than experimental animals at 35,180 and 365 days ($p<0.01$).

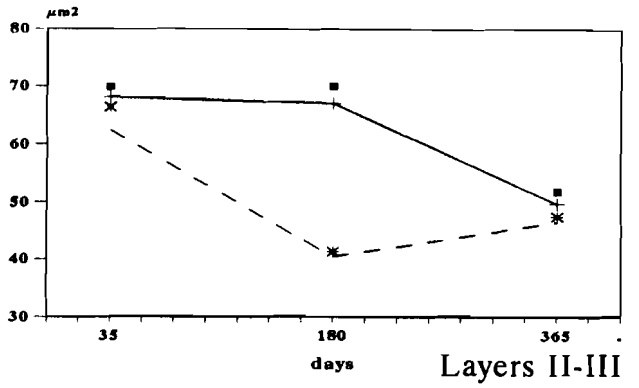
Motor activity

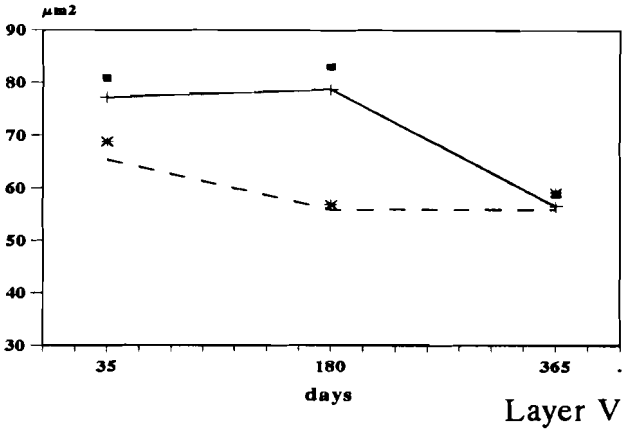
No significant differences were detected between groups and ages.

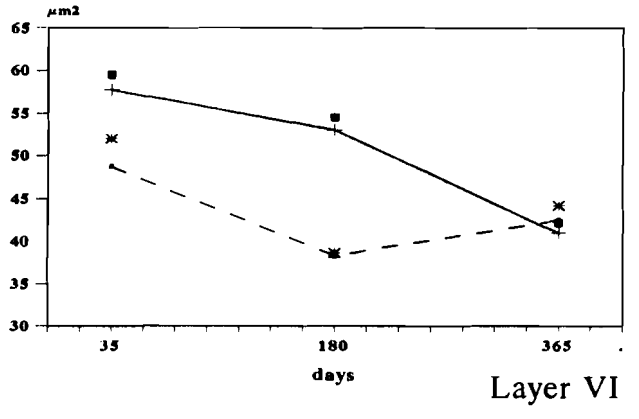
Quantitative study

At the time of sacrifice the brain volume and weight of the alcoholic animals did not show significant differences with the control animals. After dehydration, the alcoholic group showed a bigger shrinkage than the control group (18% at 35 and 365 days, and 35% at 180 days). The absolute morphometric values of the alcohol treated mice were corrected according to the greater

Fig. 1. a. Right hemisphere mouse cortex: cortical area localization. **b.** Frontal view of the cingular cortex (area 24). **c.** Frontal view of the frontal cortex (area 6). Bars: a, 300 μm; b and c, 180 μm.







