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Aniracetam and DNQX affect the acquisition of rapid tolerance to ethanol in mice

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ABSTRACT

Several studies have emphasized the role of learning in the development of rapid tolerance and have shown that glutamate-mediated neurotransmission plays an important role in this phenomenon. Since the AMPA/kainate receptor system is directly involved in plasticity mechanisms, the influence of this receptor system on rapid tolerance induced by ethanol was studied using the rotarod. In the first experiment, mice were pretreated with aniracetam, an agonist of AMPA/kainate receptors, 30 min before ethanol (2.75 g/kg; IP) treatment, and tested on the rotarod. After 24 h, the groups were tested on the rotarod under ethanol treatment. Aniracetam facilitated the acquisition of rapid tolerance to ethanol. In the second experiment, mice received DNQX, a competitive antagonist of the AMPA receptor, 30 min before ethanol treatment (3 g/kg) and submitted to the rotarod. This dose of ethanol produced tolerance per se. Groups were tested under ethanol treatment (1.75 g/kg) after 24 h. DNQX blocked rapid tolerance to ethanol. Using a similar protocol, the third experiment showed that DNQX blocked the aniracetam-induced facilitation of rapid tolerance to ethanol. Our results show that aniracetam facilitates whereas DNQX blocks ethanol tolerance, suggesting that the non-NMDA receptors are involved in this phenomenon.

1. Introduction

Prolonged exposure to ethanol is clearly in compass with tolerance development (Kalant et al., 1971). This event is intimately connected with other plasticity processes (Kalant, 1998). Tolerance mechanisms appear to be relevant to the development of ethanol abuse and dependence, because they can promote an attenuation of the aversive effect of this drug with regard to its rewarding effects, thus encouraging the use of escalating doses (American Psychiatric Association, 1994).

There are many forms of tolerance, and rapid tolerance to ethanol has been observed in the response to a second dose given 8–24 h after a single previous exposure to ethanol (Crabbe et al., 1979; da Silva et al., 2001). Two other types of tolerance are the chronic one that develops gradually over days or weeks of ethanol administration (Kalant et al., 1971; Littleton et al., 1980), and the acute tolerance that develops with a single exposure to ethanol (Kalant et al., 1971; Khanna et al., 2002) Acute tolerance probably represents an innate adaptive response (Chandler et al., 1998), and has a potential value as a predictor of vulnerability to alcoholism (Schuckit, 1986; Schuckit and Smith, 1997) whereas rapid and chronic tolerances are adaptive responses generally perceived as a consequence of repeated drug exposure.

Moreover knowledge on the mechanism of tolerance to ethanol has an important role in the study of alcoholism because of its value as a model of neuroadaptative processes (Kalant, 1998) and its possible contribution to alcohol consumption (Waller et al., 1983) and dependence (Altman et al., 1996). Some evidence from neuronal culture studies has indicated that chronic exposure to ethanol can potentiate excitotoxicity (Chandler et al., 1993; Iorio et al., 1993).

Glutamate is directly involved in this process and plays a major role in excitatory neurotransmission in the CNS (Del Rio et al., in press; Ferreira et al., 1992; Molz et al., 2008). Glutamate receptors are present in several brain structures having a wide structural diversity. These receptors are also classified according to their electrophysiological and pharmacological characteristics in subtypes: NMDA (*N*-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid), Kainate and metabotropic receptors (Brugger et al., 1990). Accumulating evidence suggests that neurophysiologic and pathologic effects of ethanol are mediated, at least in part, through the glutamatergic system (Karolewicz et al., 2008). The NMDA receptor is the most characterized constituent of the glutamate system; these receptors are among those with the highest affinity that ethanol targets in the brain (Evans et al., 2007; Grant and Lovinger, 1995; Hendricson et al., 2007), and the role of the NMDA receptor in the development of tolerance and sensitization to ethanol (Trujillo and Akil, 1995) is well described. Recent evidence also suggests the interaction of ethanol with the AMPA/kainate system (Pickering et al., 2007; Vaglenova et al., 2008). Several studies have shown that the AMPA/kainate system has an important modulatory influence on different systems. For instance, agonists and antagonists at this receptor system were shown to influence cognitive functions (Bast et al., 2005; Derkach et al., 2007; Himori and Mishima, 1994; Lynch and Gall, 2006), nociception (Cheng and Chiou, 2006), anxiety (Allison and Pratt, 2006), cell proliferation in diverse CNS regions (LoTurco et al., 1995), seizure threshold (Porter et al., 2006), anti-proliferative effects in retina

