BENEFICIAL EFFECT OF CHRONIC NIMODIPINE TREATMENT ON BEHAVIORAL DYSFUNCTIONS OF AGED RATS EXPOSED TO PERINATAL ETHANOL TREATMENT

MARKEL, E; FELSZEGHY, K; LUITEN, PGM; NYAKAS, C

Published in:
Archives of Gerontology and Geriatrics

DOI:
10.1016/0167-4943(95)00653-3

ELSEVIER

Archives of Gerontology and Geriatrics
21 (1995) 75–88

ARCHIVES OF GERONTOLOGY AND GERIATRICS

Beneficial effect of chronic nimodipine treatment on behavioral dysfunctions of aged rats exposed to perinatal ethanol treatment

Éva Markel[a], Klára Felszeghy[a], Paul G.M. Luiten[b], Csaba Nyakas*[a,b]

[a]*Central Research Division and Department of Clinical and Experimental Laboratory Medicine, Haynal University of Health Sciences, 1389 Budapest, POB 112, Hungary*
[b]*Department of Animal Physiology, University of Groningen, 9750 AA Haren, POB 14, The Netherlands*

Received 19 September 1994; revised version received 7 April 1995; accepted 10 April 1995

Abstract

The long-term effects of prenatal and early postnatal ethanol exposure were assessed in adult (5-month), aged (24-month), and senescent (30-month) rats on non-aggressive intermale social behavior, and on black-white discrimination and spatial learning behaviors. Furthermore, the effects of chronic application of the Ca^{2+} channel blocker nimodipine, which reportedly improves behavioral function in aging, were studied on the ethanol-induced behavioral deficits during aging. The results showed that the perinatal alcohol treatment suppressed social behavior by reducing the frequency and duration of social interactions at all ages. Black-white discrimination behavior and appetitively motivated learning in a hole-board were also markedly disturbed. Several measures of social and spatial learning behaviors of ethanol-exposed rats revealed progressive functional decline with aging. Chronic oral treatment with nimodipine improved the social activity and normalized the cognitive behavioral capabilities of aged and senescent rats exposed to ethanol. We concluded that: (1) the behavioral disabilities caused by perinatal ethanol toxicity are persistent in the rat lifespan and become more pronounced with aging; and (2) administration of nimodipine in the aging period improves, with a long-lasting efficacy, the ethanol-induced behavioral dysfunctions in aged rats.

Keywords: Nimodipine; Ethanol; Development; Aging; Social and cognitive behavior

* Corresponding author, Tel.: (36-1) 129 0222; Fax: (36-1) 120 8246.

0167-4943/95/$09.50
SSDI 0167-4943(95)00653-3

1. Introduction

The growing brain in the perinatal period is highly vulnerable to endogenous and exogenous noxious factors. Damage to the developing nervous system induced by hypoxia or the neurotoxin ethanol, may result in long-lasting deficits in brain functions, leading to impaired adaptive behavior and regulation of pituitary-adrenocortical activity (Abel, 1979; Riley et al., 1979; Anandam et al., 1980; Bond, 1981; Markel et al., 1986a, 1986b; Vorhees and Fernandez, 1986; Nyakas et al., 1989, 1990; Luiten et al., 1992; Nyakas et al., 1994). In these studies, the deficit in learning and memory functions was one of the marked lasting effects, suggesting that animals subject to perinatal brain damage may be used as animal models to study permanent cognitive deficiencies. Until recently, the vast majority of behavioral neuroteratology studies reported functional abnormalities in young adult animals, but provided no information on the consequences of developmental derangement on the aging process. In one of our recent papers (Nyakas et al., 1994), profound deleterious effects of prenatal chronic hypoxia on discriminative learning capacity were assessed during the aging period, while emotional behaviors of these rats were also clearly disturbed in a conditioned fear situation. It was assumed that the functional deficits seen in young ages may become more overtly expressed with aging. Consequently, therapeutic intervention in the early aging period might alleviate 'accelerated aging' and life-spanning learning disability, which results from early brain damage.

Most neurodegenerative diseases are strictly age-related disorders, in which aging is an important risk factor in the manifestation and progression of these diseases. This notion assumes that beyond the genetically determined malfunctioning of specific neurochemical pathways, chronically-disturbed cellular regulatory processes may act in concert to accelerate the neuropathological events. Among the more generally acting cellular dysfunctions, the intracellular Ca^{2+} ($[Ca^{2+}]_i$) overload has recently received considerable attention. The $[Ca^{2+}]_i$ concentration increases with age (Landfield, 1987; Khachaturian, 1989), and the acute and chronic derangement of $[Ca^{2+}]_i$ levels by hypoxia, ischemia and neurotoxicity, with increasing incidence present during aging, threatens brain functioning, including cognitive performance. This view is supported by animal studies showing that chronic treatment with nimodipine, a 1,4-dihydropyridine L-type calcium channel antagonist, exerts a beneficial effect on motor performance, as well as on cognitive behaviors in aging rodents like rat and rabbit (Deyo et al., 1989; Disterhoft et al., 1989; Schuurman and Traber, 1989; Thompson et al., 1990; De Jong et al., 1993). Human studies have also been initiated employing Ca^{2+} channel blockers to alleviate cognitive dysfunction in elderly patients (Crook, 1989).