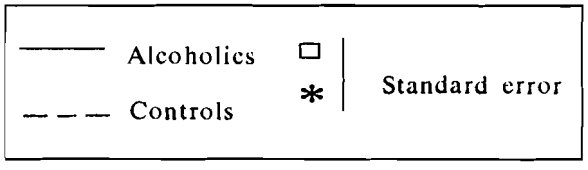
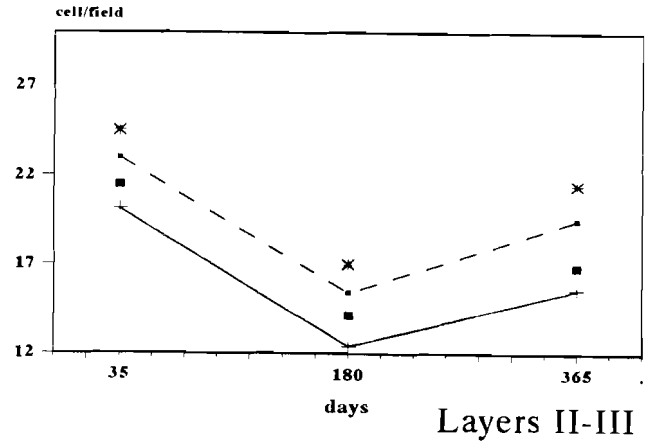
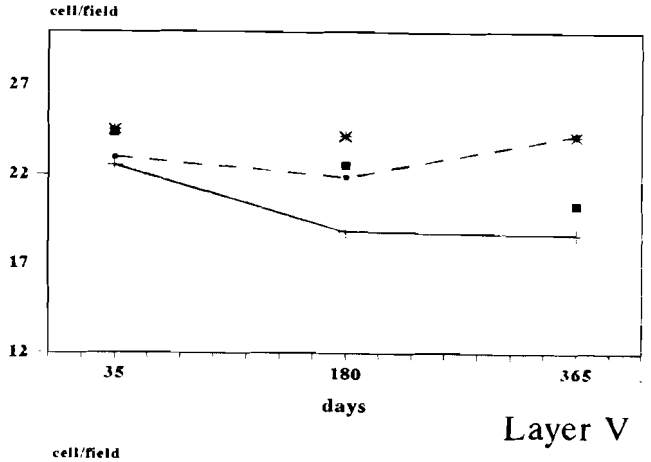
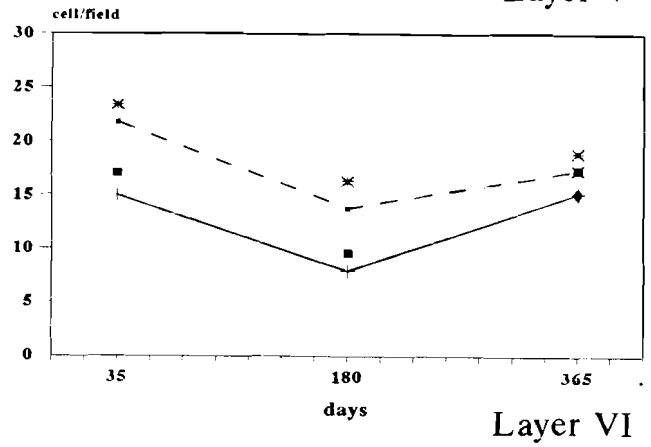


Fig. 2. Nuclear area of the cingular cortex in the different control and experimental groups of mice.







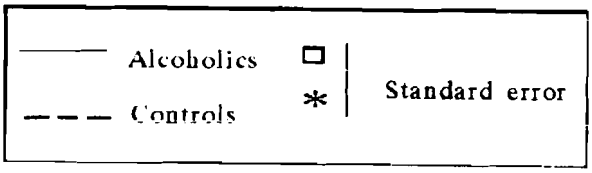


Fig. 3. Cellular density of the cingular cortex in the different control and experimental groups of mice.

brain shrinkage, in accordance with the method described by Uylings et al. (1986).

Area 24 (Fig. 1a,b)

Nuclear area (Fig. 2)

In the control group, layers II-III and VI showed a significant decrease of neuronal nuclear area from the 35th to the 180th day ($p<0.01$).

In the alcohol-treated animals, nuclear area diminished with age in all layers (II-III, V and VI) between the 35th and 365th day ($p<0.01$).

Values of nuclear area seemed to be higher in all layers of the experimental group compared to values of control animals. The difference was significant at 180 days in layers II-III ($p<0.01$) and 35 and 180 days in layers V and VI ($p<0.01$).

Cellular density (Fig. 3)

Cellular density diminished with age in the control group. The reduction was significant in layers II-III and VI between 35 and 180 days ($p<0.01$), while in layer V it was significant between 35 and 365 days ($p<0.01$).

In the experimental group, cellular density also diminished with age but the difference was only significant between the 35th and 180th day in layers II-III and VI ($p<0.01$). In layer VI the cellular density increased again at the 365th day, compared to the 180th day ($p<0.01$).

Comparing cellular density of both groups of animals, alcohol-treated mice showed lower values than control mice at the 35th day, but only in layer VI ($p<0.05$).

Area 6 (Fig. 1a,c)

Nuclear area (Fig. 4)

Nuclear area showed only a significant reduction in layer II-III of the control group between the 35th and 180th day ($p<0.01$).

In the alcohol-treated animals, nuclear area decreased in all layers between the 35th and the 365th day ($p<0.01$ in layer II-III and V; $p<0.05$ in layer VI).

