INTRODUCTION

Chronic ethanol exposure causes overactivity of noradrenergic neurons in the central and the peripheral nervous system (Pohorecky, 1974; Ahtee and Svartström-Fraser, 1975; Sjöquist et al., 1983; Jaatinen and Hervonen, 1994). The prolonged noradrenergic overactivity may contribute to the morpho-functional degeneration of the peripheral sympathetic nervous system, which has been reported in chronic alcoholics (Novak and Victor, 1974; Eisenhofer et al., 1985) and experimental animals chronically exposed to ethanol (Jaatinen et al., 1993; Jaatinen and Hervonen, 1994).

The overactivity of the noradrenergic nervous system is a central mechanism in the pathophysiology of the ethanol-withdrawal syndrome (Pohorecky, 1974; Hawley et al., 1985; Linnoila, 1987; Nutt and Glue, 1990), as reflected by the typical ethanol-withdrawal symptoms, such as anxiety, tremor, hypertension, and tachycardia. There is some evidence that ageing may increase the severity of the withdrawal reaction during repeated withdrawal episodes (Maier and Pohorecky, 1989). Ethanol metabolism has been shown to decrease with advancing age (Hahn and Burch, 1983; Sancho-Tello et al., 1988) and aged animals may be more sensitive to the acute effects of ethanol (Ott et al., 1985).

The locus coeruleus (LC) is the principal source of noradrenergic projections to most areas of the brain and it is one of the most widely projecting nuclei in the central nervous system (CNS) (Foote et al., 1990).
et al., 1983; Loughlin and Fallon, 1985). The LC has been suggested to be an important centre for maintaining homeostasis within the CNS and it has been linked with a variety of physiological regulatory processes, including regulation of sleep and wakefulness, attention, orientation, learning and memory, stress, nociception, and autonomic and endocrine functions (Aston-Jones et al., 1991).

Alterations in LC neuronal number and morphology have been reported in certain neurodegenerative diseases, such as Parkinson’s disease and Alzheimer’s disease (Gaspar and Gray, 1984; Chan-Palay and Asan, 1989; Chan-Palay, 1991; Mavridis et al., 1991). Both ageing and chronic alcohol consumption have been shown to impair noradrenergic function in the CNS (Prahan, 1980; Hoffman and Tabakoff, 1985). The results of previous studies on ageing and alcohol-induced neurodegeneration have been rather contradictory. Some studies have shown a reduced number of neurons in the LC after chronic alcohol consumption (Mayes et al., 1988; Arango et al., 1994; Lu et al., 1997) and age-related neuronal loss in the LC has also been reported (Vijayashankar and Brody, 1979; Mann, 1983; Yoshinaga, 1986). Other studies have found no age- or ethanol-induced neuronal loss in the LC (Goldman and Coleman, 1980; Halliday et al., 1992; Baker et al., 1994; Mouton et al., 1994).

Ethanol withdrawal may have an important role in the generation of ethanol-induced neuro-pathology. Previous studies have shown that intermittent ethanol exposure with repeated withdrawal periods may be more harmful to neurons than continuous exposure (Lundqvist et al., 1995; Lundqvist, 1997; Jaatinen et al., 1997). This study was designed to investigate the effects of ageing and a 5-week intermittent ethanol exposure on LC morphology and on the severity of the ethanol-withdrawal syndrome.

MATERIALS AND METHODS

Animals and treatment

Forty-eight male Wistar rats, both young (3–4 months) and old (29–30 months), were used in this study. The rats were divided into six groups: (1) a non-treated control group of young rats (young control, n = 6); (2) a sucrose-fed control group of young rats (young sucrose, n = 7); (3) an ethanol-fed group of young rats (young EtOH, n = 11); (4) a non-treated control group of old rats (old control, n = 6); (5) a sucrose-fed control group of old rats (old sucrose, n = 7) and (6) an ethanol-fed group of old rats (old EtOH, n = 11). Rats were housed individually under standard conditions (22 ± 1°C, 13 h light/11 h dark cycle with lights on between 08:00 and 21:00). The animals were carefully habituated to handling before the experiment was started. This study was a part of a larger experiment, where the effects of dexmedetomidine on ethanol-induced withdrawal symptoms and neurodegeneration were also studied (Riihioja et al., 1999).