With the present experiments, the effects of perinatal ethanol exposure on latent orientation, social and learning behaviors were studied in rats 5, 24 and 30 months old. An animal model was set up showing life-spanning cognitive and social behavioral impairment due to perinatal ethanol treatment. This model was used to study the short and long-term effects of chronic nimodipine treatment, occurring at the advanced period of aging, on the functional recovery from behavioral deficits. The results showed that chronic oral treatment with nimodipine improved the cognitive

and other behavioral capabilities of perinatally ethanol-exposed rats during aging. Furthermore, the beneficial effect of this Ca^{2+} channel blocker proved to be long-lasting since it remained observable up to the age of 30 months, despite the cessation of drug treatment.

2. Materials and methods

2.1. Animals and treatments

Albino female Wistar rats (200–280 g body wt) were paired with males, and vaginal smears were taken daily to assess conception. The sperm-positive day was considered as the first day of gestation (G1).

Ethanol treatment started at G11 and was continued during lactation until the 21st postnatal day. The ethanol-exposed dams received a drinking solution containing 16 v/v ethanol. Pair-fed animals were used as controls, supplied with normal tap water. Food and liquid consumptions were measured daily, while the body weight of dams was controlled every other day to assure proper experimental conditions for pair-feeding. As a result of pair-feeding, no significant differences in body weights were found between the ethanol-exposed and control dams. After delivery, eight pups were reared per nest in the control and five pups in the ethanol-exposed groups to reach a better equilibrium in body growth. Indeed, no significant differences could be obtained in the body weight gain of pups between groups. After weaning, the rats were kept six animals per cage under standard laboratory conditions and regular cycles of dark and light periods (light on from 6.00 a.m. to 8.00 p.m.)

At the age of 5 months, only two groups were tested: offspring from control and ethanol-exposed mothers. The chronic nimodipine (BAY E 9736) treatment was started at the age of 23 months. In fact, the ethanol-treated group was divided into two parts, and half of the ethanol-treated animals received nimodipine. Nimodipine was administered via food pellets, to which nimodipine was added at a concentration of 1000 ppm (1 mg/g food; Sniff, Soest, Germany). In conclusion, the following three groups were formed at the age of 23 months: (1) offspring from control pair-fed mothers; (2) offspring from ethanol-exposed rats, fed with normal food; and (3) offspring from ethanol-exposed rats supplied with 1000 ppm nimodipine food.

2.2. Behavioral tests

Male offspring were used in the behavioral tests, which were performed between 8.00 and 12.00 a.m. The same groups of animals were tested at three ages in the rat lifespan: at 5 months (young adult), at 24 months (aged), and at 30 months (senescence). The sequence of the different behavioral tests was as follows: social behavior was tested first, followed by either a food-rewarded spatial learning task or a black-white discrimination learning test.

2.2.1. Social behavior

The social behavior of rats was studied by using a procedure originally designed

by Schuurman and Traber (1989). The non-aggressive social interactions were assessed between two male rats from the same treatment-group, but living in different home cages. The test box consisted of a circular arena covered with sawdust, surrounded by a 35 cm high wall and dimly lit from above by a bulb of 25 W. After placing the rats into the test box, the latency to start social interactions was measured in seconds. The frequency (incidence) and the duration (s) of different social behavioral patterns were recorded for a 5-min observation period afterwards. The following social interactions were recorded: body hair inspection, anogenital inspection, crawling, mounting, following, grooming partner, nibbling, play fighting and aggressive grooming. For statistical evaluation, the sum of scores was processed.

2.2.2. Learning behavior

2.2.2.1. Black-white discrimination learning. The method described originally by Isaacson et al. (1988) was applied with modifications. Briefly, the experimental chamber, which measured 25 × 50 × 25 cm (width × length × height), was equipped with a stainless steel grid floor. The chamber was divided into two equal parts by covering all sides of one half of the box with white cardboard and the other half with black cardboard. During three daily 'preshock' sessions, the rats were allowed to move freely for 90 s in the chamber and the dark preference behavior, i.e. time spent in the dark compartment of the box was measured. On day 4, three 40-s learning trials were given. During each trial, the rat spent 20 s in the white and 20 s in the dark half of the chamber, while a plastic insert was placed between the two compartments of the box. The divider had a black and a white side, and was inserted in such a way that the white and dark halves of the apparatus were entirely separated from each other. While animals were in the dark half of the box, two electric footshocks (0.8 mA, 3 s each), with an intershock interval of 15 s, were delivered during each 20-s trial period. Thus, the animals received a total of six electric footshocks in the course of the three trials. During two daily 'post-shock' sessions, 24 and 48 h after the learning trials, the dark preference was recorded in seconds throughout a 90-s observation period. In these retention tests, the barrier was removed and the rats could explore the entire arena of the test box.

