Psicológica (2011), 32, 347-365.

Intermittent voluntary ethanol drinking during periadolescence impairs adult spatial learning after a long abstinence period in rats

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Although previous findings point to the long-term impact of ethanol exposure during periadolescence on hippocampal-dependent learning tasks, comparisons considering different onset and exposure periods during this developmental range of ages are still needed. The aim of this experiment was to determine whether intermittent voluntary chronic ethanol consumption onset at different ages, beginning either in pre-adolescence or adolescence, differentially produces impairment in the hidden-platform-water-maze-task performance persisting in adult rats after a 27-day-long period of abstinence. The performance of two groups of adult Wistar rats of both sexes, whose ethanol drinking onset was at postnatal day 19 (preadolescent) or 28 (adolescent), was compared with that of an adult control non-ethanol exposed group. The results indicated that voluntary intermittent ethanol drinking during the periadolescent period caused dramatic long-term detrimental effects in female rats which were unable to learn. Male rats were also impaired during the initial training blocks, the impact being greater in the group exposed during adolescence, but they exhibited no differences with the non-ethanol exposed control group by the end of training (block 6) and in a probe trial. These data support a greater vulnerability in females during periadolescence and point to adolescence as an especially sensitive period during male development to the long-term detrimental effects of ethanol in learning.

* Acknowledgments: This research was supported by CICYT grants #PSI2008-03933 and #PSI2009-10627 (MICINN, Spain) as well as grant #HUM-02763 (Junta de Andalucia, Spain), partially funded by FEDER. The authors are grateful to Mr. Irin D. Evans for language editing of the manuscript. Correspondence: Milagros Gallo and Ana Díaz, Departamento Psicología Experimental y Fisiología del Comportamiento, Universidad de Granada, Campus Cartuja, Granada 18071, Spain; Fax: + 34 958240664; E-mails: mgallo@ugr.es; anadiaz@correo.ugr.es

Detrimental effects of ethanol on learning ability in adulthood have been reported following both acute and chronic ethanol treatments in adult rats (Brunell & Spear, 2006; Lukoyanov, Sá, Madeira, & Paula-Barbosa, 2004; Santucci, Cortes, Bettica, & Cortes, 2008). Especially, hippocampal dependent learning seems to be highly susceptible to ethanol impact, the search for a hidden platform in the water maze being one of the most extensively applied tasks to examine this (Acheson, Richardson, & Swartzwelder, 1999; Boulobard, Lelong, Daoust, & Naassila, 2002; Lukoyanov, Andrade, Dulce Madeira, & Paula-Barbosa, 1999; Santucci, et al., 2004; Santucci et al., 2008). Moreover, it has been long assumed that ethanol exposure during early developmental stages induces more robust pernicious consequences on learning capabilities than adult treatments.

In fact, in addition to the reported harmful effects on brain and cognition of prenatal and neonatal ethanol administration (Berman & Hannigan, 2000; Molina, Spear, Spear, Menella, & Lewis, 2007), adolescence has been proposed as a particularly vulnerable developmental period for ethanol´s deleterious effects on learning (Barron et al., 2005); the evidence however from animal models at present is not conclusive (see Chin, Van Skike, & Matthews, 2010, for a discussion on the topic). It has also been reported that ethanol administration during adolescence leads to long-term cognitive deficits that may persist into adulthood (Barron et al., 2005; Girard, Xing, Ward, & Wainwright, 2000; Schulteis, Archer, Tapert, & Frank, 2008; Siciliano & Smith, 2001; Sircar & Sircar, 2005; Sircar, Basak, & Sircar, 2009). Nevertheless, most of these studies have applied forced ethanol administration, for example intraperitoneal (Sircar & Sircar, 2005; Sircar et al., 2009), intragastric (Girard et al., 2000), via vapor inhalation (Schulteis et al., 2008) or forced drinking with ethanol being the only fluid available (Siciliano & Smith, 2001). To our knowledge there are no previous reports using voluntary intermittent ethanol consumption, which would be more relevant as a model of the human adolescent pattern of drinking behavior in which periods of ethanol consumption are segmented between periods of abstinence (Masten, Faden, Zucker, & Apear, 2009; Chin et al., 2010).

Other relevant issues that may lead to confusion in this field are related to the temporal limits of adolescence. According to Spear (2000), adolescence in rats extends from the postnatal day 28 (PN28) to PN42 if a strict criterion is applied, even though the boundaries are difficult to establish due to individual differences. Accordingly, a bulk of results has pointed to the emergence during this period of learning and memory functions requiring a mature hippocampus. It has been reported a maturational deficit in preadolescent rats younger than 25 days of age in the

spatial abilities required for learning the relationship between the hidden platform and distal cues (Manrique, Molero, Cándido, & Gallo, 2005). Furthermore, it has also been suggested that different functions of context cues in learning and memory show different developmental courses, thus emerging during the adolescence the contextual specificity of latent inhibition in a variety of aversive learning tasks, such as odor-aversive conditioning (Yap & Richardson, 2005), and taste aversion learning (Manrique, Gámiz, Morón, Ballesteros, & Gallo, 2009). Additionally, adolescent rats exhibit peculiar learning features, such as an enhanced disposition to learn about context in fear conditioning tasks that it is not seen in infants or in adults (Esmorís-Arranz, Mendez & Spear, 2008). Thus, adolescence can be envisaged as a sensitive period for hippocampal-dependent tasks. Consistent with the hippocampal neurophysiology (White & Swartzwelder, 2004), it is conceivable that alcohol drinking during adolescence alters the hippocampal activity during a developmental sensitive period, leading to long-lasting modifications of the hippocampal function, thus impairing adult learning abilities that are emerging during this period.