The young and old EtOH groups were given intragastric feeds of a solution containing 25% ethanol (Spiritus fortis, diluted in distilled water) and 5% (w/v) sucrose three times a day for 4 days. After every intoxication period, there was a 3-day ethanol-withdrawal period. The 4-day ethanol exposure period and 3-day withdrawal period were repeated five times. Immediately before each ethanol feeding, the severity of intoxication of each rat was evaluated using a standardized, seven-level intoxication scale (Hemmingson et al., 1984; Clemmesen et al., 1988; Riihioja et al., 1997a,b). The dose of ethanol was adjusted individually according to the level of intoxication. The aim was to keep the animals on intoxication level 3 (clearly impaired walking, impaired elevation of abdomen and pelvis) or 4 (slowed righting reflex, no elevation of abdomen and pelvis). The sucrose-fed control animals were given intragastric feeds of isocaloric sucrose correspondingly three times a day. The non-treated control animals were given food ad libitum, and the sucrose-fed control animals were given food according to the consumption of the ethanol-treated groups.

There was no difference between the initial weights of the different treatment groups of the young animals (mean ± SD) (339 ± 28 g in the young control group, 333 ± 19 g in the young sucrose group, 334 ± 29 g in the young EtOH group), or the old animals (515 ± 42 g in the old control group, 513 ± 48 g in the old sucrose group, 511 ± 39 g in the old EtOH group).

Blood-ethanol concentrations

Blood-ethanol concentration (BEC) was determined by gas chromatography (Eriksson, 1973).
from a capillary blood sample (0.05 ml), which was taken from the tip of the tail of the ethanol-exposed rats during the last 2 days of the fourth week of ethanol exposure. The blood samples were taken before (BEC-0) and 1 h after (BEC-1h) the morning dose of ethanol. For technical reasons, the ethanol samples were taken on two consecutive days. On both days, equal numbers of animals of both age groups were handled. When the blood samples were taken from the animals, the intoxication levels of the animals were also measured. The blood samples were taken only once to avoid additional stress to the animals.

The BEC/intoxication score ratio [BEC-1h/ intoxication score (0–6) at the time of blood sampling] was used to measure the sensitivity of the animals to the intoxicating effects of ethanol. The development of ethanol tolerance was estimated by calculating the intoxication score/ cumulative ethanol dose ratios [weekly sum score of the intoxication ratings (points)/weekly ethanol dose (g/kg)].

Measurement of the ethanol-withdrawal symptoms

The withdrawal symptoms were rated every week 11, 14, 18, 22, 26, 36, 40, 48, and 62 h following ethanol withdrawal. The onset of the withdrawal rating was timed according to previous studies, where the first withdrawal symptoms were seen 8–11 h after the last dose of ethanol (Riihioja et al., 1997a,b). The withdrawal reaction was assessed using a standardized four-item scale, as described in detail previously (Hemmingsen et al., 1984; Clemmesen et al., 1988; Riihioja et al., 1997a,b). The four items used (rigidity, tremor, irritability, and hypoactivity) have been shown to be the most specific markers of ethanol-withdrawal reaction in the rat (Clemmesen et al., 1987, 1988). Each of the four items was rated 0–3, and the sum score of the four items over the withdrawal period was used in the statistical analysis. Furthermore, the animals were observed for possible spontaneous motor seizures.