The comparison between both groups showed only a significantly higher nuclear area size in experimental animals in layer II-III at the 180th day ($p<0.01$).

Cellular density (Fig. 5)

In the control animals, cellular density decreased with age from the 35th to the 365th day ($p<0.01$ in layers II-III and V, and $p<0.05$ in layer VI).

In the alcohol-treated mice, cellular density decreased from the 35th to the 180th day in all layers ($p<0.05$ in layer II-III and VI; $p<0.01$ in layer V).

Although the alcoholic group tended to show lower values of cellular density than control animals in all layers, the difference was only statistically significant in layer V at the 35th and 180th day ($p<0.01$).

Discussion

We have detected lower values of body weight in the alcoholic group, and this could be expression of a general metabolic effect of alcohol ingestion (Thomson et al., 1983; Victor et al., 1989), but could also be related to the reported effects of alcohol intake on area postrema and dorsal vagal complex, as these structures are related to the parasympathetic system and regulation of body weight (Castañeyra Perdomo et al., 1991; Bañuelos Pineda et al., 1995). Another factor to be considered is the decalcifying effect on bone metabolism caused by alcohol (Carlsson et al., 1974).

It is clear that considerable and rapid changes can take place in the dendritic arborisation of neurons of the Central Nervous System, as an expression of neuronal plasticity (Berry, 1985). These changes are usually associated with alterations in neuronal body size (Harper et al., 1987). Studies in animals (Tavares et al., 1983) and humans (Ferrer et al., 1984) have revealed changes in the dendritic arborisation of cerebellar Purkinje cells after prolonged ethanol consumption.

It has been reported that prolonged administration of alcohol to well-fed mice can cause memory deficits (Arendt et al., 1988). In our study the cingular cortex, one of the brain structures that has been implicated in memory and attention, shows higher values of the nuclear area of its neurons at the 35th and 180th day, in all layers of the alcohol-treated mice, compared to the values of the control group. This agrees with the above reported alterations of memory. The changes that we detect in neuronal nuclear sizes following chronic exposure to alcohol, could then be a manifestation of affection of neuronal plasticity. We found that ethanol caused an increase in the nuclear surface in the cingular cortex at 180 days in layers II-III and at 35 and 180 days in layers V and VI, while in the frontal cortex the increase was only seen at 180 days in layers II-III. Thus, the changes occurring in the nuclear area of the neurons might be due to mechanisms for adaptation to alcohol toxicity and to the reduction in the number of dendritic ramifications (Cragg and Phillips, 1983). Another possible factor to be considered in these changes is a direct susceptibility of the cells to alcohol, which has been reported for layers V and VI of the enthorrinal cortex (Ibañez et al., 1992) and motor area 6 of mice (Ferres Torres et al., 1985).

On the other hand, it has also been reported that the hippocampus, a structure that plays a crucial role in memory processes (Olton et al., 1979), is affected by prolonged alcohol consumption (Riley and Walker, 1978; Walker et al., 1980). As the cerebellum is connected to the frontal cortex, and the hippocampus to the cingular cortex, the effects that we describe here could also be someway related to these connections as