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development (Martins et al., 2006), increased protein expression in auditory system (Xu et al., 2007), and discriminative stimuli of abused drugs (Jackson et al., 1996). The modulatory action of the AMPA agonist aniracetam on synaptic responses can be altered by the induction of long-term potentiation (Kolta et al., 1998), characterized as an increase in the synapse efficacy that has been suggested to be the cellular basis of memory (Bashir and Collingridge, 1992; Kandel, 2004; Mapelli and D'Angelo, 2007; Pastalkova et al., 2006). Chronic ethanol treatment when synergistically combined with an AMPA agonist can cause an increased expression of NMDA and AMPA subunit proteins characterizing an adaptive mechanism to the inhibition caused by ethanol at these sites (Chandler et al., 1998). These effects can be associated to enhanced calcium activity in cerebellum Purkinje neurons (Netzeband et al., 1999). The cerebellum constitutes one of the main encephalic targets involved in ethanol induced motor incoordination (Botta et al., 2007; Carta et al., 2006).

Although the involvement of the NMDA receptor system in ethanol tolerance is well documented (Khanna et al., 2002, 1997; Neznanova et al., 2000), there is a lack of studies on the influence of the AMPA/kainate receptor system on this process. Thus, the purpose of the present study was to verify whether the activation or the blockade of the AMPA/kainate system would affect the development of rapid tolerance to the motor incoordinating effect of ethanol in mice.

2. Materials and methods

2.1. Animals

Adult male Swiss mice (2–3 month old), weighing 23–33 g from the Universidade Federal de Santa Catarina colony were used. The animals were housed in groups of 15 per cage and were kept under a controlled light–dark cycle (lights on from 06:00 h to 18:00 h) and temperature (23±1 °C). They had free access to food and water. The animals were tested between 13:30 h and 17:30 h in order to minimize circadian influences. All procedures were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Drugs

Ethanol, analytical grade, obtained from Merck Laboratory (Rio de Janeiro, Brazil) was prepared by dilution in 0.9% NaCl (saline) to the concentration of 14% w/v. Aniracetam (an AMPA/kainate agonist) and DNQX (6,7 dinitroquinoxaline-2,3-dione) (an AMPA antagonist) were purchased from Research Biochemical International (Natick, MA). DNQX (1, 2.5, 5 and 10 mg/kg) was prepared in saline, while aniracetam (7.5, 10, 15, 22.5 and 30 mg/kg) was prepared in 4% Tween in saline. Control group of the aniracetam treated group were treated with a solution of saline in Tween 4% in the same proportions of aniracetam treated group. Reagents for determination of blood ethanol levels were obtained from Sigma Chemical Co. (St Louis, MO). All drugs were freshly prepared.

2.3. Rotarod test

Motor impairment was measured on rotarod apparatus (Rotamex-V-EE/85) controlled by a computational system (Columbus Instruments Computer-Counter Interface; USA). Animals were trained under continuous acceleration (1 rpm/s) in 1-minute sessions. Whenever the animal dropped off the rotating bar, it received a footshock (0.5 mA). The speed at which the animal dropped off the rotating bar was taken as the performance score. Animals that did not reach a stable baseline (at least 20 rpm) in 10 trials were not considered for analysis. The animals that presented performance between 20 and 40 rpm were selected for the experiment. About 90% of the animals usually reach the criteria. After the selection, experimental and control groups (n=10) were arranged according to their body weight and mean performance during the last training session. With this procedure, animals presented similar basal values in all groups.