However, drawing conclusions on the particular relevance of adolescence as a critical period for the long-term effects of ethanol requires investigating previous preadolescent stages. This seems to be of particular relevance given the prevalence of underage alcohol consumption in humans, which is considered as a developmental problem (Masten et al., 2009). To our knowledge, there are no studies comparing the adulthood-persisting detrimental effects of various voluntary ethanol drinking onset ages during periadolescence on spatial learning abilities in order to identify a potential sensitive period. Thus, we have taken advantage of a rodent model of voluntary intermittent ethanol drinking developed in our laboratory (Garcia-Burgos, González, Manrique, & Gallo, 2009; Garcia-Burgos, Manrique, Gallo, & González, 2010) following that of Spanagel & Holter (1999) in order to compare the effect of ethanol drinking onset during pre-adolescence (PN19) with that of adolescence (PN28) on the performance in the hidden–platform-water-maze task during adulthood. A non ethanol exposed adult group served as control. It should be stressed that it is a model of voluntary ethanol consumption with water and food always being simultaneously available and by no means a model of ethanol addiction, as shown by our previous results. The rats were part of the subjects used in a previous study aimed at exploring the effect of the developmental period on early voluntary intermittent alcohol consumption and withdrawal. Thus, groups of different ages (including pre-adolescence and adolescence) have been compared along a first 10-day ethanol availability exposure period

followed by a 7-day abstinence period (Garcia-Burgos et al., 2009). Additionally, the groups received a number of subsequent ethanol availability and abstinence episodes (Garcia-Burgos et al., 2010). According to the recommendations for reduction of the number of animals used in research (European Communities Council Directive of 24 November 1986; 86/609/EEC, article 7.3), we have considered of great value to assess their performance on the hidden-platform navigation task during adulthood, after a 27-day-long abstinence period. We hypothesize that if the adolescence covers a critical window period for inducing a long-lasting ethanol detrimental impact on spatial learning ability, the group PN28 should exhibit greater adult impairments in the acquisition of the hidden-platform-water-maze task than group PN19, with both being impaired in comparison with a control non-ethanol exposed group. Additionally, since there are scarce data regarding sex-dependent vulnerability to ethanol-induced effects during adolescence (Siciliano & Smith, 2001; Sircar et al., 2009) the groups included both males and females in order to explore this issue.

METHOD

Subjects. Forty-three Wistar rats (21 male and 22 female) were assigned to 3 groups: two exposed to ethanol at different postnatal days (PN), including PN19Et (7 males and 7 females) and PN28Et (6 males and 7 females), and a control non-exposed group PN90Ctrl (8 males and 8 females). Food and tap water were available *ad libitum* in the home cage throughout the behavioral procedure. The animals were maintained in a 21±1ºC temperature controlled vivarium on a 12 hr light-dark cycle (lights on at 8:00 am). As required by the experimental design in order to record fluid consumption, animals belonging to the groups PN19Et and PN28Et were individually housed during the alcohol exposure period. Subjects in PN90Ctrl were individually housed from their arrival to the lab well before the beginning of the behavioral procedure. All the experimental procedures were approved by the University of Granada Animal Research Ethics Committee, and in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Procedure and apparatus. The PN19 and PN28 groups received four phases of ethanol exposure (Figure 1).

During these phases they had continuous simultaneous access to four bottles containing water and 5%, 10%, and 20% (v/v) ethanol solutions. Ethanol solutions were prepared with tap water and 96% ethanol apt for

consumption (Ethanol 96% v/v PA-ACS, Panreac Química SAU, Barcelona, Spain). In all (see Table 1), intake was recorded for 55 days in animals initiating ethanol drinking in preadolescence (PN19Et) and for 46 days in those animals whose exposure began in adolescence (PN28Et). Further details can be found in Garcia- Burgos et al. (2009; 2010).

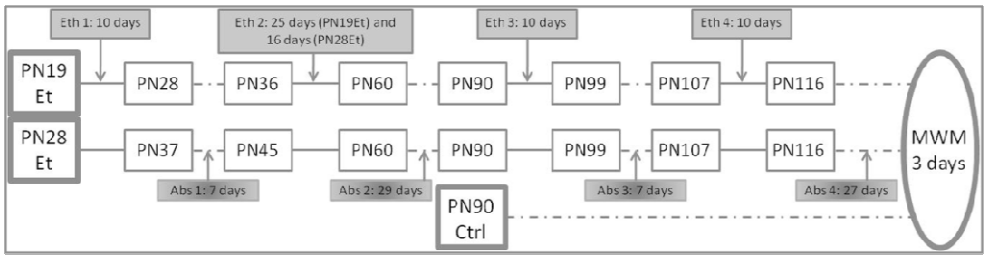

Figure 1. Schematic representation of the four ethanol exposures and withdrawal phases applied to PN19Et and PN28Et groups before the behavioral procedure (MWM; Morris Water Maze) which was similar in the three groups: PN19Et, PN28Et and PN90Ctrl.