2.2.2.2. Spatial learning in a hole-board test. The apparatus described by Oades (1981) was applied to study food-motivated spatial learning behavior. This apparatus consists of a rectangular arena containing 16 equidistant holes, 13 cm apart. The arena was surrounded by a 35 cm high wall. The rats were deprived of food, but water was available ad lib. For 3 days before testing, the animals were fed only with 5 g food, once per day in their home cage, and the loss in body weight was recorded by daily measurements. On the 4th and 5th days, the rats were habituated to the experimental box for 20 min each day, and consumed their food in the test arena. During these sessions, all holes contained an attainable portion of food (40 mg cheese). One hour after each session, the animals received a complementary portion of standard laboratory food in the home cage to maintain their body weight at a certain percentage of the original body weight. The aimed weight loss varied according to the age of the animals in order to compensate for motivational differences (Van der Staay et al., 1990). The young adult rats (aged 5 months) were kept at 90% of

their free feeding weight, the aged and senescent animals were kept at 85% and 82.5%, respectively.

In the learning phase of the test, from days 6 to 10, food could be reached in a randomly established pattern of four out of 16 holes. In the remaining non-baited holes, the food was hidden by perforated plastic disks, serving as a false bottom in the holes, which sufficed in applying equal olfactory stimuli for all holes. Each daily session consisted of seven trials, with intertrial intervals of 30–40 s. The rats were trained to collect food from the fixed set of four baited holes, and the number of visited food-baited holes (FH) and non-food-baited holes (NFH) were counted until all four pieces of food were collected, or when 10 min had elapsed, whichever event occurred first. A hole visit was scored when the nose of a rat turned to the edge of hole, or was placed in it (Oades, 1981). A total of 35 acquisition trials was given during 5 consecutive days.

Learning capability was expressed by assessing working memory (WM) and reference memory (RM). These measures of learning behavior and the mean intervisit interval (IVI) were calculated as described by others earlier (Van der Staay et al., 1990). Namely, the WM ration was defined as: number of food rewarded visits/number of visits and revisits to the baited set of holes. The RM ratio was defined as: number of visits and revisits to the baited set of holes/number of visits and revisits to all holes. The mean IVI was determined by dividing the time between the first and the last visits in a trial by: number of visits − 1.

2.3. Statistics

The statistical analysis of the behavioral data was carried out by ANOVA with and without repeated measures (STATS program). For comparison of two groups, the post hoc *t*-test was used according to the same program.

3. Results

3.1. Social behavior

The latency, duration and frequency of social interactions in rats aged 5, 24 and 30 months are shown in Fig. 1. As a result of ethanol exposure, the rats showed a delayed latency to start social behavioral activity at all three ages. The frequency and duration of social interactions were diminished between the pairs of ethanol-treated rats as compared to age-matched controls, and this effect could also be observed in all ages.

After a 3-week treatment with nimodipine, the different measures of social behavior of 24-month old ethanol-exposed rats became comparable to controls, except delayed latency, which was only partially normalized. The beneficial effect of nimodipine treatment remained present, even 6 months later at the age of 30 months. Although at the senescent age of 30 months, the rats were somewhat more active to display social interactions than at the age of 24 months, the frequency of social interactions showed a clear age-related reduction.

