ETHANOL FACILITATION OF SHORT-TERM MEMORY IN ADULT RATS WITH A DISTURBED CIRCADIAN CYCLE

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Abstract — The aim of this study was to evaluate the effect of 3-month ethanol treatment on olfactory social memory test performance using two inter-exposure intervals [30 min: short-term recognition (STR); or 120 min: long-term recognition (LTR)] in adult rats with a disturbed circadian cycle (DCC). Ethanol treatment both in ethanol-prefering and -non-prefering groups improved the STR task compared to control rats. However, LTR procedure triggered the opposite tendency. Moreover, no differences between control rats with DCC and those with normal diurnal rhythm in STR and LTR paradigms were observed. Our results suggest that, under some conditions, alcohol facilitates short-term memory in adult rats.

INTRODUCTION

Generally, in chronic alcoholics the morphological, neurophysiological and biochemical changes in the central nervous system (CNS) are associated with cognitive deficits (Eckardt et al., 1996; Parsons, 1998; see also the review by Fadda and Rossetti, 1998). The mechanism of the action initiated by ethanol (EtOH) on both recent and long-term memory has not as yet been fully elucidated. The results of experimental research indicate that EtOH induces particularly significant changes in the memory and learning processes in fetuses and young animals (Omoto et al., 1993; McKinzie et al., 1994; Pauli et al., 1995; Kelly and Tran, 1997; Nagahara and Handa, 1997; Tomlinson et al., 1998; Krahl et al., 1999). Research on the effects of administration of EtOH to pregnant female rats showed that their offspring not only suffered from learning process disturbances, but they also exhibited degenerative changes of cerebral tissue, particularly of cerebral cortex, hippocampus and cerebellum (Greene et al., 1992; Hekmatpanah and Haghhighat, 1994; Krahl et al., 1999). Yet, the results of research on the effects of chronic EtOH administration on memory processes and learning processes in adult rats often led to contradictory conclusions (Blokland et al., 1993; Car et al., 1994; Sasaki et al., 1995; Steigerwald and Miller, 1997; Krahl et al., 1999). Discrepancies may be attributed to different methods used in each study, including the amounts and duration of EtOH treatment, as well as the specificity and sensitivity of the tasks used to test learning and memory.

One of the tests used for research on memory processes and learning processes in experimental animals is the social memory test (Thor and Holloway, 1982; Dantzer et al., 1987; Perio et al., 1989; Popik et al., 1992; Benelli et al., 1995; Griffin and Taylor, 1995; Prast et al., 1996; Kelly and Tran, 1997; Argyriou et al., 1998; Popik and van Ree, 1998; Reid et al., 1999; Kogan et al., 2000). This test is based on the ability of animals to discriminate other animals by following chemosensory cues, which are the primary stimuli permitting such discrimination (Carr et al., 1976; Thor and Holloway, 1982). It can be assumed that this memory takes the form of short-term memory, strictly related to sensory memory (Dantzer et al., 1987). It was also confirmed that this social behaviour (interest towards the young rat expressed by the adult rat) is not a type of behaviour conditioned by instincts, because rats behave in this way when they come into contact not only directly with a young rat but also with its urine (Sawyer et al., 1984). Therefore, the introduction of the social memory test, being completely free of external enhancing stimuli, to the research on the memory of chronically EtOH-treated animals, should allow for better assessment of some cognitive processes in animals.

It is well known that one criterion for an animal model of alcohol dependence is the voluntary ingestion of ethanol (Lester and Freed, 1973). In normal populations of experimental animals, a group of EtOH ‘preferring’ animals can be distinguished (Hammoumi et al., 1997). In our previous research on different aspects of EtOH action, animals were first forced to consume ethanol and their EtOH preference was then defined by selecting ‘preferring’ animals (Mikolajczak et al., 1999). However, having adopted this method, we obtained a relatively small ratio (10–12%) of ‘preferring’ animals. Because it is known that disruption of the circadian cycle of experimental animals results in higher ratios of preferring animals (Geller, 1971; Blum et al., 1989; Lin and Hubbard, 1994), we placed animals for 24 h in completely dark compartments, throughout the whole duration of the experiment (12 weeks). This yielded higher ratios of ‘preferring’ animals (24%), compared to only 10% of rats kept in a normal circadian cycle (12 h/12 h) (P. Mikolajczak et al., unpublished data).