The Morris water-maze phase of the experiment was performed after a long period of abstinence (27 days). The behavioral procedure was based on that described in Manrique et al. (2005). The pool consisted of a 200 cm diameter and 50 cm deep circular plastic tank located in a 4 x 5 m room containing a great amount of extra-maze cues (electrophysiological instruments, posters, lights, video-camera, etc) visible to the swimming animal. The temperature of the water was maintained at 24-26 ºC. The pool was divided conceptually into four quadrants, and the 11 cm diameter circular platform was placed in a fixed location approximately 35 cm from the pool border in the centre of one of the quadrants. Each subject received 6 blocks of training (4 trials per block), applied in 2 daily sessions during 3 consecutive days. Each trial began by placing the subject into the water facing the pool wall at one of four compass conditions (east, west, north or south). The order varied randomly. Each animal was allowed to swim freely for 60 sec or until it climbed onto the platform. All of the rats spent the last 15 sec of each trial on the platform. Immediately after the last trial, the

platform was removed and a probe trial was performed. Each subject was allowed to swim during 60 sec. A video system and associated software (Noldus, EthoVision 3.1) was used to record escape latency, speed, path length and searching patterns.

RESULTS

Data on the consumption of preadolescent and adolescent groups during the first and subsequent ethanol exposure episodes are discussed in detail elsewhere (Garcia- Burgos et al., 2009; 2010). However, a 2 x 2 (Group x Sex) ANOVA on the ethanol intake during adulthood by both groups, i.e. of the ethanol episodes from PN90 to PN99, did not reveal any significant effect of Group ($F(1,23) = 0.20$; $p > .6$), Sex ($F(1,23) = 0.84$; $p > .3$) or the interaction Group x Sex ($F(1,23) = 0.20$; $p > .6$). A similar analysis of the ethanol consumption from PN107 to PN116 also yielded no significant effect of Group ($F(1,23) = 0.34$; $p > .5$), Sex ($F(1,23) = 0.33$; $p > .5$) or the interaction Group x Sex ($F(1,23) = 2.70$; $p > .1$), (see Figure 1 and Table 1). Thus, in spite of having been exposed to two previous unequal ethanol amounts during preadolescence or adolescence, the groups PN19Et and PN28Et did not differ either in the average ethanol amount consumed during adulthood. Also there were not differences between the groups in body weight at the end of the ethanol drinking period or Group x Sex interaction ($Fs<1$) . As expected, the only significant effect was that of Sex ($F(1,23) = 185.15$; $p < .01$).

Table 1. Mean (± SEM) alcohol intake (g/kg) during exposure periods before the Morris Water Maze. Numbers in parentheses are postnatal days (PN) of ethanol solutions availability.

Groups	Sex	N	Exposure				Days of total exposure
			1	2	3 (PN90-99)	4 (PN107-116)	
PN19Et	M	7	12.2 ± 3.5 (PN19-28)	4.2 ± 0.9 (PN36-60)	2.7 ± 0.7	3.1 ± 1.1	55
	F	7	11.5 ± 2.5 (PN19-28)	6.4 ± 2.0 (PN36-60)	3.0 ± 0.9	2.4 ± 0.6	55
PN28Et	M	6	6.9 ± 1.5 (PN28-37)	3.7 ± 0.8 (PN45-60)	2.1 ± 0.6	1.7 ± 0.6	46
	F	7	8.3 ± 3.2 (PN28-37)	4.9 ± 1.5 (PN45-60)	3.0 ± 0.7	3.0 ± 0.7	46

Concerning the statistical analyses of the performance in the hidden-platform–water-maze task, for brevity only the results concerning path length (distance) are reported since this variable takes into account both latency and speed. In any case, the results using these latter variables are consistent with those of distance.

During acquisition along the 6 blocks of trials, a 3 x 2 x 6 (Group x Sex x Block) analysis of variance (ANOVA) yielded significant main effects of Group, ($F(2,37) = 19.71$; $p < .01$) and Block ($F(5,185) = 8.56$; $p < .01$), but not of Sex ($p > .3$). All interactions, except Group x Sex, ($F(2,37) = 2.60$; $p > .08$), were significant, Group x Block ($F(10,185) = 8.22$; $p < .01$), Sex x Block ($F(5,185) = 3.01$; $p < .05$) and Group x Sex x Block ($F(10,185) = 2.68$; $p < .05$).

To analyze the triple interaction, several two-way ANOVAs were performed. First, we analyzed the effects of groups and blocks for male and female rats separately.

