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Alcohol tolerance in rats submitted to different periods of chronic and acute ethanol intake

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The development of tolerance to the effects of ethanol is not uniform and may vary according to the actual and previous pattern of consumption. In this experiment we assessed body temperature and the recovery of two reflexes after a high dose of ethanol in rats submitted to chronic and acute ethanol consumption. Animals were previously submitted to chronic or acute alcohol consumption from postnatal day 21 until postnatal days 56 and 84. On the testing days, the animals received a single dose of 25% ethanol (5 g/kg, i.p.) or the same amount of saline solution. The results showed that animals were affected in the day 56 to a greater extent than in the day 84 by chronic heavy consumption of ethanol solution. With moderate and acute ethanol consumption, the 56-day-old animals developed greater tolerance. However, tolerance was not developed for the motor-impairing effects, since all groups required a long time to recover reflexes.

Tolerancia al alcohol en ratas sometidas a diferentes períodos de consumo agudo y crónico de etanol. El desarrollo de tolerancia a los efectos del alcohol no es uniforme, y suele variar según el patrón de consumo previo y actual. En este trabajo se evaluó la temperatura corporal y el tiempo de recuperación de dos reflejos tras el consumo crónico y agudo de elevadas dosis de etanol. Previamente, los animales bebieron alcohol de forma crónica o aguda desde los 21 hasta los 56 y 84 días de edad. Durante los días de evaluación, los animales recibieron una única dosis de etanol al 25% (5 g/kg, i.p.), o la misma cantidad de solución salina. Los resultados mostraron una mayor afectación a los 56 días de consumo crónico elevado de etanol respecto a los 84 días. Con un consumo moderado o agudo de etanol, los animales de 56 días desarrollaron una mayor tolerancia. Sin embargo, esta tolerancia no se observó en cuanto a los déficits motores, dado que todos los grupos necesitaron un largo período de tiempo para recuperar los reflejos.

Alcoholism is a human disorder that causes a great number of social, family and psychological problems in our society. Traffic accidents are the main cause of death among young people and many of these have been caused by high alcohol consumption. Impaired judgment and increased reaction time are most often cited as the causes of alcohol-related traffic accidents (Gómez Fraguela, Luengo Martín and Romero Triñanes, 2002; Naranjo and Bremner, 1993). Laboratory psychopharmacologic studies indicate that doses of ethanol up to 1 g/Kg are associated with a general slowing down of psychomotor and cognitive performance such as reduced information-processing capacity or impaired tracking performance in real and simulated driving tasks (Hindmarch, Kerr and Sherwood, 1991; Maylor and Rabbitt, 1993). Nowadays, young people drink alcohol at an early age and consume large amounts in very short periods of time. Most people begin drinking in adolescence and many recent studies have indicated that adolescence may represent a period of development of great sensitivity to ethanol and that, in this period, behavioral and neuronal aspects are more affected than in adulthood (Rodríguez Franco, Padilla Muñoz, Caballero and Rodríguez, 2002; De Bellis et al., 2000; Acheson, Richardson and Swartzwelder, 1999; Pyapali, Turner, Wilson and Swartzwelder, 1999; Swartzwelder, Richardson, Markwiese-Foerch, Wilson and Little, 1998; Markwiese, Acheson, Levin, Wilson and Swartzwelder, 1998; Swartzwelder, Wilson and Tayyeb, 1995).

One component of alcoholism that is commonly studied is the development of alcohol tolerance after chronic or acute alcohol consumption. According to DSM-IV (American Psychiatryc Association, 1994), alcohol tolerance is the decrease in ethanol effects with regular consumption of the same dose or the necessity to drink more alcohol to experience the same effects. However, the development of tolerance is not uniform and the effects of ethanol decrease to different extents and in different time periods. Previous studies in adult rats have shown that tolerance to the effect of ethanol on body temperature develops prior to tolerance to motor-impairing effects (Pohorecky, Brick and Carpenter, 1986). One of the characteristic response to administration of moderate to high doses of ethanol is a decrease in body temperature. Ethanol-

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induced hypothermia has been utilized extensively in the ethanol research field for several decades as a measure of ethanol's acute effects, and as a model system for the study of ethanol tolerance (Crabbe, Feller, Terda and Merrill, 1990; Crabbe, Janovsky, Young, Kosobud, Stack and Rigter, 1982).