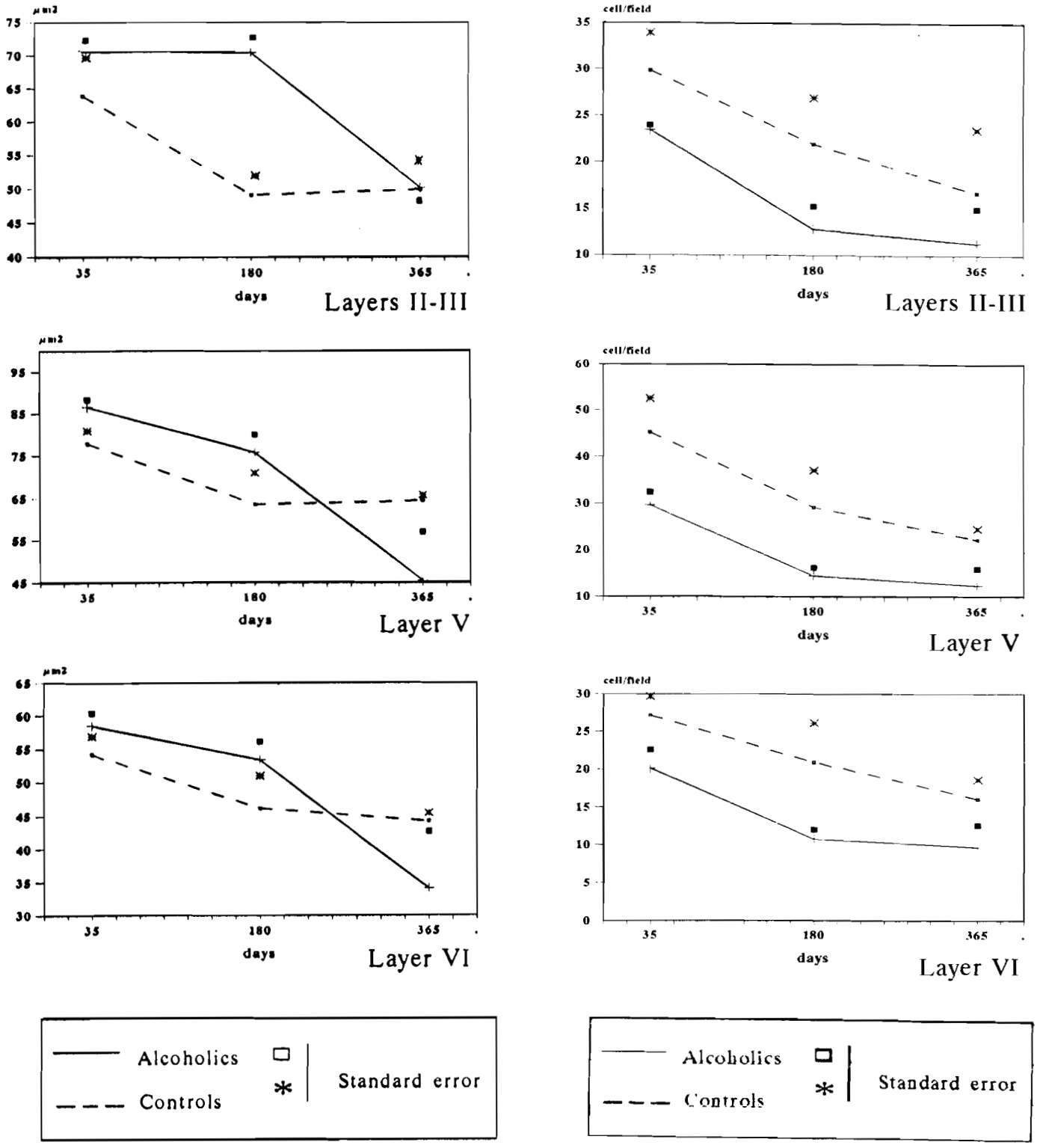


Fig. 4. Nuclear area of the frontal cortex in the different control and experimental groups of mice.

Fig. 5. Cellular density of the frontal cortex in the different control and experimental groups of mice.

multiple alcohol-triggered alterations have been described for both the cerebellum (Chu, 1983) and the hippocampus (Grupp, 1980; Mancillas et al., 1986).

Anderson et al. (1983) have reported variations in the number of neurons during aging. This agrees with our results here, which show a decrease in the cellular density with age in practically all layers of both motor and cingular areas in the control and experimental groups.

Although several authors have failed to find statistical differences in the number of neurons between alcoholic and control humans (Harper et al., 1987; Krill and Harper, 1989), we have found here that cellular density is lower in the alcohol-treated mice in layer VI of the cingular cortex at 35 days and in layer V of the motor cortex at 35 and 180 days, suggesting a decrease in the number of neurons, as the nuclear area of the neurons at these same ages tends to be even higher than in the control animals in the above mentioned layers of both cortexes.

We can conclude that aging produces a decrease in the number of neurons and a decrease in nuclear area sizes in both studied cortical areas and in both control and alcohol-treated mice. Additionally, chronic alcohol ingestion in mice produces, in both cingular area 24 and frontal motor area 6, a reduction in the number of neurons and an increase in nuclear area size at 35 and 180 days in comparation with the values in control animals. This could be related to a functional compensation mechanism, the increased nuclear size being a reflection of greater cellular activity (Hildebrand, 1980; Srebro et al., 1988). However, this functional compensation mechanism begins to fail with age and/or alcohol exposure time: after one year of alcohol treatment, the nuclear area size of the experimental group tends to fall, becoming even lower than that of the control group. Both motor and cingular areas seem to be morphologically sensible to the effects of aging and chronic alcohol exposure, which could explain some of the memory and motor dysfunctions produced by both factors.

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