It is known that administration of EtOH to rats during pre- and postnatal periods results in impairment of social memory after the animals have reached adulthood (Kelly and Tran, 1997; Reid et al., 1999). However, to our knowledge, there is no information on the effect of alcohol treatment on this type of memory in adult rats, so it seemed interesting to examine the effect of chronic EtOH (3 months) treatment on social recognition task performances in adult rats, selected on the basis of their preference for EtOH and kept on a 24-h dark cycle.

MATERIALS AND METHODS

Animals and alcohol intake

The experiments were performed on male Wistar rats ~2 months old at the beginning of the alcohol intake experiment.

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All animals were housed individually in their home cages, kept on a 24-h dark cycle (disturbed circadian cycle: DCC) under constant ambient conditions (20 ± 2°C), and a relative humidity of 65%, and forced to drink only 12% EtOH for 2 months (-9 g/kg/day) (forced alcohol drinking period). During the next 4 weeks (preferring period), animals were presented with a free choice paradigm between tap water and 12% (w/w) EtOH solution (Lin and Hubbard, 1994; Mikolajczak et al., 1999). This procedure led us to distinguish two groups of EtOH-treated animals: (1) rats with a mean intake of EtOH exceeding 50% of their total fluid, i.e. ‘preferring’ (PRF; 7.2 ± 0.4 g EtOH/kg/day, n = 12); (2) rats for whom alcohol solution constituted less than 50% of total fluid intake, i.e. ‘non-preferring’ (NPF; 1.4 ± 0.3 g EtOH/kg/day, n = 22).

Additionally, for comparative purposes, throughout the whole period of chronic ethanol treatment, two EtOH-naive control groups of animals received only tap water: (3) with DCC (CD, n = 16); (4) with normal circadian cycle [kept on a reversed 12 h/12 h night/day cycle (lights on 19.00-7.00: CN, n = 15)].

There were no statistically significant differences in daily total fluid intake (ml ± SD) between all investigated animals [PRF, 35.0 ± 2.6; NPF, 33.5 ± 1.9; CD, 30.3 ± 1.5; CN, 32.8 ± 1.4; one-way analysis of variance (ANOVA): F(3,64) = 1.05, P > 0.1]. Throughout the process of chronic alcohol treatment all the animals had free access to standard laboratory food [LaboFeed B (LSM); Feeds and Concentrates Production Plant, Poland, PN-ISO 9001:1996]. The average body weights (g ± SD) of animals (5-month-old) after the preference period were as follows: PRF, 418 ± 10; NPF, 425 ± 11; CD, 423 ± 11; CN, 449 ± 10. There were no statistical differences in the body mass of rats [one-way ANOVA F(3,64) = 1.44, P > 0.1].

Effect on spontaneous locomotor activity: the actimeter

Locomotor activity was conducted using the ‘PAN’ licensed activity meter (Poland) by placing animals in the centre of the apparatus and recording their activity by electromechanical counters (Mikolajczak et al., 1999). The data were expressed as signals corresponding to spontaneous movements during 5 min. The experiments assessing the sedative activity were conducted after the preference study period between 09.00 and 15.00 in a dimly illuminated, soundproof room.

Social memory test

After the preference period and after assessment of locomotor activity, all groups of rats (PRF, NPF, CD, CN) were presented with social memory paradigms. Social memory paradigms based on olfactory recognition (Thor and Holloway, 1982; Sawyer et al., 1984) which allowed the measurement of short-term memory conditions with short-term (STR paradigm) or long-term (LTR paradigm) recognition procedures were used in this study (Thor and Holloway, 1982; Dantzler et al., 1987; Griffin and Taylor, 1995; Taylor et al., 1999). Every adult rat was investigated through 3 consecutive days. Briefly, an adult rat was presented to a juvenile (~30 days old, 50 g) male rat (social stimuli) for 5 min and total social-investigatory behaviour (defined as being proximally orientated to juvenile rat (J) or having direct contact while sniffing, following, nosing, grooming, pawing or generally inspecting any body surface of the juvenile by the adult) was measured with a hand-held cumulative timer to the nearest 0.1 s (T1).