Many models of alcoholism use chronic self-administration to evaluate the effects of intake whereas other models prefer acute administration to determine the punctual effects of ethanol. However, many social drinkers, especially the younger ones, present two different drinking patterns: chronic regular consumption and, occasionally, acute intake of higher doses. Moreover, the amount of ethanol consumed chronically and the pattern of intake may determine the intensity of effects on drinkers. For this reason, the present study was designed to determine the alcohol tolerance to body temperature and motor-impairment effects of ethanol in rats submitted to chronic and acute alcohol consumption after a high dose of ethanol that may be considered as a high acute intake. We assessed body temperature and the recovery of two reflexes after a high dose of ethanol in rats submitted to acute and chronic alcohol consumption with two alcoholic solutions during two periods of time.

Material and methods

Subjects

A total of 128 male Wistar rats, weighing 240-305 g were used in the experiment. They were housed individually in a temperature- and humidity-controlled environment with a 12 hours light/dark cycle (8:00 - 20:00) and the ambient temperature was 24±3ºC. During the experiment, animals had free access to food and liquid. Rats were randomly assigned to one of four experimental groups. (I) Control rats, animals without experimental manipulation (CTR), (II) rats chronically administered a high dose of ethanol (20%, v/v) (ALCH), (III) rats receiving chronic treatment with a moderate dose of ethanol (10%, v/v) (ALCM), and (IV) rats submitted to acute intake of ethanol (ALCA). Each of these groups was then divided into two subgroups, one of which was evaluated on postnatal day 56 (PD-56) and the other group on postnatal day 84 (PD-84). Then, four groups of sixteen animals were evaluated when the animals submitted to chronic alcohol intake reached the highest dose of ethanol solution (10% or 20% in the PD-56), and the remaining four groups were studied four weeks later.

Alcohol consumption procedure

Alcohol-treated rats (ALCH and ALCM) had free access to an aqueous ethanol solution (Panreac, Barcelona, Spain) as the only available liquid source from 21 days old to the end of experiment (postnatal days 56 and 84). In ALCH group, the administration was gradual, starting with 2% (v/v) in the first week after the 21st postnatal day with a doubling dosage each week until it reached the 16% (v/v). In the following week, the dose was 20% (v/v) until the end of the experiment. In the ALCM group, the administration was 2%, 4%, 6%, 8% each week, and 10% until the end of the experiment. Animals first received the ethanol solution at postnatal day 21 to avoid rejection due to the taste of ethanol (Capretta, Petersik and Stwart, 1975) and so they would consume similar amounts of liquid as control animals (Table 1). This model provokes the progressive alcohol consumption of the animals and produced hepatic lesions in the animals after nine months similar to those observed in alcoholic liver disease in humans (García-Moreno, Corcuera, Conejo, Martín, Gómez and Alonso, 1998). Finally, animals from ALCA group received 2 gr/Kg of ethanol in saline solution (25%, v/v) once per week by intraperitoneal injection (IP). This procedure does not cause dehydration or loss of electrolytes in animals (Tang and Falk, 1986). On the day of the evaluation (PD-56 or PD-84) the animals received a single dose of ethanol solution administered intraperitoneally (5 g/Kg, 25% sol. v/v) or the same amount of saline solution to evaluate its effects on the animals' body temperature and motor behaviour. We used this dose and route of administration because they produced sedation (Swartzwelder et al., 1998). We observed that the most frequent dose range used in similar experiments ranges between 1.5 and 3.2 g/Kg (Khanna, Kalant, Weiner and Shah, 1992; Lomax, Bajorek, Chesarek and Chafee, 1980); however, we also found works that used higher doses (Swartzwelder et al., 1998; Le, Khanna and Kalant, 1984). We opted for a high dose to ensure a sedative effect and also because we thought that a higher dose would facilitate identification of differences in previous consumption pattern.