2.4. Blood ethanol assay

Groups of animals were pretreated with different doses of AMPA/kainate receptor agonist (aniracetam) or antagonist (DNQX) 30 min before ethanol treatment (1.75 g/kg). Blood samples were collected from the animals by direct tail puncture 5 min after ethanol administration. Blood ethanol concentration was evaluated enzymatically based on ethanol conversion to acetaldehyde by the action of alcohol dehydrogenase (Poklis and Mackell, 1982).

3. Experimental design

3.1. Experiment 1: effect of aniracetam on the development of rapid tolerance to ethanol-induced motor impairment

On day 1, trained mice were divided into 10 groups (n=20) in order to receive pretreatment with saline (5 groups) or aniracetam (1 group per dose) in doses of 7.5; 10.0; 15.0; 22.5 or 30.0 mg/kg given by oral route (o.r.). This treatment regimen was chosen for the detection of facilitation for tolerance development. After 30 min, each group was further divided in two subgroups in order to be injected with ethanol (1.75 g/kg) or saline by intraperitoneal (IP) route. Thus, 20 groups of ten mice each were obtained. Before the first injection and 5, 15 and 30 min. later, the degree of motor impairment was assessed (rotarod test) in all animals as described above. Two hours after saline or ethanol injection, the animals received an additional dose of saline or EtOH (1.0 g/kg), in order to complete 2.75 g/kg. The procedure of administering EtOH in two doses (1.75+1.0 g/kg) was employed because previous experiments showed that a total dose of 2.75 g/kg on day 1 is insufficient to produce a reliable rapid tolerance on day 2 (Bare et al., 1998). Mice were then returned to their home cages. After 24 h, all animals including controls received EtOH (1.75 g/kg, IP) and 5, 10 and 15 min later, they were tested on the rotarod to evaluate rapid tolerance.

3.2. Experiment 2: effect of DNQX on the development of rapid tolerance to ethanol-induced motor impairment

The protocol used was similar to experiment 1. On day one, eight groups of trained mice (n=20 each) were divided in order to receive pretreatment with saline (4 groups) or DNQX (one group per dose). Doses of 1.0; 2.5; 5.0 or 10.0 mg/kg of DNQX were given by IP injection. After 30 min, each group was further divided into two groups in order to receive ethanol (1.75 g/kg) or saline by IP route. Each animal was tested 5, 15 and 30 min later on the rotarod apparatus as described above. Two hours after saline or ethanol injection, the animals received an additional dose of saline or EtOH (1.25 g/kg), in order to complete 3.0 g/kg. Two divided doses of ethanol (1.75+1.25 g/kg) were used because previous experiments showed that a total dose of 3 g/kg was required on day 1 to produce a reliable rapid tolerance on day 2. Because doses greater than 1.75 g/kg may not always fall into the linear rapid tolerance of the dose–response curve, the choice of this dose allows us to compare results across days (Bare et al., 1998). Mice were then returned to their home cages. After 24 h, all animals including controls received EtOH (1.75 g/kg) and 5, 10 and 15 min later were tested on the rotarod to evaluate rapid tolerance.

3.3. Experiment 3: interaction between DNQX x Aniracetam on the development of rapid tolerance to ethanol-induced motor impairment

On day 1, 80 trained mice were randomly divided into two groups of 40 animals each. One group was injected (IP) with DNQX (5 mg/kg) and the other group received the same volume of saline at zero time.

Each of the groups was further subdivided into two subgroups. At 30 min, one of the subgroups of each group received aniracetam (o.r.) (15 mg/kg), while the other subgroup received vehicle. Finally, all of the subgroups were divided again into two subgroups each, making eight subgroups. At 60 min, four of the eight subgroups received ethanol (1.75 g/kg, IP), while the remaining subgroups received saline. Five, 15 and 30 min later, each animal was tested on the rotarod apparatus as described above. Two hours after saline or ethanol injection, the animals received an additional dose of saline or EtOH (1.0 g/kg), in order to complete 2.75 g/kg. Mice were then returned to their home cages. After 24 h, all animals including controls received EtOH (1.75 g/kg IP) and 5, 10 and 15 min later, they were tested on the rotarod to evaluate rapid tolerance.