Thus, two 3 x 6 (Group x Block) ANOVAs were conducted on the distances to reach the platform for each sex. Mean distances swam by the different groups to reach the platform in each block of training are depicted in Figure 2 (A, males; B, females).

Regarding male rats (Figure 2A), the main effects of Group ($F(2,18) = 22.20$; $p < .01$), and Block ($F(5,90) = 11.34$; $p < .01$) were significant, as well as the Group x Block interaction ($F(10,90) = 5.99$; $p < .01$). Several one-way ANOVAs with group as the between groups factor and LSD post hoc tests were performed to analyze the differences among groups in each training block (see Table 2, Male, by block).

Regarding female rats (see Figure 2B) the 3 x 6 (Group x Block) ANOVA yielded a significant effect of Group ($F(5,19) = 3.75$; $p < .05$), Group x Block interaction ($F(10,95) = 5.03$; $p < .01$) but no effect of Block ($F(5,95) = 1.15$; $p = .3$). Table 2 (Female, by block) shows the results from the several one-way ANOVAs performed for each block and the LSD post hoc test to explore differences among groups.

To determine differences during the acquisition-learning phase, the main goal of the study, group and sex effects were analyzed along blocks of training by several repeated measured ANOVAs and LSD post hoc tests. Concerning males (Table 2, Male, along blocks), a significant decrease in path length was evident in PN90Ctrl group by block 3 when compared with both block 1 and 2, whereas the distance did not decrease in groups PN19Et and PN28Et until blocks 4 and 5 if we compare them with block 3.

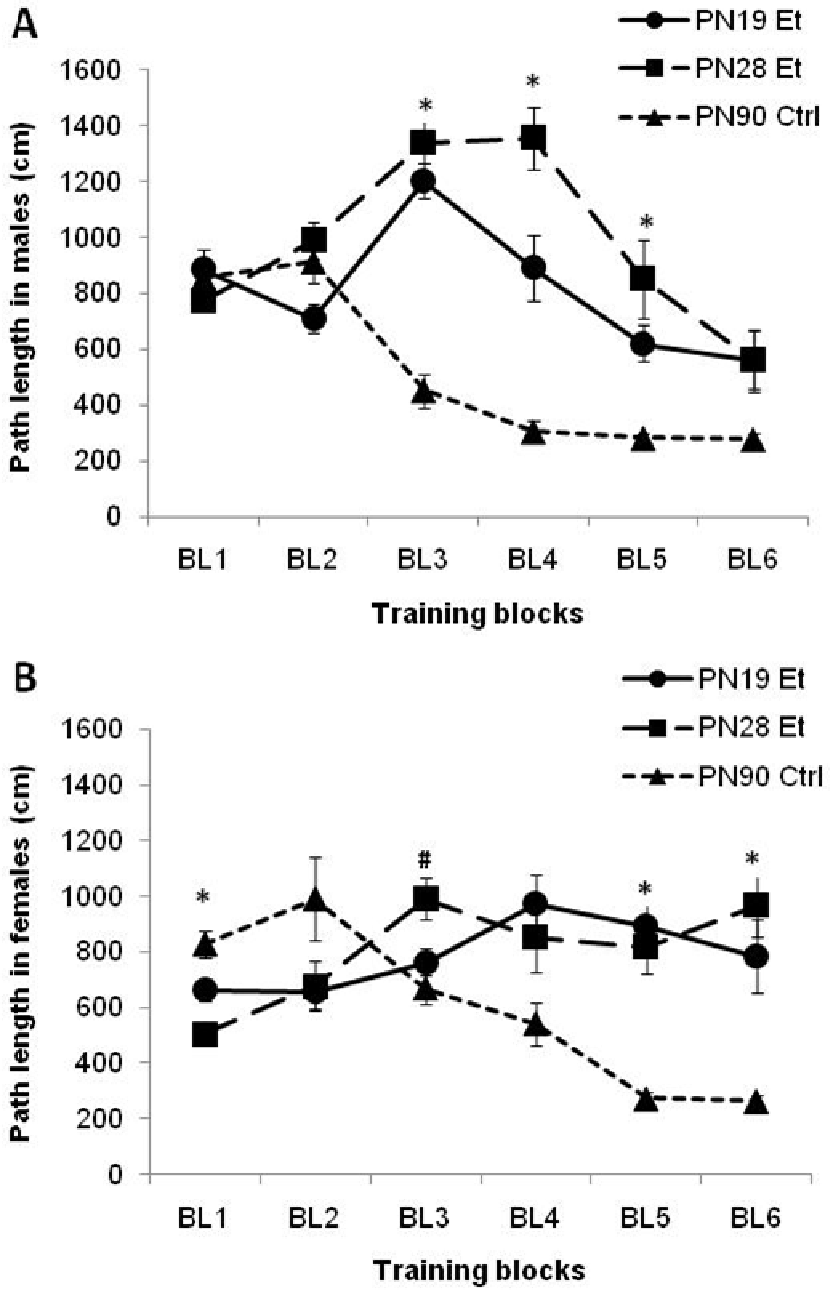

Figure 2. Mean (±SEM) distance to reach the platform during acquisition for male (A) and female (B) rats in each group (* p< 0.01 and # p< 0.05).