Body temperature (expressed in degrees centigrade)

From three days before evaluation, we recorded daily the colonic temperatures of all rats in the morning (between 9'00 and 10'00 a.m.) using a digital thermometer OMRON MC-38 (Omron Matsusaka Co Ltd. Japan). On the testing day, we also measured the colonic temperatures of animals immediately before they received the intraperitoneally injection of ethanol. After this, the colonic temperatures were measured again 90 and 180 minutes after the IP injection to determine possible temperature changes.

Reflexes

We also tested two righting reflexes every 15 minutes after IP ethanol injection until the animals reached the criterion of reflex recovery. First we evaluated the floor-righting reflex, the ability of each animal to right itself (all legs on the floor) within 3 seconds after being placed on its back. Reflex was recovered when the animal successfully righted itself twice consecutively. We then evaluated the fall-righting reflex. When the rat is dropped upside down from a 40 cm height onto a soft surface, it turns during the fall and lands on all four legs. This reflex was recovered when the animal landed correctly twice consecutively.

Changes in body temperature after IP (expressed in degrees centigrade) and the time spent to recover reflexes (expressed in minutes) were recorded. Statistical analyses corresponded to a three-way analysis of variance to assess the significance of the duration of intake (*Period*, PD-56 or PD-84), the pattern of alcohol intake (*Group*, acute or moderate and high chronic) and IP injection (*IP*, ethanol or saline). To establish differences between the groups the Student-Newman-Keuls Method was used.

In summary, the animals consumed alcohol chronically or in an acute manner over five or nine weeks. On the day of the evaluation the animal's body temperature was measured and then they received an intraperitoneal injection of ethanol. After, the body temperature was measured again and two types of reflex behaviour were evaluated to see if the animals had developed tolerance to the reduction in body temperature and alteration in the motor behaviour caused by ethanol.

Results

Table 1 shows the changes in body temperature (Mean ±SE) of all the groups of animals at 90 and 180 minutes after the IP. We performed the statistical analysis with the values measured at 90 minutes because the behaviour of the data at 180' was very similar to those obtained at 90', then, statistical differences were similar in both periods (Fig. 1 and 2). The Three-way ANOVA showed significant effects of the factors *group* ($F_{3,112}$= 15.52, P<0.001), *IP* ($F_{1,112}$= 266.40, P<0.001) and the interaction between them ($F_{3,112}$= 16.48, P<0.001). We also found a significant interaction among the three variables ($F_{3,112}$= 6.07, P<0.001), and between the variables *period* and *group* ($F_{3,112}$= 5.58, P= 0.001) but not between *period* and *IP*. The animals that received saline IP exhibited little change in body temperature in both the PD-56 and PD-84 groups. However, the animals administered ethanol IP showed great variations in body temperature and with differences among all the groups. In the PD-56 animals, the greatest variations in body temperature were found in the CTR and ALCH groups that differed significantly from the ALCM and ALCA groups (P<0.05) (Figure 1). No differences were observed between ALCM and ALCA or between CTR and ALCH. This means that the animals from the ALCM and ALCA groups presented the highest tolerance to acute ethanol administration. In contrast, as expected, animals from the CTR group exhibited a low tolerance but, surprisingly, this was similar to the tolerance level observed in animals that received chronic alcohol consumption with the high concentration solution. The CTR, ALCM and ALCA groups of

Table 1

Parameters (mean±SEM) evaluated in the animals in PD-56 and PD-84. Liquid consumption is expressed in ml/Kg/day and ethanol consumption in gr/Kg/day