Tissue preparation and histological procedures

The rats were killed by decapitation during deep sodium pentobarbital anaesthesia (60 mg/kg, i.p.). After decapitation, the brains were removed quickly. The cerebellar peduncles were transected and the brainstem was separated by a transverse cut rostral to the inferior colliculi. The brainstem was further cut in two mid-sagittally. The tissue was fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS: pH 7.4) at 4°C for 24 h and then incubated in 10, 20, and 30% sucrose in PBS to avoid cryodamage. The right side of the LC of each brainstem was used for morphometric analysis. The brainstem was sectioned sagittally at 8 μm in a cryostat. Six serial sections were collected on one slide for cresyl violet staining and the next two sections on another slide for immunohistochemistry. A total of eight to 12 series of eight sections were obtained from each rat. The noradrenergic nature of the neurons was ensured by immunohistochemical demonstration of tyrosine hydroxylase (TH) by the avidin–biotin–peroxidase procedure (ABC reaction) (Hsu et al., 1981).

Locus coeruleus quantitative analyses

The stereological study was performed using an Olympus Vanox-t microscope (Olympus Inc., Tokyo, Japan), a Hamamoto ARGUS-10 image processor and a video monitor. The morphometric analysis was blind for age and treatment. From the Nissl-stained (cresyl violet) sections, the first and the last sections containing LC proper neurons were identified. The reference section on the first slide was chosen at random and the next section was the look-up section. The volume of the LC proper (V_{(ref)}) was estimated by using the point counting method and the Cavalieri principle (Gundersen and Jensen, 1987). The volume of the LC proper was obtained from the following equation: $V_{(ref)} (\text{mm}^3) = \text{total number of grid points hitting the LC proper} \times \text{the area associated with each point corrected for magnification (0.0088 mm}^2) \times \text{section thickness (0.008 mm)} \times \text{the number of sections in each series (eight sections)}$.

The numerical density of LC neurons ($N_v$) was estimated by the physical disector method (Sterio, 1984; Gundersen et al., 1988; West, 1994) on the Nissl-stained sections. The numerical density was calculated as follows: $N_v (\text{cells/mm}^3) = \text{neurons counted/total number of dissectors counted} \times \text{volume of the disector (0.0025 mm}^2 \times 0.008 \text{ mm})$. The total number of LC neurons was calculated as follows: total number of neurons $= V_{(ref)} \times N_v$.

Statistical methods

The data are expressed as means ± SD (SEM in Figs 4–6). Standard two-way ANOVA (three treatment categories and two age categories) was
used to study the overall effects of age and the treatments on the LC morphology. The group differences were further studied by Bonferroni-corrected \( t \)-tests. The post-hoc comparisons were performed between the young and old controls (age effect), between the control and sucrose groups for each age (sucrose treatment effect), and between the sucrose and EtOH groups for each age (ethanol treatment effect). If only ethanol-treated rats were studied, one-way ANOVA was used. Age differences in the vulnerability to ethanol-induced neurodegeneration were analysed by ANCOVA with ethanol dose (g/kg), intoxication score, and withdrawal symptom score as a covariate. ANOVA for repeated measurements was used to analyse the differences between the age groups in total weekly ethanol doses (g/kg), the intoxication levels, the intoxication score/ethanol dose ratios and ethanol-withdrawal symptoms. ANCOVA was used to analyse differences between the age groups in the severity of ethanol-withdrawal symptoms with ethanol dose and intoxication score as covariates. ANCOVA for repeated measurements was used to analyse the differences between the age groups in the BECs with the day of blood sampling as a covariate. A probability of less than 0.05 was considered to indicate a significant difference between group means (\( P < 0.01 \) when five pairs of means were compared).

**RESULTS**

The weekly course of the body weight (g) and food intake (g) in each treatment group is shown in Table 1. During the 5-week ethanol exposure period, the weight of animals in the ethanol-treated groups of both ages decreased (–30 ± 20 g in the young EtOH group, –84 ± 20 g in the old EtOH group) (means ± SD). In the control groups of both ages, the weight of the animals increased (+63 ± 8 g in the young control group, +6 ± 20 g in the old control group). In the young sucrose group, the weight of the animals slightly increased (+5 ± 22 g), but in the old sucrose group, the weight of the animals decreased (–46 ± 16 g).