Table 2. Summary of the triple interaction Group x Sex x Block analyses. Fs values and multiple comparisons following post hoc LSD tests coming from several ANOVAs performed on the distances swam to reach the platform in each block of training by adult male and female rats exposed to ethanol at different ages (PN19Et, PN28Et), and non exposed PN90Ctrl.

Males	**By block**	Block 3	F(2,18) = 27.34; p < .01 PN90Ctrl < PN19Et = PN28Et
		Block 4	F (2,18) = 16.29; p < .01 PN90Ctrl < PN19Et < PN28Et
		Block 5	F(2,18) = 6.33; p < .05 PN90Ctrl < PN19Et = PN28Et
	Along blocks	PN19Et	F(5,30) = 3.73; p < .01 bl1=bl2; bl2 <bl3; bl3>bl5, bl6; bl4=bl5, bl6; bl5=bl6
		PN28Et	F(5,30) = 3.73; p < .01 bl1= bl2; bl3>bl1, bl5, bl6; bl4>bl5, bl6; bl5=bl6
		PN90Ctrl	F(5,35) = 22.41; p < .01 bl1= bl2 > bl3, bl4 = bl5 = bl6
Females	**By block**	Block 1	F(2,19) = 3.75; p < .05 PN90Ctrl > PN28Et = PN19Et
		Block 3	F(2,19) = 3.68; p < .05 PN28Et > PN90Ctrl = PN19Et
		Block 5	F(2,19) = 10.62; p < .01 PN90Ctrl < PN19 = PN28
		Block 6	F(2,19) = 7.480; p < .01 PN90Ctrl < PN19 = PN28
	Along blocks	PN19Et	F(5,30) = 1.07; p = .39 (no significant)
		PN28Et	F(5,30) = 2.38; p = .06 (marginally significant) bl1 < bl3 = bl4 = bl5 = bl6
		PN90Ctrl	F(5,35) = 9.05; p < .01 bl1, bl2 > bl4, bl5, bl6; bl2 > bl3 ; bl4 > bl5; bl5 = bl6

There were no differences between the groups at the end of training. It should be emphasized that PN28Et appeared to show a greater impairment than PN19Et, since that group swam significantly longer distances than PN19Et in block 4, exhibiting both ethanol exposed groups longer path lengths than PN90Ctrl control group.

Regarding females (Table 2, Female, along blocks), the results indicate that only females in PN90Ctrl group reduced the distances swam to reach the platform from block 2, reaching the learning asymptote by block 5. Female rats in groups PN19Et and PN28Et did not show any evidence of learning.

Additionally, there were significant Sex x Block interactions both in group PN19Et, ($F(5,60) = 2.55$; $p < .05$), (males swam longer distances than females on block 3; $p < .01$) and group PN28Et, ($F(5,55) = 3.45$; $p < .001$), males swam longer distances than females in block 1, ($F(2,18) = 22.20$; $p < .001$), and 3 ($F(2,19) = 3.75$; $p < .05$). There were no differences related to sex in group PN90Ctrl.

In summary, the exploration of the triple interaction showed that males learnt in the PN90Ctrl group, while both male groups PN28Et and PN19Et swam longer distances and exhibited a delayed acquisition, these effects being more evident for group PN28Et. Females also learnt in the PN90Ctrl group, but not in groups PN19Et and PN28Et. Thus, exposure to ethanol both in pre-adolescence and adolescence impaired the performance during acquisition in both sexes. The effect was more pronounced in males at the beginning of the acquisition process during the initial blocks of trials in which they swam longer distances than females, and especially in group PN28Et, which exhibited a worse performance than group PN19Et in block 4. The longer pathways swam by males in block 3 reflected higher speed during the second training day, mainly during the fourth morning block, since no increase in latencies to reach the platform was found (data not reported). However, a decreasing curve from block 3 to block 6 can be seen in both groups of males exposed to ethanol, thus reflecting spatial learning. The group PN19Et swam shorter distances in block 4 ($p = .08$), block 5 ($p < .01$) and block 6 ($p < .01$) than that recorded in block 3, with no differences between blocks 5 and 6 ($p > .7$). The PN28Et swam shorter distances in block 5 and block 6 ($p < .05$), than those of both block 3 and block 4, and no differences were seen between the blocks 5 and 6 ($p > .1$). Moreover, distances swam in block 6 were marginally shorter than those swam in block 1 ($p = .06$) in group PN19Et and shorter than those swam in block 2 ($p < .05$) in group PN28Et. However, females belonging to PN19Et and PN28Et groups showed no evidence of learning; swimming longer distances than PN90Ctrl during the last blocks of trials (see Table 2).