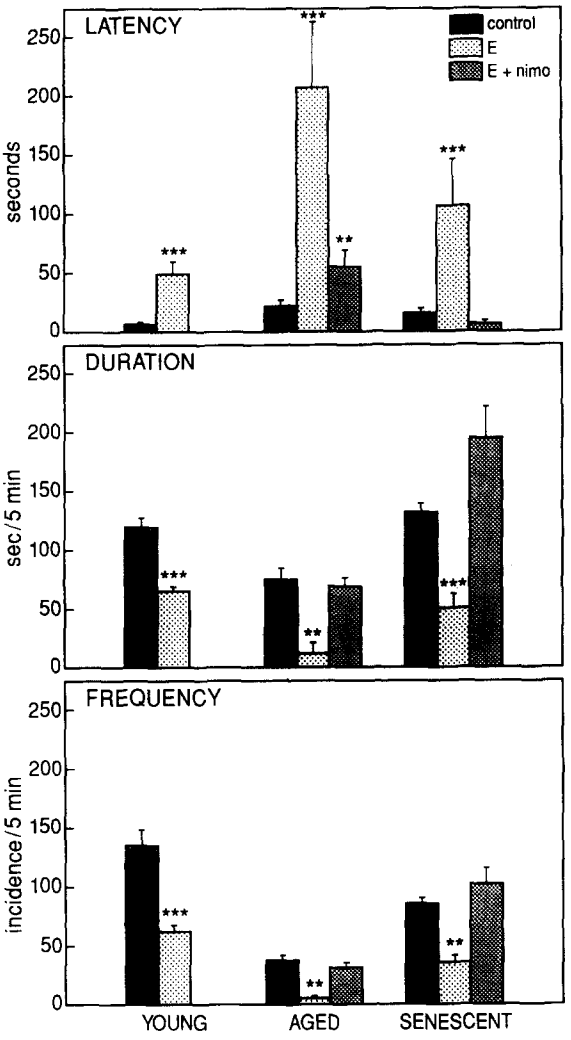

Fig. 1. Social behavior of young (5-month), aged (24-month) and senescent (30-month) rats, following perinatal ethanol exposure and the beneficial effect of chronic nimodipine administration. Upper panel: latency to start social interactions; middle panel: duration of social interactions; bottom panel: frequency of behavioral interactions. Groups are: control (pair-fed), ethanol-exposed (E), and ethanol-exposed but nimodipine treated (E + NIMO). Six pairs of rats were used in each group. Statistically significant differences from controls: $^{*}P < 0.05$, $^{**}P < 0.02$, $^{***}P < 0.01$ (post hoc t-test).

3.2. Learning behavior

The learning capability of animals was tested by using two behavioral paradigms: black-white discrimination and food-rewarded spatial learning. The results found in the black-white discrimination learning test are summarized in Fig. 2. Before foot-

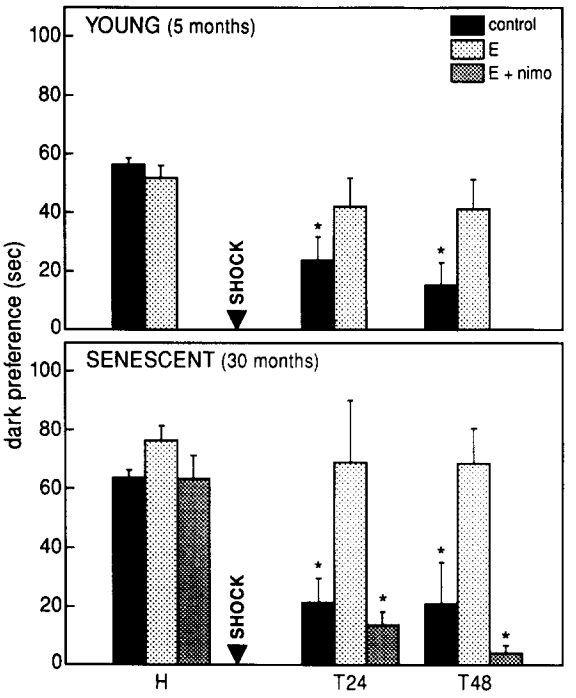

Fig. 2. Effects of ethanol and nimodipine treatments on discrimination behavior in a black-white experimental box. The effect of ethanol pretreatment was tested in the young age (upper panel) and that of nimodipine treatment in the senile age (lower panel). Before footshocks ('shock'), dark preference was measured throughout three daily sessions during habituation (H). After 'shock', the time spent in the black compartment was measured 24 (T24) and 48 (T48) h later. Groups are: control ($n = 12$); ethanol-exposed (E, $n = 12$); and ethanol-exposed and nimodipine treated (E + NIMO, $n = 5$). $^{*}P < 0.01$ versus own preshock (H) values (ANOVA with repeated measures was followed by paired t-tests).

shock, both young and senescent, irrespective of the treatments, spent about two-thirds of their time, i.e. about 60 s from the 90-s observation period, in the black half of the box. Following footshocks, the shocked young (upper panel) and senescent (bottom panel) control rats showed a significant dark-avoidance, as compared to the ethanol-exposed animals. In fact, the ethanol-exposed rats did not show any sign of discrimination between the black (shocked) and the white compartments. The learning capacity of the 30-month old control rats was very comparable to that of young controls. The avoidance response of control groups was equally present 24 and 48 h after footshocks. Those ethanol-exposed rats, which were treated with nimodipine at the age of 23 months for 3 weeks, were able to perform the task to the level of control animals by the age of 30 months. Therefore, chronic nimodipine treatment exerted a long-term improvement in the learning capability of ethanol-intoxicated aged rats.