**Blood-ethanol concentration**

The BECs on the 2 days of blood sampling are shown in Table 2. ANCOVA for repeated measurements revealed no difference between the young and old animals in the BECs (\( P = 0.473 \) for age effect). There was a difference in BECs between the two consecutive days (\( P = 0.000 \) for the covariate, i.e. the day of blood sampling). The difference in BECs between the days was due to the different intoxication levels of the animals and thus different ethanol doses given to the animals on each day.

One-way ANOVA revealed no difference between the age groups in the BEC-0/intoxication score ratio (\( P = 0.948 \)) or in the BEC-1h/intoxication ratio (\( P = 0.948 \)).

**Table 1. Course of body weight and food intake during the 5-week intermittent ethanol treatment**

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young control (n = 6)</td>
<td>339 ± 28</td>
<td>343 ± 46</td>
<td>373 ± 25</td>
<td>394 ± 24</td>
<td>402 ± 23</td>
</tr>
<tr>
<td>Old control (n = 6)</td>
<td>515 ± 42</td>
<td>520 ± 41</td>
<td>522 ± 42</td>
<td>531 ± 39</td>
<td>521 ± 34</td>
</tr>
<tr>
<td>Young sucrose (n = 7)</td>
<td>339 ± 20</td>
<td>328 ± 21</td>
<td>331 ± 23</td>
<td>338 ± 23</td>
<td>345 ± 23</td>
</tr>
<tr>
<td>Old sucrose (n = 7)</td>
<td>516 ± 41</td>
<td>490 ± 50</td>
<td>482 ± 51</td>
<td>477 ± 51</td>
<td>473 ± 50</td>
</tr>
<tr>
<td>Young EtOH (n = 11)</td>
<td>332 ± 29</td>
<td>315 ± 24</td>
<td>313 ± 23</td>
<td>314 ± 21</td>
<td>317 ± 17</td>
</tr>
<tr>
<td>Old EtOH (n = 9)</td>
<td>499 ± 35</td>
<td>478 ± 39</td>
<td>466 ± 37</td>
<td>456 ± 36</td>
<td>446 ± 32</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Food intake (g)</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young control (n = 6)</td>
<td>160 ± 0</td>
<td>140 ± 0</td>
<td>140 ± 0</td>
<td>138 ± 4</td>
<td>129 ± 13</td>
</tr>
<tr>
<td>Old control (n = 6)</td>
<td>157 ± 5</td>
<td>140 ± 0</td>
<td>130 ± 15</td>
<td>137 ± 7</td>
<td>134 ± 9</td>
</tr>
<tr>
<td>Young sucrose (n = 7)</td>
<td>113 ± 11</td>
<td>50 ± 15</td>
<td>67 ± 15</td>
<td>72 ± 20</td>
<td>72 ± 19</td>
</tr>
<tr>
<td>Old sucrose (n = 7)</td>
<td>83 ± 23</td>
<td>54 ± 16</td>
<td>57 ± 12</td>
<td>62 ± 10</td>
<td>58 ± 16</td>
</tr>
<tr>
<td>Young EtOH (n = 11)</td>
<td>101 ± 18</td>
<td>56 ± 23</td>
<td>71 ± 19</td>
<td>74 ± 16</td>
<td>68 ± 15</td>
</tr>
<tr>
<td>Old EtOH (n = 9)</td>
<td>80 ± 16</td>
<td>62 ± 21</td>
<td>65 ± 13</td>
<td>76 ± 14</td>
<td>73 ± 17</td>
</tr>
</tbody>
</table>

Values are means ± SD. Numbers of rats (n) are indicated in parentheses.
score ratio \( (P = 0.173) \). Pearson’s analysis of correlation showed a positive correlation between BEC-1h and the intoxication score in the young animals \( (r = 0.65, P = 0.044) \) and the old animals \( (r = 0.92, P = 0.001) \). One rat from each age group was excluded from the analysis, because they were not able to walk in spite of very low BECs, i.e., their intoxication levels could not be estimated reliably.