3.4. Statistical analysis

Statistica 6 for Windows, Statsoft, Inc. software (StatSoft Inc., Tulsa, OK, USA) was used to perform the statistical analysis. The difference between the baseline and maximum impairment scores provided the maximum percentage of motor impairment induced by ethanol. Data were analyzed using analysis of variance ANOVA, with pretreatment and treatment as independent variables, according to the protocol. *Post hoc* comparisons were performed using Tukey's test. Values of $p<0.05$ were considered significant. In experiments 1, 2, and 3, data are presented as mean±SEM, according to:

$$\text{Maximum percent of motor impairment} = \frac{(\text{baseline score})-(\text{test score})}{\text{baseline score}} \times 100$$

4. Results

4.1. Experiment 1: effect of aniracetam on the development of rapid tolerance to ethanol-induced motor impairment

The results obtained in experiment 1 are depicted in Fig. 1 (panels A, B, C, D, and E). Motor impairment was not significantly reduced in all saline+ethanol (SE) groups on the second day of the experiment, suggesting that the dose of 2.75 g/kg ethanol (1.75+1.0 g/kg) is insufficient to produce rapid tolerance. Two-way ANOVA indicated a significant overall effect of treatment (Fig. 1A: $F_{(1,36)}=2.51$; $p>0.05$; Fig. 1B: $F_{(1,36)}=3.32$; $p>0.05$; Fig. 1C: $F_{(1,36)}=101.91$; $p<0.0001$; Fig. 1D: $F_{(1,36)}=11.43$; $p<0.002$; Fig. 1E: $F_{(1,36)}=2.36$; $p>0.05$). The groups treated with aniracetam (10, 15 or 22.5 mg/kg) before ethanol treatment on day 1 showed reduced motor impairment when compared to the controls (SE), suggesting that these animals developed tolerance on day 2. Two-way ANOVA revealed the effect of pretreatment (Fig. 1B: $F_{(1,36)}=4.60$; $p<0.04$; Fig. 1C: $F_{(1,36)}=102.54$; $p<0.0001$; Fig. 1D: $F_{(1,36)}=5.51$; $p<0.03$).