The acquisition of visiting the correct set of baited food-holes, with a decreasing amount of errors in the appetitively-motivated spatial learning test, is shown in the

next two figures. Working memory (Fig. 3) and reference memory (Fig. 4) performances were calculated separately. The measure of WM represents the percentage of all visits to the baited set of holes that had been reinforced with food. The measure of RM expresses the number of visits to the baited set of holes as a percentage of the total number of visits to all holes. Fig. 3 shows that the WM of rats was not influenced by the perinatal ethanol treatment, when tested at young age and at the age of 24 months (upper and middle panels, respectively). At the age of 30 months, how-

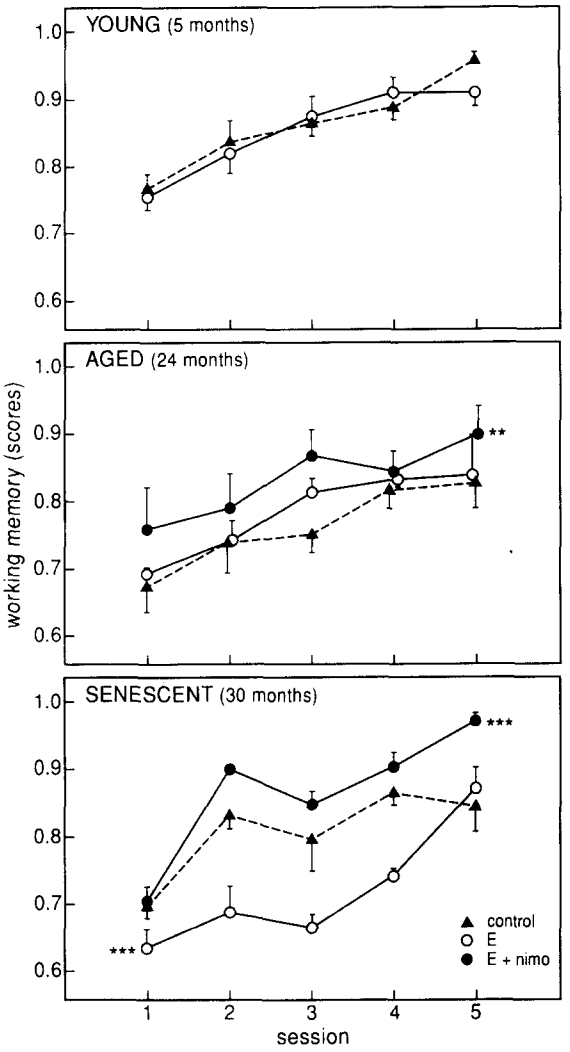

Fig. 3. Working memory (WM) performance in a hole-board spatial learning test after alcohol (E) and nimodipine (E + NIMO) treatments. The different age-groups are: young (5-month), aged (24-month) and senescent (30-month). WM score: number of food rewarded visits/number of visits and revisits to the baited set of holes. Five rats were trained in each group. $^{***}P < 0.01$, $^{**}P < 0.02$ vs. controls (ANOVA with repeated measures).

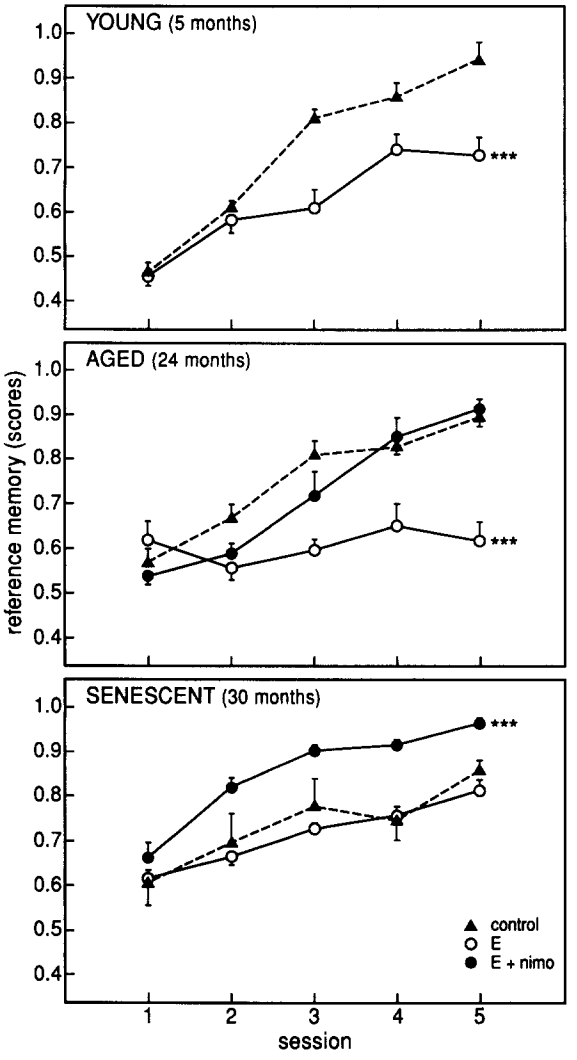

Fig. 4. Reference memory (RM) performance in a hole-board spatial learning test after alcohol (E) and nimodipine (E + NIMO) treatments. RM score: number of visits and revisits to the baited set of holes/number of visits and revisits to all holes. Data are from five rats per group. $^{***}P < 0.01$ vs. controls (ANOVA with repeated measures).

ever, the performance of ethanol-exposed animals was inferior to controls. ANOVA with repeated measures showed a significant treatment effect: $F[1,7] = 44.56$, $P < 0.001$ (see bottom panel).