Next, after 30 min (STR paradigm) the same procedure with the same juvenile rat (known JR) was repeated (T2; day 1). The next day, for evaluation of non-specific effects of the STR paradigm, an unknown new juvenile (unknown JR) was exposed to the same adult rat during a second exposure (T2; day 2). On day 3, during T1 and T2, the same juvenile rat (known JR) was used as a social stimulus, but the inter-exposure interval was 120 min (LTR paradigm). Test values are expressed as the ratio of time spent on investigation during T2 divided by T1 (ratio of investigation duration: RID) (Prast et al., 1996; Argyriou et al., 1998). Any aggressive behaviour between animals (i.e. biting, kicking and fighting) was considered an immediate cause for terminating the experiment and excluding the data from the analysis.

All social investigations were conducted in home cages of adult rats between 09.00 and 15.00 in a dimly illuminated, soundproof room.

After every behavioural experiment, the EtOH-treated animals had free access to food, water and EtOH; in the case of control rats food and water were freely available.

Statistical analysis

The results obtained were expressed as the arithmetic means ± SEM. The statistical assessment of the EtOH effects kept in DCC were carried out using one-way ANOVA on locomotor activity or social investigations of the three different groups of rats (PRF, NPF and CD) followed by the least significance difference (LSD) test, when values of ANOVA reached P ≤ 0.05. ANOVA with replication was used for the assessment of specificity of the STR procedure by comparison of the results of PRF, NPF and CD groups after known JR vs unknown JR presentation as the repeated measure. The statistical analysis of DCC effect (CD vs CN) on locomotor activity or social memory was carried out by using one-way ANOVA.

RESULTS

In the first experiment, when testing the sedative action of chronic ethanol treatment on the basis of the assessment of rats’ locomotor activity, it was noticed that the observed values for PRF (151 ± 7) or NPF (152 ± 5) did not statistically differ when compared to CD (140 ± 7) [ANOVA: F(2,47) = 0.16; P > 0.1] (Fig. 1). Control rats with normal diurnal rhythm CN (115 ± 17) had lower values of locomotor activity in comparison to CD animals; however, the differences were not statistically significant [F(1,29) = 1.97; P > 0.1].

The results of influence of chronic EtOH treatment on olfactory social memory test performance in adult rats with DCC are shown in Figs 2 and 3. It was found that chronic EtOH treatment both in PRF and NPF groups led to better fulfillment of the STR tasks (the lower the RID, the better the memory) compared to that for CD rats (Fig. 2) [ANOVA: F(2,47) = 3.28; P < 0.05] and RID values were lower for PRF (0.47 ± 0.06) and NPF (0.56 ± 0.07), in comparison to CD (0.78 ± 0.13) (LSD test: P < 0.05 and P < 0.06, respectively). However, no differences between NPF and PRF animals were found using the STR procedure. No significant differences were found when an unknown JR was exposed to the adult rats of PRF (0.99 ± 0.11), NPF (1.18 ± 0.13) or CD (1.20 ± 0.13) groups during the second contact (Fig. 2) [ANOVA: F(2,47) =
0.10; \( P > 0.1 \). This suggests that the observed effect of chronic EtOH treatment is specific: comparing the effect of an unknown JR to a known JR, the ANOVA values were large [\( F(1,47) = 23.1, P < 0.001 \)]. Using the LTR procedure (Fig. 3), it was observed that RID values for PRF (0.76 \pm 0.09) and NPF (0.87 \pm 0.11) rats were higher than in CD (0.64 \pm 0.06) animals, but there were no statistically significant differences between these parameters [ANOVA: \( F(2,47) = 1.40, P > 0.1 \)]. Moreover, no significant differences between RID values of the CD and CN groups in STR (0.78 \pm 0.13 and 0.80 \pm 0.10, \( P > 0.1 \)) and LTR (0.64 \pm 0.06 and 0.69 \pm 0.09, \( P > 0.1 \)) paradigms were observed (Figs 2 and 3, respectively).