ANOVA for repeated measurements revealed that both age and time had an effect on the intoxication score/ethanol dose ratio, and the development of tolerance was different in each age group (see Fig. 1).

The course of the ethanol exposure and the withdrawal reaction

Two rats died during the 5-week ethanol exposure period, both from the group of ethanol-fed old rats. In both cases, no gross pathology was found in the autopsy and thus the apparent cause of death was ethanol intoxication. Thus, 20 rats were used for the statistical analysis of ethanol-withdrawal symptoms, 11 rats in the young EtOH group and nine rats in the old EtOH group.

The course of the total ethanol doses (g/kg) and the intoxication levels (points) during the 5-week ethanol exposure period is shown in Table 2. ANOVA for repeated measurements showed a statistically significant difference between the young EtOH group and the old EtOH group in the total dose of ethanol during the 5-week period, and the dose of ethanol increased with time during the experiment \( (P = 0.000 \text{ for group effect, } P = 0.000 \text{ for time effect, } P = 0.582 \text{ for interaction}) \). Despite the smaller ethanol doses/kg body wt in the old EtOH group, intoxication level in the old animals was higher than in the young animals \( (P = 0.039 \text{ for group effect}) \). The intoxication level decreased with time during the 5-week period \( (P = 0.000 \text{ for time effect, } P = 0.042 \text{ for group } \times \text{ time interaction}) \).

The course of the ethanol-withdrawal symptoms in the young and old EtOH groups during the 5-week period is shown in Fig. 2. ANOVA for repeated measurements showed no statistically significant difference between the age groups in the severity of ethanol-withdrawal symptoms \( (P = 0.126 \text{ for group effect}) \). The severity of the symptoms increased with time during the experiment \( (P = 0.000 \text{ for time effect, } P = 0.155 \text{ for group } \times \text{ time interaction}) \). None of the rats exhibited epileptic seizures.

ANCOVA revealed no statistical difference between the young and old rats in the severity of the ethanol-withdrawal syndrome, when the total dose of ethanol \( (P = 0.275 \text{ for group difference}) \), or the intoxication level \( (P = 0.989 \text{ for group difference}) \) was used as a covariate. However, the level of intoxication had an influence on the severity of the ethanol-withdrawal symptoms \( (P = 0.023 \text{ for the covariate}) \).
Qualitative observations

Virtually all the LC neurons were TH-positive. The LC neurons of the ethanol-exposed rats were smaller than in the control rats and the typical rostro-caudal orientation of the neurons (Czranna and Molliver, 1980; Lu et al., 1997) was disturbed (Fig. 3). These changes were more obvious in the old ethanol-exposed rats, but were also seen in the young EtOH rats.

Locus coeruleus morphology

The LC volumes and LC neuronal densities are shown in Figs 4 and 5 respectively. Treatment, contrary to ageing, had an effect on the volume of the LC ($P = 0.969$ for age effect, $P = 0.005$ for treatment effect, $P = 0.415$ for interaction) (Fig. 4). The post-hoc comparisons showed no statistically significant differences between the groups in LC volume.

Both ageing and the treatments had an effect on LC neuronal density ($P = 0.026$ for age effect, $P = 0.000$ for treatment effect, $P = 0.096$ for interaction) (Fig. 5). The neuronal density was decreased in the old EtOH group compared with the old sucrose group, while there was no significant difference between the young EtOH and the young sucrose groups.