Nimodipine significantly improved WM right after chronic treatment at the age of 24 months (ANOVA with repeated treatment: $F[1,7] = 30.95$, $P < 0.01$ versus control group). The same treatment prevented the decline of performance of ethanol-

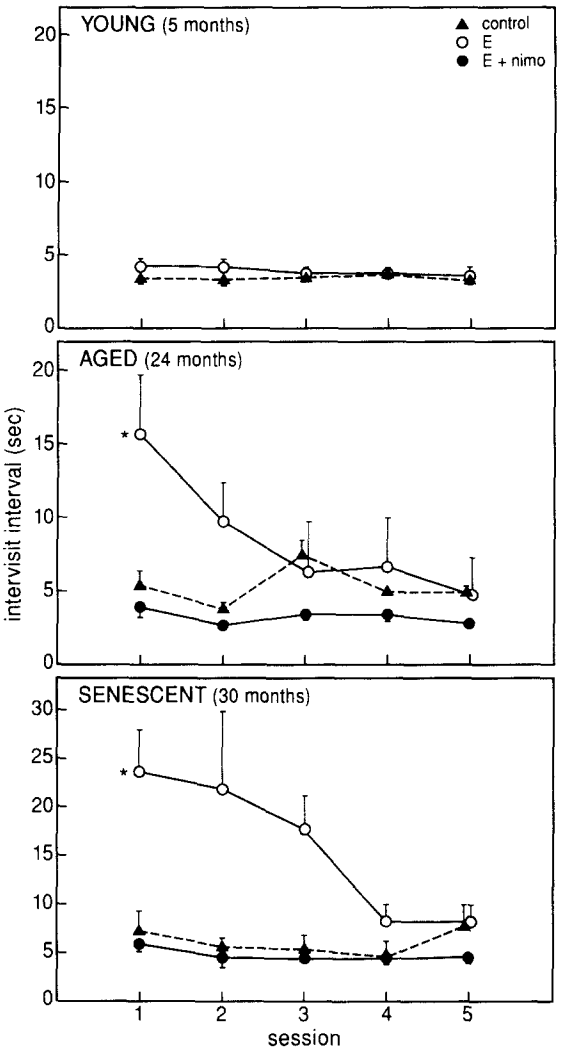

Fig. 5. Mean intervisit intervals (IVI) obtained in young, aged and elderly rats after treatments with ethanol (E) and nimodipine (E + NIMO) during performing a hole-board spatial learning task. Each group contained 5 animals. $*P < 0.05$ vs. controls (ANOVA with repeated measures).

exposed rats at the age of 30 months. In fact, the performance of nimodipine-treated elderly rats was superior to controls (ANOVA: $F[1,7] = 17.90$, $P < 0.001$).

The results found after RM calculations are shown in Fig. 4. Unlike WM performance, the RM performance of ethanol-exposed rats was significantly attenuated in the young and in the 24-month old animals (ANOVA, $P < 0.001$), while not being different from controls in senescence. The drug treatment compensated the ethanol-

induced deficit seen in the 24-month old aged rats. Interestingly, the performance of the E + NIMO group at the age of 30 months was superior to controls $F[1,7] = 10.74$, $P < 0.001$). This result confirmed again that the chronic nimodipine treatment in the aging period exerted a lasting effect which was still detectable 6 months after terminating the drug treatment.

In the hole-board spatial learning test, the perinatal ethanol treatment markedly influenced the measure of mean IVI in the aged and senescent rats (see Fig. 5). Both aged and senescent ethanol-exposed rats spent more time visiting holes and complete trials than did controls or ethanol-exposed, but nimodipine-fed animals ($F[1,8] = 3.25$, $P < 0.05$ vs. control and $F[1,7] = 6.86$, $P < 0.05$ vs. control, respectively, see middle and bottom panels of Fig. 5). The elongated IVI seen in the ethanol-exposed rats gradually disappeared in the course of repeated sessions, which shows that the behavioral disturbance was most accentuated at the beginning of the test.

4. Discussion

The present data showed that perinatal exposure to ethanol caused a long-term disturbance in social and learning behaviors of rats. In each investigated behavioral category, the ethanol-exposed rats displayed abnormalities in their performance. Social interactions were reduced and cognitive ability was seriously impaired compared to controls in all ages investigated. In some behavioral measures, the functional deficit was more pronounced in the advanced ages than in the young adult age, pointing to the possibility that aging might be an accentuating or precipitating factor. The alterations in behavioral activities were compensated by chronic treatment of aged rats with the Ca^{2+} channel blocker drug, nimodipine.

Combined prenatal and early postnatal ethanol intoxication suppressed social behavior and decreased the number of social interactions at 5, 24 and 30 months of age. It was also found that, with aging, the duration of intermale social behavior declined. This latter finding confirms earlier observations, indicating that aging is accompanied by an attenuation of social interactions (Schuurman and Traber, 1989), and specifically, a decrease in social attention towards the partner (Spruijt, 1991).