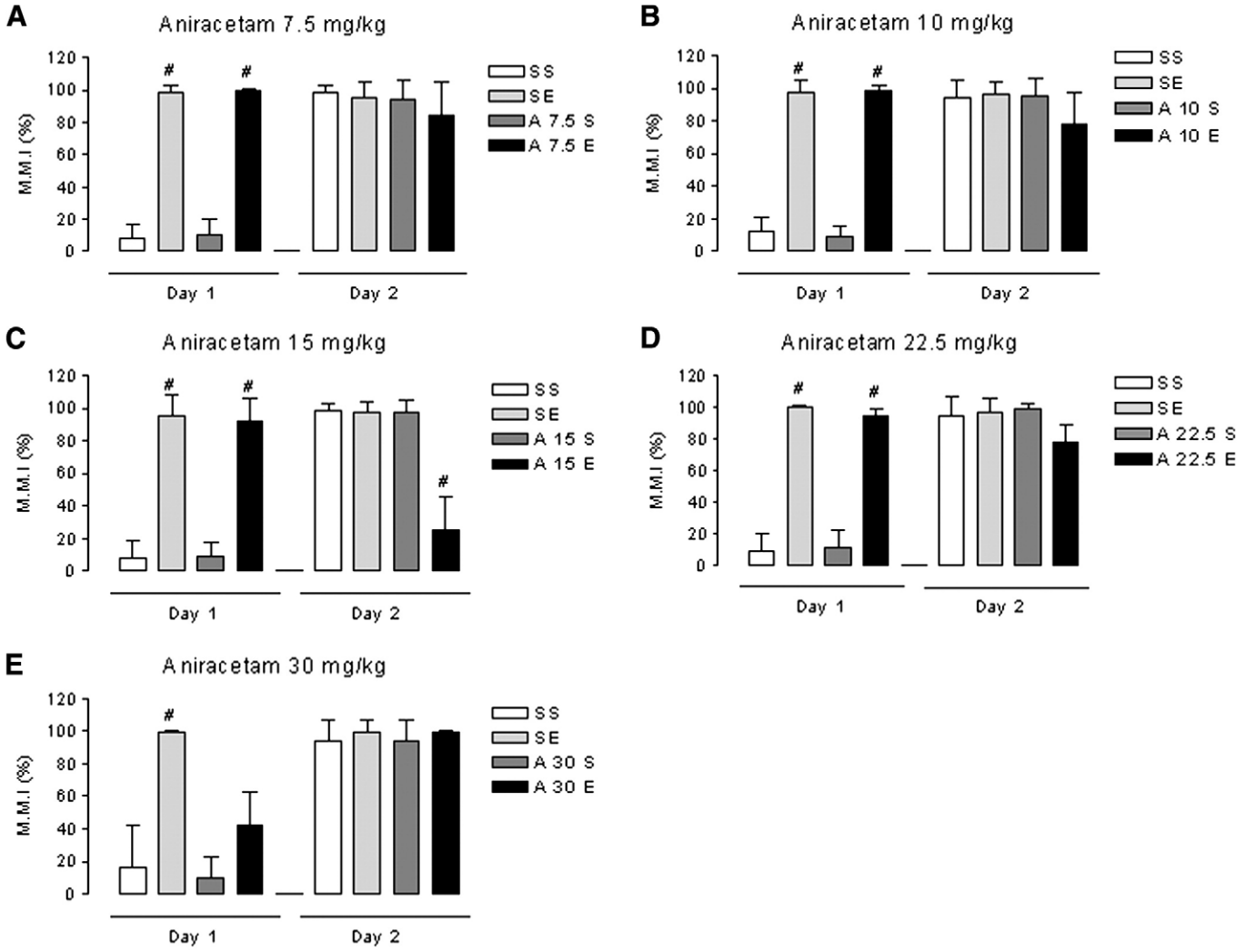

Fig. 1. Development of rapid tolerance to ethanol-induced motor impairment by aniracetam pretreatment. Ten groups of mice received saline followed by ethanol or saline, and another ten groups received aniracetam (7.5, 10.0, 15.0 22.5 and 30 mg/kg) o.r. 30 min prior to saline or ethanol 1.75 g/kg, IP, on day 1. Two hours later, the animals received an additional dose of saline or ethanol (1.0 g/kg, IP), in order to complete 2.75 g/kg. Rapid tolerance to ethanol was assessed on day 2, 30 min after all groups received a challenge dose of ethanol (1.75 g/kg, IP). Results shown are means±SEM of 10 animals per group. # $p<0.05$ compared to respective control (Tukey's test).