(A) YOUNG ANIMALS (PD-56)				
Group	**Total liquid**	**Ethanol**	**Var body temp 90'/180'**	**Reflexes floor/fall**
CTR-A	170.42±6.24	–	-2.78±0.33/-2.44±0.30	289.58±19.61/478.88±12.2
CTR-S	162.59±8.05	–	-0.25±0.06/-0.05±0.20	15.00±0.00 / 15.00±0.00
ALCM-A	178.63±13.42	13.93±1.27	-1.19±0.22/-0.91±0.22	289.20±41.45/432.68±23.59
ALCM-S	168.34±9.16	13.14±1.15	-0.47±0.07/-0.17±0.04	15.00±0.00 / 15.00±0.00
ALCH-A	163.85±11.23	25.58±1.97	-2.96±0.41/-2.80±0.42	432.60±23.37/443.75±45.56
ALCH-S	164.57±15.42	25.68±3.07	-0.22±0.06/-0.08±0.03	15.00±0.00 / 15.00±0.00
ALCA-A	169.47±10.52	0.53±0.11[a]	-0.76±0.21/-0.66±0.24	276.70±19.53/429.30±25.58
ALCA-S	174.26±12.41	0.59±0.08[a]	-0.06±0.04/ 0.00±0.00	15.00±0.00 / 15.00±0.00
(B) ADULT ANIMALS (PD-84)				
Group	**Total liquid**	**Ethanol**	**Var body temp 90'/180'**	**Reflexes floor/fall**
CTR-A	115.11±6.33	–	-2.91±0.13/-2.75±0.12	347.30±32.37/479.53±9.81
CTR-S	119.78±8.44	–	-0.36±0.05/-0.06±0.03	15.00±0.00 / 15.00±0.00
ALCM-A	120.48±6.84	9.19±1.23	-1.46±0.26/-1.25±0.27	352.08±51.18/389.93±34.58
ALCM-S	112.81±7.31	9.03±1.01	-0.45±0.05/-0.20±0.04	15.00±0.00 / 15.00±0.00
ALCH-A	125.26±9.14	18.14±1.27	-1.70±0.24/-1.36±0.27	388.88±44.20/438.38±4023
ALCH-S	125.26±6.49	16.52±1.77	-0.21±0.05/-0.11±0.06	15.00±0.00 / 15.00±0.00
ALCA-A	113.64±8.21	0.63±0.44[a]	-1.35±0.16/-1.31±0.19	262.06±20.55/387.62±35.66
ALCA-S	118.38±10.51	0.64±0.39[a]	0.00±0.00/ 0.00±0.00	15.00±0.00 / 15.00±0.00

[a] *one day per week*

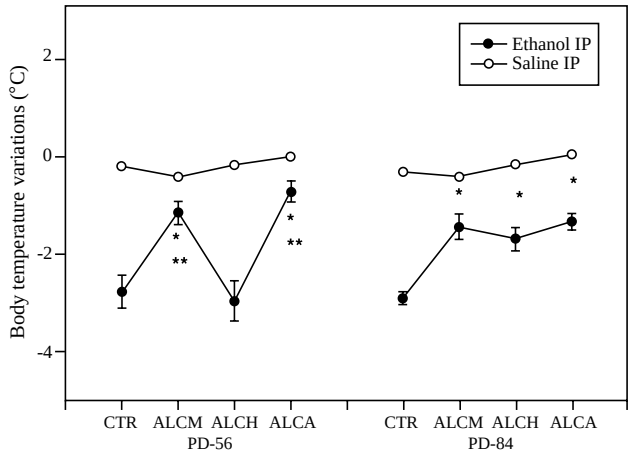

***Figure 1**. Ethanol-induced hypothermia in the 56 and 84 postnatal days 90 minutes after IP injection. (*) Significant differences compared to CTR group. (**) Significant differences compared to ALCH group*

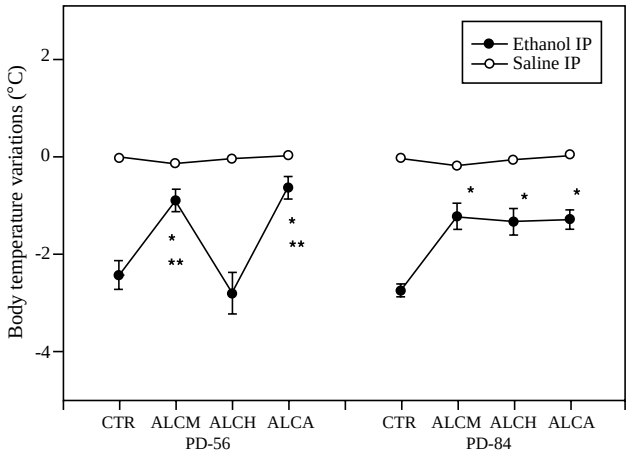

***Figure 2.** Ethanol-induced hypothermia in the 56 and 84 postnatal days 180 minutes after IP injection. (*) Significant differences compared to CTR group. (**) Significant differences compared to ALCH group*