The major behavioral findings of this study point to the serious learning and memory deficits seen after perinatal ethanol exposure in the rat. The discrimination learning deficit was present in both ages investigated, i.e. 5 and 30 months. The ethanol-exposed offspring also showed memory impairment in the appetitively-motivated spatial learning task (hole-board test). Depending on the age, WM or RM was disturbed. The aged and senescent ethanol-exposed rats showed a remarkable behavioral disturbance and uncertainty at the beginning of the learning phase of the hole-board test, reflected by an increased mean IVI time. This kind of behavioral deficit was not present in young ethanol intoxicated animals, showing that functional abnormalities may become overt only during aging and that the reduced behavioral adaptability may be at least partly compensated in young animals.

A number of behavioral neuroteratological examinations in rat revealed that perinatal alcohol exposure leads to learning and memory disturbances at adult ages (Abel, 1979; Riley et al., 1979; Anandam et al., 1980; Bond, 1981; Vorhees and Fer-

nandez, 1986; among others). A lifespanning disturbance in cognitive behavior has also been reported (Janicke and Coper, 1993). In this latter paper, it has also been shown that the impairment in learning performance was more pronounced in the senile phase of life of ethanol-exposed rats.

The ultimate purpose of the present experiments was to study the pharmacologic effect of chronic nimodipine treatment in aged rats, showing behavioral disturbances in their young age due to perinatal alcohol exposure. In fact, an animal model was created, in which the ethanol-pretreated rats displayed cognitive and other behavioral abnormalities. The Ca^{2+} antagonist treatment counteracted the behavioral effects of ethanol-exposure. The social, discriminative and spatial learning behaviors of aged and senile alcohol-exposed rats, treated chronically with nimodipine, has been normalized. Occasionally, like in the case of reference memory performance, the nimodipine-treated rats were even superior to controls. Based on these observations, one may conclude that the behavioral deficits seen after perinatal exposure to ethanol are not irreversible. Furthermore, it may also be assumed that the ethanol-treated rats preserved a considerable amount of functional capacity to compensate for the inferior behavioral performances. It is assumed that the chronic nimodipine treatment supported these functional compensatory processes.

As far as the underlying mechanism of nimodipine effect during aging might be concerned, a more balanced cellular Ca^{2+} homeostasis can be proposed. This view is supported by observations showing that Ca^{2+} influx in hippocampal neurons probably increases with age, since L-like Ca^{2+} currents and Ca^{2+}-dependent potentials are increased in aged hippocampal neurons in vitro (Landfield, 1993). A number of findings in other laboratories evidenced that chronic nimodipine treatment has a beneficial effect on cognitive performance in aged rodents, like rabbits (Deyo et al., 1989; Disterhoft et al., 1989; Thompson et al., 1990) and rats (Levere and Walker, 1993; Ingram et al., 1994). Nimodipine exerted a beneficial effect on age-related memory disfunction in monkeys (Moss and Rosene, 1993). In the present study, we showed that nimodipine was beneficial to compensate ethanol-induced long-term behavioral dysfunction in aged and elderly rats.

Acknowledgements

Supported by Grant No.448 of the Ministry of Welfare in Hungary. Furthermore, C.N. was supported by a fellowship from the Netherlands Science Foundation (NWO), while staying at the University of Groningen.

References

Abel, E.L. (1979): Prenatal effects of alcohol on adult learning in rats. Pharm. Biochem. Behav., 10, 239–243.

Anandam, N.T., Strait, T. and Stern, J.M. (1980): In utero ethanol retards early discrimination learning and decreases adult responsiveness to ethanol. Teratology, 21, 25A.

Bond, N.W. (1981): Prenatal alcohol exposure in rats: A review of its effects on offspring activity and learning ability. Aust. J. Physiol., 33, 331–344.

Crook, T.H. (1989): Strategies for treating Alzheimer's disease and age-associated memory impairment. In: Diagnosis and Treatment of Senile Dementia, pp. 234–242. Editors: M. Bergener and B. Reisenberg. Springer, Berlin.

De Jong, G.I., Nyakas, C., Schuurman, T. and Luiten, P.G.M. (1993): Aging-related alterations in behavioral activation and cerebrovascular integrity in rats are dose-dependently influenced by nimodipine. Neurosci. Res. Commun., 12, 1–8.

Deyo, R.A., Straube, K.T. and Disterhoft, J.F. (1989): Nimodipine facilitates associative learning in aging rabbits. Science, 243, 809–811.

Disterhoft, J.F., Deyo, R.A., Black, J., De Jonge, M., Straube, K.T. and Thompson, J.T. (1989): Associative learning in aging rabbits is facilitated by nimodipine. In: Nimodipine and Central Nervous System Function: New Vistas, pp. 209–225. Editors: J. Trabers and W.H. Gispen. Schattauer, Stuttgart.