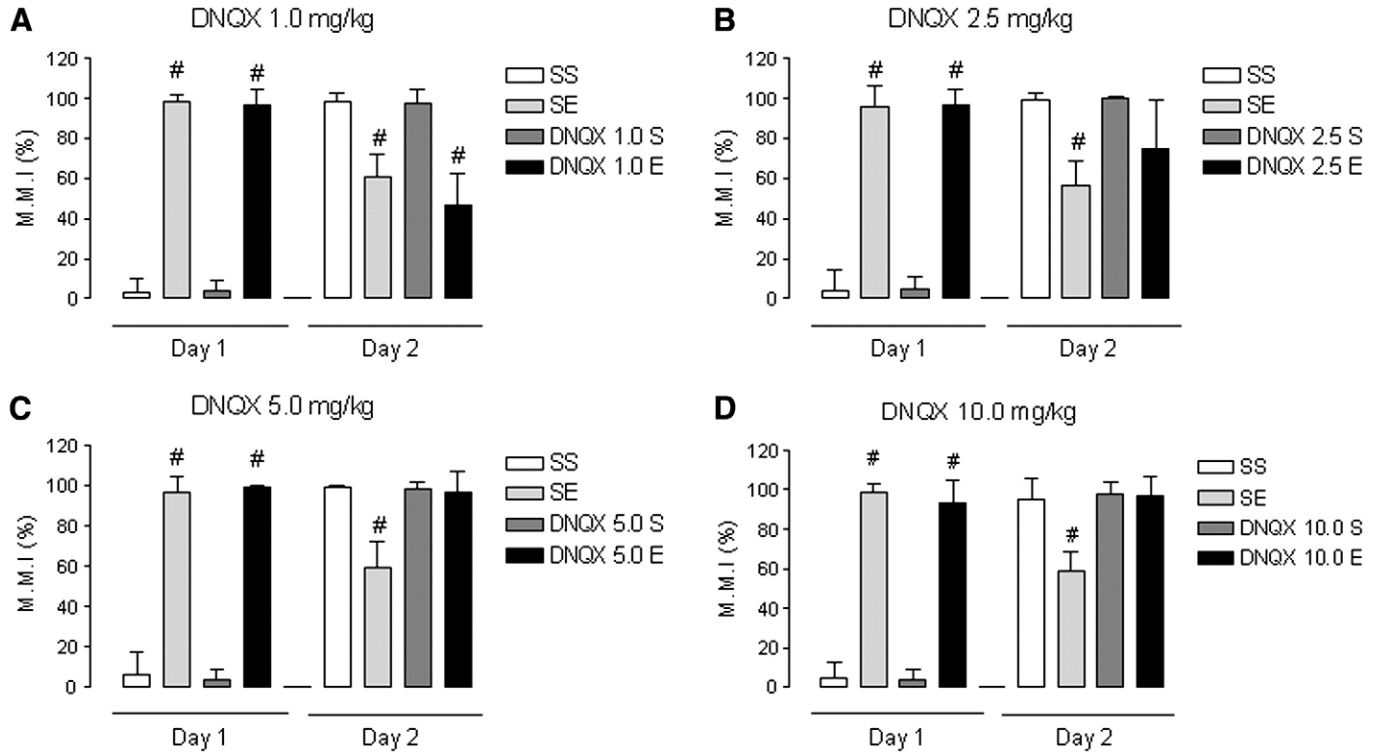

Fig. 2. Blockade of rapid tolerance to ethanol-induced motor impairment by DNQX pretreatment. Eight groups of mice received saline followed by ethanol or saline, and another eight groups received DNQX (1.0, 2.5, 5.0 or 10 mg/kg), IP, 30 min prior to saline or ethanol 1.75 g/kg, IP, on day 1. Two hours later, the animals received an additional dose of saline or ethanol (1.25 g/kg, IP), in order to complete 3 g/kg. Rapid tolerance to ethanol was assessed on day 2, when all groups received a challenge dose of ethanol (1.75 g/kg, IP). Results shown are means ± SEM of 10 animals per group. # $p<0.05$ compared to respective control (Tukey's test).

Interactions between pretreatment and treatment were also significant (Fig. 1B: $F_{(1,36)}=5.60$; $p<0.02$; Fig. 1C: $F_{(1,36)}=94.81$; $p<0.0001$; Fig. 1D: $F_{(1,36)}=14.53$; $p<0.0005$). *Post hoc* analysis indicated that aniracetam (10.0, 15.0 or 22.5 mg/kg) facilitated the development of rapid tolerance (Tukey's test). Aniracetam in doses of 7.5 and 30 mg/kg did not affect the performance of animals treated with ethanol (Fig. 1A and E respectively). On day 1, all groups treated with ethanol associated with saline or Aniracetam showed significant differences compared to their respective control groups (SS and AS). Moreover, the group treated with Aniracetam in doses of 30 mg/kg plus ethanol (AE) showed reduced motor incoordination on day 1 when compared to saline + ethanol group (SE). Two-way ANOVA showed that the effect of treatment [$F_{(1,36)}=111.44$, $p<0.0001$] and pre-treatment and treatment were significant [$F_{(1,36)}=21.72$, $p<0.0001$]. *Post hoc* comparisons confirmed that motor impairment was significantly reduced when the SE was compared with AE groups on the first day of the experiment.