Both age and the treatments had an effect on the number of LC neurons ($P = 0.021$ for age effect, $P = 0.000$ for treatment effect, $P = 0.314$ for interaction) (Fig. 6). Post-hoc comparisons showed that the total number of LC neurons was decreased in the old EtOH group compared with the old sucrose group, but there was only a tendency towards a reduced LC neuronal number in the young EtOH group compared with the young sucrose group (uncorrected $P = 0.040$). There was also a tendency towards a reduced neuronal number in the old sucrose group compared with the old control group (uncorrected $P = 0.054$). ANCOVA revealed no age differences in the sensitivity to ethanol-induced neurodegeneration when ethanol dose (g/kg) ($P = 0.992$ for group difference), intoxication ($P = 0.883$) and withdrawal symptoms ($P = 0.792$) were used as covariates.

DISCUSSION

The results of this study showed that a 5-week intermittent ethanol exposure reduced the LC total neuronal numbers and LC neuronal density in old animals, compared with the age-matched, sucrose-fed control animals. Previous studies have
produced somewhat contradictory results on the effects of chronic ethanol exposure in the LC. Lifelong ethanol consumption has been shown to reduce the total neuronal number of the LC of alcohol-preferring AA (Alko, Alcohol) rats by 26–30% and alcohol non-preferring ANA (Alko, Non-Alcohol) rats by 30% only in females (Lu et al., 1997; Rintala et al., 1998). In human alcoholics, no significant loss of TH-positive neurons in the LC was seen compared with age-matched controls.

Fig. 3. Sagittal sections from the locus coeruleus (LC) of an old control rat (A), an old sucrose-fed rat (B), and an old ethanol-exposed rat (C).

(D), (E), and (F) represent details of the same sections at a higher magnification. In the ethanol-exposed rat, the LC neurons were smaller and the rostro-caudal orientation of the neuronal somata was slightly disturbed compared to the control rat. Bar = 50 μm throughout.
Another study found no difference in the number of LC neurons between chronic alcoholics with Wernicke’s encephalopathy and age-matched controls (Halliday et al., 1992). Between 30 to 70 years of age, alcoholics have been shown to have 12–51% fewer neurons than controls of the same age (Arango et al., 1993, 1994, 1996). Possible explanations for the discrepancy between the different studies may be differences in age, sex or ethanol-induced organ complications between the groups studied, but also methodological differences.

Thus far, there is no general agreement about the effect of ageing on LC neuronal numbers. Many previous studies have suggested that ageing causes degeneration of neurons in the LC (Vijayashankar and Brody, 1979; Mann, 1983; Yoshinaga, 1986; Chan-Palay and Asan, 1989, 1994). A significant neuronal loss has been documented in the human LC after 60 years of age; the extent of LC neuronal loss reported has varied from 27–37% to 40–50% (Vijayashankar and Brody, 1979; Marcyniuk et al., 1989; Chan-Palay and Asan, 1989). However, in a recent study (Mouton et al., 1994), no age-related neuronal loss was found in human LC up to 72 years of age. No age-related loss of LC neurons was found in Fischer 344 rats up to 30 months of age (Goldman and Coleman, 1980), or in alcohol-prefering AA rats up to 24 months of age (Lu et al., 1997; Rintala et al., 1998). The results of the present study showed that there was no difference between the young and old non-treated controls in the total LC neuronal numbers, volume or density.

Previously, ageing has been reported to increase vulnerability to ethanol-induced damage in the central (Ritzman and Springer, 1980) and peripheral (Jaatinen and Hervonen, 1994) nervous system. In the present study, the post-hoc comparisons suggested that, in the old rats, ethanol exposure significantly reduced LC neuronal numbers compared with the sucrose group, but in the young EtOH rats there was only a tendency towards reduced LC neuronal numbers. However, ANCOVA showed no age differences in the sensitivity to ethanol-induced neurodegeneration when ethanol dose, intoxication or the withdrawal symptom

![Fig. 4. Locus coerules volume in the different groups.](image)

![Fig. 5. Neuronal density of the locus coerules in the different groups.](image)

![Fig. 6. Total number of locus coerules neurons in the different groups.](image)
score were used as covariates. The difference in the LC neuronal loss between the age groups may thus be due to the differences in the intoxication levels.