PD-84 animals exhibited a similar behavior to the PD-56 ones. However, animals from the ALCH group presented an increased tolerance level and reached a similar level as the other alcoholic groups (Figure 2). With the PD-84 animals, no significant differences were observed among alcoholic groups but all differed significantly from the CTR group ($P<0.05$).

With regard to floor righting reflex, we found significant effects of the factors *group* ($F_{3,112}= 9.67$, $P<0.001$), *IP* ($F_{1,112}= 852.61$, $P<0.001$) and the interaction between them ($F_{3,112}= 9.67$, $P<0.001$). All the animals that received saline IP reached the criterion of recovery at the first test (15'). However, the PD-56 animals that received ethanol IP needed significantly more time to recover the reflex ($P<0.05$). Among these animals, the ALCH group required more time than the other groups ($P<0.05$)(Figure 3). In the PD-84 animals, no significant differences were found among ethanol IP groups but these differed significantly from saline IP groups ($P<0.05$) that reached the criterion at 15'. In the fall righting reflex, only the *IP* factor had a significant effect ($F_{1,112}= 1.47$, $P<0.001$). Both the PD-56 and PD-84 animals that received saline IP reached the criterion in the first test, whereas the animals with ethanol IP needed significantly more time to do it ($P<0.05$). In this case, no differences were found among alcoholic and control groups (Figure 4) of animals that received ethanol IP.

Discussion

Acute consumption of large amounts of alcohol causes hypothermia and loss of some reflexes among other effects. In our experiment, we found both effects but only alcoholic animals showed tolerance to hypothermia. The ability to recover reflexes after ethanol IP was greatly improved in all groups, both alcoholic and control. Those receiving saline IP reached the criteria in the first test fifteen minutes after the injection. However, those receiving ethanol IP required significantly more time. Several authors have shown that moderate doses of ethanol can alter the air righting reflex (Tracy, Wayner and Armstrong, 1999; Tracy and Wayner, 1998). The PD-56 animals from the ALCH group needed more time to recover the floor righting reflex than the other groups. However, all PD-84 groups took a similar length of time to achieve this. For the fall righting reflex, all groups of PD-56 and PD-84 animals required a similar time. The air righting reflex is a more complex task than the straightening reflex and is acquired postnatally while straightening is present, although imperfectly, at birth (Pellis, Pellis and Whishaw, 1996; Pellis and Pellis, 1994; Pellis, Pellis and Nelson, 1992).

With our data we can deduce that these animals did not exhibit alcohol tolerance and that PD-56 animals were more impaired than adults ones by acute intake after chronic high alcohol consumption. A probably explanation is that the dose administered was too high (5 gr/Kg) and that with a lower dose we could found significant differences in developing tolerance among groups. However, all alcoholic animals developed different degrees of alcohol tolerance to hypothermia after acute consumption. These results agree with other studies that found differences in developing tolerance to hypothermia or sedative effects of ethanol (Swartzwelder el al., 1998; Pohorecky el al., 1986).

The PD-56 animals that consumed 10% ethanol chronically or those that consumed acutely developed a great tolerance to ethanol-induced hypothermia. However, the PD-56 animals that consumed 20% ethanol chronically did not exhibit tolerance and the ethanol-induced hypothermia was similar to that of the animals from the control group. The PD-84 animals from the CTR, ALCM and ALCA groups showed a similar level of hypothermia to the PD-56 ones. However, the PD-84 animals that received 20% ethanol (ALCH) showed less hypothermia than the young ones. This could mean that with this dose of chronic ethanol consumption the PD-84 animals developed alcohol tolerance after acute consumption whereas the PD-56 animals did not. During adolescence, alcohol intake may cause development of higher and faster tolerance than in adulthood (Swartzwelder el al., 1998; Pohorecky et al., 1986). However, the period of alcohol intake before the test could be an important factor since the young animals, assessed on postnatal day 56, consumed alcohol for five weeks whereas the adult animals, assessed on PD-84, consumed it for nine weeks.