DISCUSSION

The tests in our experiment were carried out on chronically EtOH-treated rats which, from the beginning of the experiment, remained in complete darkness for 24 h in order to disrupt their diurnal cycle, a procedure which increases the number of ethanol-prefering animals (Geller, 1971; Blum et al., 1989; Lin and Hubbard, 1994). Moreover, the ‘preferring’ rats (PRF), kept in conditions of DCC, consumed more ethanol (7.2 \pm 0.4 vs 4.7 \pm 0.5 g/kg/day) (P. Mikolajczak et al., unpublished data).

With respect to EtOH-drinking pattern and variations in body weight of the investigated rats, there were no statistically significant differences in daily total fluid intake and body weight among all investigated animals. Therefore, it can be concluded that the animals used in our study were not dehydrated and probably not undernourished. However, in the model of experimental alcoholism that we have adopted, we obtained two groups of rats differing in voluntary EtOH intake. We believe that the alcohol intake observed in our PRF group (7.3 \pm 0.3 g/kg/day) is in agreement with the dose postulated to obtain dependence during chronic treatment in rats (~7 g/kg/day) (Hammoumi et al., 1997). Moreover, these PRF rats differ from NPF in some EtOH-induced behavioural paradigms such as in their period of EtOH-induced sleep-time (Mikolajczak et al., 1995).

It has to be emphasized that the sedative component of action of EtOH can be omitted in this study. As far as the locomotor activities are concerned, there were no statistically significant differences between the analysed groups of rats (Fig. 1), which is in agreement with observations by others (McMillen et al., 1998). It is also possible to conclude that, probably because of the continuous availability of EtOH during the experiments, there were few, if any, withdrawal symptoms influencing the rats’ activity. Moreover, because it is known that EtOH-withdrawn rats show an impairment of their cognitive performances (Lukoyanov et al., 1999), probably associated

Fig. 1. Locomotor activities of preferring (PRF), non-prefering (NPF) and control (CD) adult rats kept on a 24-h dark cycle (DCC) or control animals with reversed normal diurnal cycle (CN). Data are expressed as means \pm SEM. ANOVA for CD, PRF, NPF: \( F(2,47) = 0.16, P > 0.1 \); ANOVA for CN and CD: \( F(1,29) = 1.97, P > 0.1 \).

Fig. 2. Changes of short-term recognition (STR) task in preferring (PRF), non-prefering (NPF) and control (CD) adult rats kept on a 24-h dark cycle (DCC) or control animals with reversed normal diurnal cycle (CN).

Test values are expressed as the ratio of time spent on social investigation (ratio of investigation duration: RID; see the Materials and methods section). Data are expressed as means \pm SEM. ANOVA for CD, PRF, NPF (white columns = known JR): \( F(2,47) = 3.28, P < 0.05 \); ANOVA for CD, PRF, NPF (grey columns = unknown JR): \( F(2,47) = 0.10, P > 0.1 \); statistically significant versus CD group: \#P < 0.06 or \##P < 0.05 (LSD post-hoc test).

Fig. 3. Changes of the long-term recognition (LTR) task in preferring (PRF), non-prefering (NPF) and control (CD) adult rats kept on a 24-h dark cycle (DCC) or control animals with reversed normal diurnal cycle (CN).

Test values are expressed as the ratio of time spent on social investigation (ratio of investigation duration: RID; see the Materials and methods section). Data are expressed as means \pm SEM. ANOVA for CD, PRF, NPF: \( F(2,47) = 1.40, P > 0.1 \).
with excitotoxicity of increased glutamatergic transmission (Fadda and Rosetti, 1998), the presence of the withdrawal state in our EtOH-treated rats can also be excluded on account of their responses in our paradigms.

According to other researches (Arletti et al., 1997; Argyriou et al., 1998; Popik and van Ree, 1998), assessment of non-specific influences, such as impairment of alertness, was only performed by exposing an unknown JR rat to the adult rats in the case of the STR paradigm, when significantly diminished RIDs in EtOH-treated animals were found. Therefore, the elimination of any non-specific action in the LTR procedure was not necessary, because there were no statistical differences between all investigated groups.