The most obvious explanation for the age differences in intoxication levels is a difference in the distribution volume of ethanol between the young and old rats (Ernst et al., 1976), which may lead to higher BECs in the old animals, when ethanol doses are given/kg body wt. Another explanation may be a difference in ethanol metabolism between the two age groups. Ageing has been reported to decrease the rate of ethanol metabolism, mainly by decreasing the activity of ethanol- and acetaldehyde-metabolizing enzymes in the liver (Britton et al., 1984; Seitz et al., 1989, 1992).

Surprisingly, a decreased LC total neuronal number was also found in the young sucrose-treated rats, compared with the corresponding non-treated controls. One explanation for this may be the stress caused by the repeated intragastric intubations and caloric restriction. Within the noradrenergic system, the LC plays an important role in mediating the stress response, and the reactivity of the LC system in stressful situations is well documented (Stanford, 1995; Flugge, 1996). Excessive alcohol consumption has been shown to impair adaptation to repeated restraint stress in rats (Haleem, 1996). In the present study, the stress reaction related to the intragastric intubations may partly explain the neuronal loss in the sucrose- and ethanol-treated rats. According to the post-hoc comparisons, the young rats seemed to be more vulnerable to neuronal degeneration associated with repeated intragastric feedings or nutritional restriction, than the old rats.

The pathophysiological mechanisms by which chronic ethanol exposure produces neuronal damage in the LC have not been established. The ethanol-withdrawal syndrome and excitotoxic mechanisms related to it may contribute to ethanol-induced neurodegeneration. Intermittent ethanol exposure with repeated withdrawal periods has been suggested to be more damaging to neurons than continuous exposure (Lundqvist et al., 1994, 1995; Lundqvist, 1997). Previously, ageing has been reported to increase the severity of the ethanol withdrawal syndrome (Maier and Pohorecky, 1989). However, in the present study, no statistically significant difference between the age groups in severity of the ethanol-withdrawal syndrome was seen.

In both age groups, the animals had to be given increasing ethanol doses week after week to maintain the same level of intoxication during the whole experiment. This may be due to an increased ethanol tolerance of the CNS and/or an increased rate of ethanol metabolism during the 5-week exposure period. In previous studies, the rate of ethanol metabolism has been shown to be enhanced by ethanol intake for several weeks (Sancho-Tello et al., 1988). Long-lasting ethanol exposure has been reported to elicit tolerance to ethanol (LeBlanc et al., 1969; Lê and Kiijanmaa, 1988; Collins et al., 1996). Unfortunately, no repeated measurements of BECs were made, and so the effects of enhanced ethanol metabolism or tolerance to the intoxicating effects of ethanol could not be evaluated in this study.

Ageing has been reported to impair or slow down the development of tolerance to ethanol (Mayfield et al., 1992; York and Chan, 1994; Spencer and McEwen, 1997). In the present study, there was no difference between the young and old animals in ethanol tolerance when measured as the BEC/intoxication ratio at the end of the experiment. However, in terms of the intoxication score/ethanol dose ratio, the old animals seemed to be more sensitive to the intoxicating effects of ethanol at the beginning of the experiment, but during the 5-week ethanol exposure period they reached the same tolerance level as the young animals.

In summary, there was no statistical difference between the age groups in the severity of the ethanol-withdrawal reaction. In the old rats, the 5-week ethanol exposure significantly reduced LC neuronal numbers, compared with the sucrose group, but, in the young EtOH rats, there was only a tendency towards reduced LC neuronal number. However, according to ANCOVA, the difference in the LC neuronal loss between the age groups may have been due to differences in the intoxication levels. A reduction in LC neuronal numbers was also found in the sucrose-treated young animals, which may be due to the chronic stress related to the pair-feeding regimen.

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REFERENCES