These conclusions seemed to be supported by the results of the immediate probe trial without platform (Figures 3A and 3B). A 3 x 2 x 2 (Group x Sex x Quadrant) three-way ANOVA performed on the time spent in the target versus the opposite quadrant yielded significant main effects of both Group ($F(2, 37) = 4.25$; $p < .05$) and Quadrant, ($F(1, 37) = 53.97$; $p < .0001$), as well as the Sex x Quadrant interaction ($F(1, 37) = 8.57$). No other main effects or interactions were significant. Taking into account the results of the training phase, and the clear differences in performance between sex during training, especially in the last block, we thought it could be worth testing the following a priori contrasts through planned comparisons:

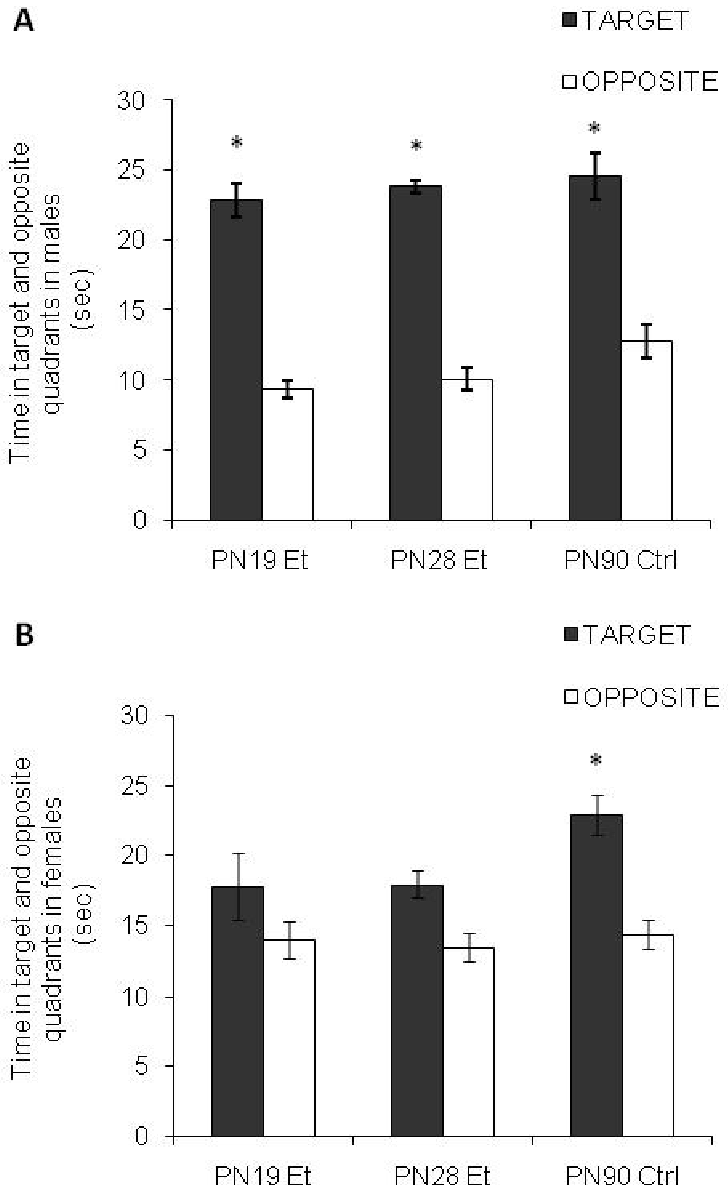

Figure 3. Mean (±SEM) time spent in the target and opposite quadrants during the probe trial for male (A) and female (B) rats in each group (* $p < 0.01$).

a) there should not be differences in performance among male groups; b) for females, only PN90Ctrl group should significantly spend more time in the target quadrant than in the opposite one, as it was the only group which showed a learning curve during training. The results confirmed the predictions since regarding males, there were no differences when comparing PN19 and PN28 groups with PN90Ctrl group ($F < 1$); moreover, in each group the rats spent more time swimming in the target quadrant than in the opposite: PN19 ($F(1, 19) = 28.54$; $p < .01$); PN28 ($F(1, 19) = 25.46$; $p < .01$); PN90Ctrl ($F(1, 19) = 24.83$; $p < .01$). On the contrary, PN19 and PN28 female groups differed when compared with PN90Ctrl ($F(1, 18) = 5.50$; $p < .05$). While group PN90Ctrl searched longer time in the target than in the opposite quadrant ($F(1, 18) = 6.41$; $p < .05$), both PN19 and PN28 groups spent a similar amount of time in each quadrant (largest $F(1, 18) < 1.55$; $p = .28$), thus evidencing absence of learning in both groups exposed to ethanol either during the preadolescent or the adolescent period.