Long-term alcohol dependence, as a consequence of alcoholic tolerance, results in neuronal adaptation that probably contributes to ethanol withdrawal-induced central nervous system excitability and,

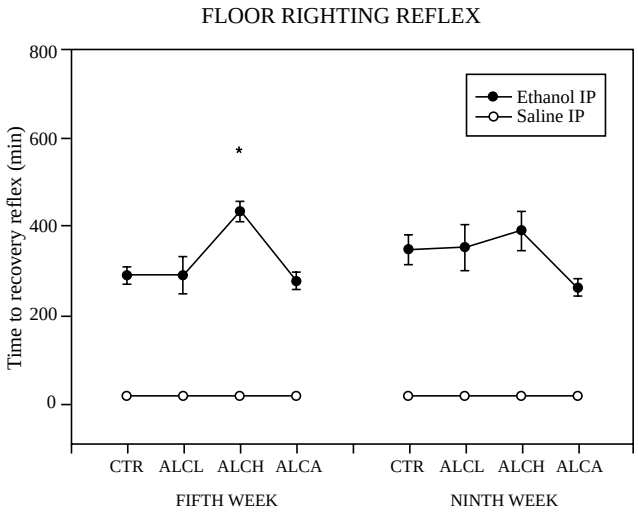

Figure 3.*Time to recover floor righting reflex in young and adult animals. (*) Significant differences compared to all other groups*

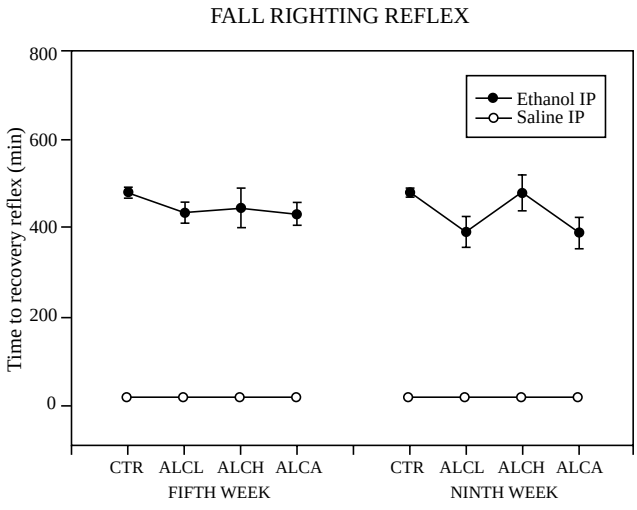

Figure 4. *Time to recover fall righting reflex in young and adult animals. No significant differences were observed among groups*

potentially, neurotoxicity. A possible explanation for the lower tolerance in ALCH PD-56 animals is that, with a high ethanol intake, the neurons need more time to develop the adaptation process but that this neuroadaptation would be possible with lower amounts of ethanol. Ethanol has potent effects on both excitatory and inhibitory neurotransmitter systems, one of which is glutamate. Chronic ethanol exposure leads to an up-regulation of NMDA receptors (Grant and Lovinger, 1995) and excitotoxicity occurs following excessive excitation of neurons through glutamate receptors.