Ingram, D.K., Joseph, J.A., Spangler, E.L., Roberts, D., Hengemihle, J. and Fanelli, R.J. (1994): Chronic nimodipine treatment in aged rats: Analysis of motor and cognitive effects and muscarinic-induced striatal dopamine release. Neurobiol. Aging, 15, 55–61.

Isaacson, R.L., Johnston, J.E. and Vargas, D.M. (1988): The effect of a calcium antagonist on the retention of simple association learning. Physiol. Behav., 42, 447–452.

Janicke, B. and Coper, H. (1993): The effects of prenatal alcohol exposure of the behavior of rats during their life span. J. Gerontol. Biol. Sci., 43, B156–B167.

Khachaturian, Z.S. (1989): The role of calcium regulation in brain aging: re-examination of a hypothesis. Aging (Milano), 1, 17–34.

Landfield, P.W. (1987): 'Increased calcium current' hypothesis of brain aging. Neurobiol. Aging, 8, 346–347.

Landfield, P.W. (1993): Calcium currents and calcium channel antagonists in the aging brain. In: Drugs in Development, vol. 2. Ca^{2+} Antagonist in CNS, pp. 241–248. Editors: A. Scriabine, R.A. Janis, and D.J. Triggle. Neva Press, Brandford.

Levere, T.E. and Walker, A. (1991): Old age and cognition: Enhancement of recent memory in aged rats by the calcium channel blocker nimodipine. Neurobiol. Aging, 13, 63–66.

Luiten, P.G.M., Van der Zee, E.A., Gáspár, E. Buwalda, B., Strosberg, A.D. and Nyakas, C. (1992): Long-term cholinergic denervation caused by early postnatal AF64A lesion prevents development of muscarinic receptors in rat hippocampus. J. Chem. Neuroanat., 5, 131–141.

Markel, É., Nyakas, C., Tal, E. and Endroczi, E. (1986a): Changes in avoidance behaviour following ethanol treatment in rats of different ages. Acta Physiol. Hung., 68, 175–181.

Markel, É., Scaccianoce, S., Korányi. L. and Endroczi, E. (1986b): Effects of arginin-vasopressin and ACTH4–10 on acquisition of active avoidance response in rats with alcohol pretreatment in prenatal and adult age. Acta Physiol. Hung., 68, 221–227.

Moss, M.B. and Douglas, L.R. (1993): Therapeutic effects of nimodipine on age-related memory dysfunction in the monkey. In: Drugs in Development, vol. 2. Ca^{2+} antagonists in CNS. Editors: A. Scriabine, R.A. Janis and D.J. Triggle. Neva Press, Branford.

Nyakas, C., Buwalda, B., Markel, É., Korte, S.M. and Luiten, P.G.M. (1994): Life-spanning behavioural and adrenal dysfunction induced by prenatal hypoxia in the rat is prevented by the calcium antagonist, nimodipine. Eur. J. Neurosci., 6, 746–753.

Nyakas, C., Markel, É., Bohus, B., Schuurman, T. and Luiten P.G.M. (1990): Protective effect of the calcium antagonist nimodipine on discrimination learning deficits and impaired retention behavior caused by prenatal nitrite exposure in rats. Behav. Brain. Res., 38, 69–76.

Nyakas, C., Markel, É., Kramers, R.J.K., Gáspár, E., Bohus, B. and Luiten, P.G.M. (1989): Effects of nimodipine on hypoxia-induced learning and memory deficits. In: Nimodipine and Central Nervous System Function: New Vistas, pp. 175–194. Editors: J.Traber and W.H. Gispen. Schattauer, Stuttgart.

Oades, R.D. (1981): Types of memory or attention? Impairments after lesion of the hippocampus and limbic ventral tegmentum. Brain Res. Bull, 7, 221–226.

Riley, E.P., Lochry, E.A. and Shapiro, N.R. (1979): Lack of response inhibition in rats prenatally exposed to alcohol. Psychopharmacology (Berl), 62, 47–52.

Schuurman, T. and Traber, J. (1989): Effects of nimodipine on behavior of old rats. In: Nimodipine and Central Nervous System Function: New Vistas, pp.195–208. Editors: J. Traber and W.H. Gispen. Schattauer, Stuttgart.

Spruijt, B.M. (1991): Progressive decline in social attention in aging rats: An information-statistical method. Neurobiol. Aging, 13, 145–151.

Van der Staay, F.J., Van Nies, J. and Raaijmakers, W. (1990): The effects of aging in rats on working and reference memory performance in a spatial hole-board discrimination task. Behav. Neurol. Biol., 53, 356–370.

Thompson, L.T., Deyo, R.A. and Disterhoft, J.F. (1990): Nimodipine enchances spontaneous activity of hippocampal neurons in aging rabbits at a dose that facilitates associative learning. Brain Res., 535, 119–130.

Vorhees, C.V. and Fernandez, K. (1986): Effects of short-term prenatal alcohol exposure on maze, activity, and olfactory orientation performance in rats. Neurobehav. Toxicol. Teratol., 8, 23–28.