DISCUSSION

The results reported confirm previous data showing that ethanol consumption has a lasting deleterious impact on spatial learning even after long abstinence periods (Santucci et al., 2008). In the present experiment, after a 27-day abstinence period, the two ethanol exposed groups exhibited acquisition learning deficits compared with the control non-ethanol exposed group. This period of abstinence was long enough to expect any effect of ethanol withdrawal to have vanished. Following chronic ethanol consumption during 25 days (Celik, Cakir, Kayir, Bilgi, & Uzbay, 2005) and 35 days (Bilgi, Tokgöz, Aydin, Celik, & Uzbay, 2003) increased serum cholinesterase activity has been observed in Wistar rats after 24 h of ethanol withdrawal, but it returned to control levels after 72 h of ethanol withdrawal. In general, research on the temporal course of ethanol withdrawal signs in Wistar rats indicates peak intensities in the range of 12 and 24 hours (Macey, Schulteis, Heinrichs, & Koob, 1996). Since the abstinence period followed four voluntary intermittent ethanol-drinking episodes from periadolescence to early adulthood (PN116), it is not possible to relate the learning impairment with a specific temporal window during development. Nonetheless, taking into account previous reports of adult impairments in conditional discrimination learning and object recognition after forced ethanol administration during adolescence following 20-day-long withdrawal periods (Pascual, Blanco, Caulli, Miñarro, & Guerri, 2007), the results support the long-term deleterious effect of periadolescence drinking on adult learning ability. An unspecific deficit on

motor ability can be excluded as a potential explanation of the adult deficits reported since no speed differences between the groups were found. Thus, the data allow us to draw several conclusions regarding the involvement of the ethanol drinking onset age in the learning deficits reported.

First, the nature of the acquisition-learning impairment found in the present study differed in males and females. While females showed no evidence of learning, a significant increase in path length by block 3 was evident in male rats. This lead to a decreasing slope during the last trials which can be considered a learning curve if block 3 is taken as the reference point. In fact, ethanol exposed males reached similar values to control non-exposed groups by the end of training, while females exhibited significantly longer path lengths during the last trials. Consistently, the non-platform probe trial indicated a different pattern of search in male and female groups. While all the male groups spent significantly longer time searching in the target than the opposite quadrant, female groups exposed to ethanol spent similar time in both quadrants. This is consistent with previous findings pointing to a greater vulnerability of females to ethanol´s deleterious impact (Barron & Riley, 1990; Kelly, Goodlett, Hulsether, & West, 1988), although there have been also reports failing to support the "female vulnerability to alcohol toxicity" hypothesis (Goodlett & Petterson, 1995). No effect of sex was seen in the control group never exposed to ethanol.

Second, adolescence may be proposed as a more vulnerable period than preadolescence in male rats regarding the deleterious effect of early alcohol exposure on spatial learning. Accordingly, PN28Et group exhibited longer distance than PN19Et taking into account block 4. Thus, the results show greater impairment by ethanol consumption during a period that covers most of the strict adolescent window that Spear (2000) located between PN28-PN42. As a matter of fact, the first ethanol availability phase for PN28Et group lasted from PN28 to PN37. On the contrary, ethanol was not available to PN19Et group during the period covering from PN29 to PN35, since it matched the first abstinence phase after the initial ethanol availability from PN19 to PN28. Thus, a greater learning impairment in PN28Et group points to adolescence as an especially sensitive period during male development to the long-term effects of ethanol. This conclusion is supported by the fact that opposite results should be expected if other variables, such as the total ethanol consumption and total duration of ethanol exposure, were critical. It should be taken into account that the use of a voluntary drinking model, closer to a natural setting, leads to unavoidable differences in ethanol intake. As it has been described in detail elsewhere (Garcia-Burgos et al., 2009) there is an inverse relationship between age and ethanol consumption. The group PN19Et thus drank higher

ethanol doses than the PN28Et group in the first period of exposure. Also, PN19Et group drank alcohol for 9 days more (55 instead of 46 days) than PN28Et, due to the need of equating the last abstinence period in both groups. Nevertheless, the results showed less impairment in PN19Et than in PN28Et group, since there were significant differences between these male groups in block 4. It seems clear that if either the dose or the length of the total ethanol consumption period had been the critical variables for the impairment induced by ethanol drinking, significant differences between both groups would have been evident in the opposite direction.

Furthermore, even though it cannot be discarded a deleterious effect of early isolation in the ethanol exposed groups on adult learning, the fact that both male groups exhibited different magnitude deficits in spite of having been subjected to identical housing conditions support a selective impact of alcohol intake on the development of learning and memory brain circuits.

In all, our results lend support to previous proposals claiming that adolescence may represent a developmentally sensitive period with respect to the effects of ethanol on neurobehavioral development (Acheson et al., 1999; Rice & Barone, 2000; Spear & Varlinskaya, 2005; White & Swartzwelder, 2004, 2005). It can be proposed that the dramatic impact of ethanol drinking during adolescence on spatial learning tasks might be related with the protracted hippocampal maturation during this developmental period. Consistently, the learning impairment induced by ethanol administration has been attributed to selective effects on brain development, especially affecting the hippocampus and related areas (Guerri & Pascual, 2010; Squeglia, Jacobus, & Tapert, 2009; Witt, 2010). In fact, ethanol administration has been proposed as a tool for inducing performance deficits similar to those produced by hippocampal lesions (Matthews & Silvers, 2004). Since the hippocampus is a late-developing brain region during ontogeny, a bulk of the available data points to a delayed functional emergence during the periadolescent period of learning abilities requiring a mature hippocampus (Stanton, 2000). Consistently, adolescent learning presents peculiar features (Manrique et al., 2009) and the ability to perform the hidden-platform-water-maze task is not well developed during this period (Manrique et al., 2005). A sensitive period during adolescence for the effects of ethanol on spatial learning is consistent also with ethanol´s effect on hippocampal neurophysiology (White & Swartzwelder, 2004; Witt, 2010). A different pattern of neuronal cell death in adolescent and adult rats after heavy episodic ethanol exposure has been reported (Crews, Brawn, Hoplight, Switzer, & Knapp, 2000). Different seizure susceptibility during ethanol withdrawal in adolescent and adult rats