The participation of NMDA receptor in tolerance development has been clearly demonstrated (Khanna, Shah and Chau, 1997; Khanna, Chau and Shah, 1996) and the pharmacological activity of the NMDA receptor may be influenced by the history of alcohol consumption (Neznanova, Blokhina, Sukhotina and Bespalov, 2000). Cheema et al. (2000) found that ethanol induces a susceptibility to apoptotic signals at low doses in the developing cerebral cortex, but ethanol itself specifically induces apoptosis at higher doses. Moreover, studies of Germani et al. (1999) suggest that chronic exposure to ethanol can influence central nervous system plasticity during development. According to these authors, low doses of ethanol may enhance neural plasticity perhaps via an increased efficacy of neurotrophic factors, whereas higher doses may negatively affect neural development also by means of the impairment of the expression of the neurotrophic factors (Germani el al., 1999). Animals from the ALCH group could have suffered from a higher neurotoxicity that would make difficult the neuroadaptation that can occur in animals from the ALCM and ALCA groups with lower alcohol consumption. Signs of severe toxicity occur at lower blood alcohol concentrations in young teenagers than in adults whereas the elimination rate of ethanol is similar in both groups (Lamminpa, 1995). Glutamate-mediated excitoxicity has been divided into two components: a rapid component associated with osmotic swelling and often immediate neuronal death, and a delayed component that occurs as a progressive degenerative process over a period of hours to days. Both processes are related to excess accumulation of calcium (Meldrum and Gartwaite, 1990; Olney, 1990; Orrenius, McConkey, Bellomo and Nicotera, 1989). Chronic ethanol consumption induces an adaptive supersensibility of NMDA receptors that can increase excitoxicity (Nagy, Muller and Laszlo, 2001; Crews, Steck, Chandler, Yu and Day, 1998; Smothers, Mrotek and Lovinger, 1997). It is possible that low ethanol intake will produce changes in synaptic plasticity that lead to the development of alcohol tolerance whereas high ethanol intake could lead to excitotoxicity and neuronal death.

The answer to this question must also be found in the $GABA_A$ receptor system since the activity of this receptor, together with the NMDA, plays an important role in both the acute as well as chronic effects of ethanol (Steffensen, Nie, Criado and Siggins, 2000; Grobin, Matthews, Devaud and Morrow, 1998). Both NMDA and $GABA_A$ receptors are composed of multiple subunit proteins, which are thought to assemble as hetero-pentameric structures that exhibit distinct properties depending upon the particular subunit composition. Chronic ethanol intake leads to the development of ethanol tolerance and dependence that is associated with a decrease in GABAergic and an increase in glutamatergic function but these effects vary across the brain (Chandler, Harris and Crews, 1998; Devaud, 2001).

Moreover, prolonged exposure to alcohol was found, with a similar model to this one (20% final alcohol) to produce a decline in the response of the hypothalamus-hypophysary-adrenal axis (HPA), related with the development of tolerance to ethanol effects (Silva, Paula-Barbosa and Madeira, 2002). Given that the HPA axis still follows a maturation process after 21 days (Vazquez, 1998), could temporarily alter the functioning of the HPA axis and this would explain that young animals could be more sensitive to the sedative effects of alcohol and would not develop tolerance until the adult age. On the other hand, the handling itself (specifically, repeated introduction of the rectal thermometer and immobilisation of the animal) can interact with the effects of alcohol, altering the hypothermia (Peris and Cunningham, 1987).

Animals from the ALCA group may present a more conditioned tolerance since the changes in hypothermia are less pronounced than in the ALCM group. This is because these animals always received the ethanol IP on the same day and in the same place. Several studies have shown that animals submitted to regular ethanol injections developed tolerance to a challenge injection when the process was carried out in the same place, whereas animals submitted to the same procedure did not when they were assessed in a different room (Duncan, Alici and Woodward, 2000; Crowell, Hinson and Siegel, 1981). However, this aspect requires further research in order to elucidate the characteristics of the conditioning process that underlies alcohol tolerance.

In our experiment, we have simulated different patterns of alcohol consumption, some of which are very common among human people. These data support the idea that young people are very sensitive to ethanol effects and that tolerance to the different effects of ethanol develops at different times. Moreover, alcoholic tolerance prior to alcoholic dependence can be developed a very short periods of alcohol consumption. Apart from individual differences, the history of alcohol consumption may determine, in part, the effect of acute consumption. We consider that young people should receive appropriate information about the risk of alcohol consumption.

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