The observation that the recognition time for the STR paradigm was shortened by chronic EtOH treatment shows that ethanol in some conditions leads to facilitation of short-term memory (Figs 2 and 3). This is in agreement with the observations that, in some EtOH-treatment protocols, long-term EtOH intake does not lead to cognitive impairment (Arendt et al., 1989; Steigerwald and Miller, 1997; Lukoyanov et al., 1999). It seems that, under certain conditions (8–20 weeks of EtOH treatment in some studies), the performances of some learning and memory tasks may sometimes be transiently enhanced by chronic exposure to EtOH (Blokland et al., 1993; Steigerwald and Miller, 1997). It is known that, depending on dose and task requirements, EtOH can facilitate or impair cognitive functions and this dichotomy can be accounted for by ETOH’s suppression of behavioural variability and processing of incidental stimuli (Devenport and Merriman, 1983; McKinzie et al., 1994). It seems that the effect of context and conditions of the stimuli used in different learning and memory tests may be responsible for obtaining either facilitation or impairment of some cognitive functions (Devenport and Merriman, 1983). However, the assessment of learning and memory differences between rodent lines selected for high- and low-volitional intake of EtOH are still under investigation (Salimov, 1999). It was postulated that selectively bred lines of rats for alcohol preference differ in many of their behavioural patterns (Overstreet et al., 1997; McMillen et al., 1998; Salimov, 1999). For example, it was shown that positive (produced by appetitive training) or aversive (produced by aversive training) conditioning procedures alter the learning ability of preferring and non-prefering rats in an opposing manner using a signalled bar-pressing task (Blankenship et al., 1998). However, in our study, there were no differences between PRF and NPF animals in social memory tests reported in the STR or LTR procedure. One of the explanations for the lack of differences may be due to the fact that, on applying the social memory test, neither aversive nor rewarding stimuli are used as cues for conditioning. It is probably true that this test, being completely void of external enhancing stimuli, utilizes the ability of the animals to discriminate other animals through chemosensory cues, which are the primary stimuli that permit such discrimination (Carr et al., 1976; Thor and Holloway, 1982). Therefore, the introduction of the social memory test to research on the memory of chronically EtOH-treated animals should allow for better assessment of some cognitive processes in animals. From the experiments that have been performed and which permitted the localization of brain structures involved in the social memory phenomenon (Letty et al., 1995), it follows that the most significant role is attributed to the amygdala, and it seems reasonable to speculate that this part of the brain is not affected by EtOH in PRF and NPF rats.

On the basis of our data, it is difficult to explain whether and how the DCC is involved in the short-term memory in PRF and NPF animals. Numerous reports have revealed a relationship between chronic EtOH intake and dysregulation in the circadian patterns of several physiological systems (Sturtevant and Garber, 1988; Hyytia and Sinclair, 1990; Madeira et al., 1997; Baird et al., 1998; Rajakrishnan et al., 1999). As mentioned earlier, total darkness developed a propensity to drink EtOH (Geller, 1971; Sinclair and Geller, 1972; Lin and Hubbard, 1994), and repeated photoperiod phase shifting led to increase of voluntary EtOH intake in animals (Gauvin et al., 1997). Indirect involvement of alteration of circadian rhythm in learning and memory processes has been postulated (Moore and Sphe, 1993); on the other hand, in view of the important role of the pineal gland in EtOH preference (Geller, 1971; Sinclair and Geller, 1972; Blum et al., 1989), the circadian-sensitive neuronal elements (such as pineal gland and suprachiasmatic nucleus) may be involved in changes in cognitive function of EtOH-treated animals. However, there are no significant differences between RID values of CN and CD animals using the STR or LTR procedures; this means that the DCC does not alter short-term memory in EtOH-naive rats. Moreover, since PRF and NPF animals did not differ in the social memory test, the inter-relationships between EtOH preference, DCC and short-term memory in rats are therefore difficult to assess from this study, but the eventual contribution of DCC in the results of social memory in EtOH-treated rats cannot be excluded.

In conclusion, our results confirm the hypothesis that, under some conditions, probably when the toxic effects of EtOH are not as yet observable, EtOH may express a positive influence, especially on short-term memory of adult rats; this corresponds with some data for humans (Dufouil et al., 1997). From our data it is difficult to explain these observations directly. In this study, chronic administration of EtOH was performed on animals commonly regarded as adults (the 8th week of life) and this may be why the adopted procedure did not result in negative effects of EtOH on the STR paradigm in both groups of chronically EtOH-treated animals.

REFERENCES