has also been described (Acheson et al., 1999). It can be envisaged that alcohol drinking during adolescence alters the hippocampal activity during a developmentally sensitive period, leading to long-lasting modifications of hippocampal function, thus impairing acquisition in the water-maze task during adulthood. However, no conclusions can be drawn from the present results about the specific ethanol-induced mechanism causing the impairment, since a variety of actions, including decreased body weight, have been reported. Although there were no differences between the groups in body weight at the end of the ethanol exposure phase, an early effect during development can not be discarded. Also the nature of the intermittent alcohol consumption does not allow us to dissociate between the potential pernicious effect on development of either ethanol intake or the abstinence periods.

We would like to stress the relevance of using experimental settings similar to those found in natural situations in order to understand the effect of ethanol drinking during development. In fact, auto-administration of extremely high ethanol doses is a typical and unavoidable feature of voluntary consumption both in young animals (Molina et al., 2007; Vetter, Doremus-Fitzwater, & Spear, 2007) and humans (Brown & Tapert, 2004; Masten et al., 2009; Windle et al., 2008). However, this pattern of ethanol intake does not necessarily lead to increased ethanol consumption in adults and it cannot be considered a model of ethanol dependence similar to those procedures including forced administration of high ethanol doses (Morris, Kelsom, Liput, Marshall, & Nixon, 2010; Santin, Rubio, Begega, Miranda, & Arias, 2000). As a matter of fact, we found no differences in the amount of ethanol drank by the PN19Et and PN28Et groups during adulthood, thus suggesting that all the groups were in similar conditions by the time of testing, at least as it relates to ethanol drinking behavior. Although it has been demonstrated that fetal or infantile ethanol administration promotes adolescent and adult ethanol drinking (Spear & Molina, 2005), our results indicate no effect of periadolescent voluntary drinking on mean ethanol intake during adulthood.

At present, there is great concern about underage use of alcohol and its consequences for development given the high rates of risky drinking patterns during periadolescence in humans (Masten et al., 2009; Matthews, 2010; Witt, 2010). It is also becoming clear that different stages during human pre-adolescence and adolescence should be addressed independently as underage alcohol drinking is a developmental phenomenon (see the issue of Alcohol Research and Health, 2009, 32(1) devoted to it). Therefore, animal research on this topic should benefit from voluntary intermittent drinking models of human ethanol-use patterns in order to understand its

origin and consequences. Using such a model, our results show complex long-term effects of periadolescent ethanol intake on adult spatial learning, with females being more vulnerable than males, while males still exhibit a sensitive period covering adolescence.

RESUMEN

El consumo voluntario intermitente de etanol durante la periadolescencia deteriora el aprendizaje espacial en ratas adultas después de un largo periodo de abstinencia. Aunque hallazgos previos indican que la exposición a etanol durante el periodo periadolescente ejerce un impacto a largo plazo sobre la ejecución de tareas de aprendizaje dependientes del Hipocampo, se carece de comparaciones que tengan en cuenta diferentes periodos de inicio y duración de la exposición dentro del rango de edades incluidas en esta etapa del desarrollo. El objetivo del presente experimento fue determinar si el inicio a diferentes edades, bien en la preadolescencia bien en la adolescencia, produciría un deterioro persistente observable en ratas adultas después de un periodo de abstinencia de 27 días sobre la ejecución de la tarea de búsqueda de plataforma oculta en el laberinto acuático. Para ello se comparó la ejecución de dos grupos de ratas Wistar adultas de ambos sexos que habían iniciado el consumo bien el día postnatal 19 (preadolescentes) bien el día postnatal 28 (adolescentes) con la ejecución de un grupo control adulto no expuesto a etanol. Los resultados indicaron que el consumo voluntario intermitente de etanol durante el periodo periadolescente causó un dramático efecto a largo plazo en las ratas hembras, las cuales fueron incapaces de aprender. Las ratas macho mostraron también deterioro durante los bloques de ensayos iniciales, siendo mayor el impacto en el grupo expuesto durante la adolescencia, pero no difirieron del grupo control sin exposición a etanol al final del entrenamiento (bloque 6), ni en un ensayo de prueba. Estos datos sugieren una mayor vulnerabilidad en las hembras durante la periadolescencia y presentan la adolescencia como un periodo especialmente sensible en el desarrollo de los machos para los efectos perniciosos del etanol sobre el aprendizaje adulto.

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(Manuscript received: 29 July 2010; accepted: 25 November 2010)