4.2. Experiment 2: effect of DNQX on the development of rapid tolerance to ethanol-induced motor impairment

The results of this experiment are shown in Fig. 2. Motor impairment was significantly reduced in all saline+ethanol (SE) groups on the second day of the experiment, after receiving ethanol treatment, suggesting the development of tolerance. Two-way ANOVA revealed a significant overall effect of treatment (Fig. 2A: $F_{(1,36)}=201.60$; $p<0.0001$; Fig. 2B: $F_{(1,36)}=60.75$; $p<0.0001$; Fig. 2C: $F_{(1,36)}=60.29$; $p<0.0001$; Fig. 2D: $F_{(1,36)}=42.01$; $p<0.0001$). *Post hoc* analysis showed the development of rapid tolerance (Tukey's test). The groups treated with DNQX in doses of 5.0 and 10.0 mg/kg before ethanol treatment on day 1 showed blockade of rapid tolerance on day 2. Two-way ANOVA revealed the effect of pretreatment (Fig. 2C: $F_{(1,36)}=45.16$; $p<0.0001$; Fig. 2D: $F_{(1,36)}=50.45$; $p<0.0001$). Interactions between pretreatment and treatment were significant (Fig. 2C: $F_{(1,36)}=48.93$; $p<0.0001$; Fig. 2D: $F_{(1,36)}=38.22$; $p<0.0001$). *Post hoc* analysis revealed a significant dose-dependent blockade of rapid tolerance by DNQX in doses of 5.0 and 10.0 mg/kg (Tukey's test). DNQX in doses of 1.0 and 2.5 mg/kg did not block rapid tolerance (Fig. 2).

4.3. Experiment 3: interaction between DNQX and Aniracetam in the development of rapid tolerance to ethanol- induced motor impairment

Results are shown in Fig. 3. On day 1, mice injected with ethanol 2.75 g/kg (1.75+1.0) showed the expected motor impairment response, and the administration of aniracetam or DNQX did not significantly affect the motor impairment responses. On day 2, motor

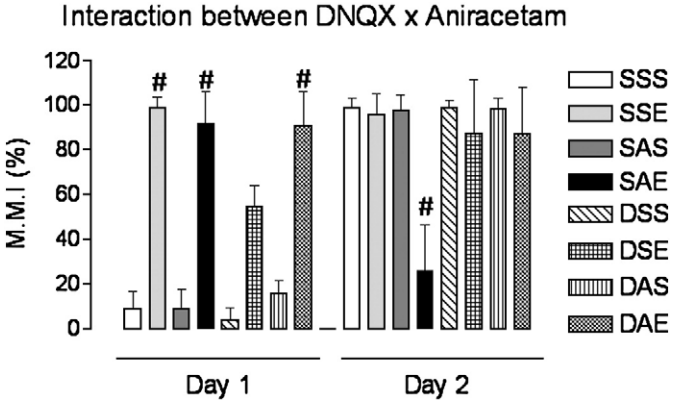

Fig. 3. Effects of the interaction between aniracetam and DNQX pretreatments on the development of rapid tolerance to ethanol-induced motor impairment. Eight groups received saline or DNQX (5 mg/kg, IP) 30 min before the administration of saline or aniracetam (15.0 mg/kg, o.r). Saline or ethanol (1.75 g/kg, IP) was administered 30 min after the last treatment, on day 1. Two hours later, they received an additional dose of ethanol (1.0 g/kg, IP), in order to complete 2.75 g/kg, and the control group received saline. Rapid tolerance to ethanol was assessed on day 2, when all groups received a challenge dose of ethanol (1.75 g/kg, IP). Results shown are means ± SEM of 10 animals per group. # $p<0.05$ compared to respective control (Tukey's test).

incoordination was not significantly reduced in the group saline–saline–ethanol (1.75 g/kg IP) (SSE). ANOVA indicated a significant effect of treatment ($F_{(1,72)}=61.72$; $p<0.0001$), and *post hoc* analysis did not indicate the development of rapid tolerance (Tukey's test).

Confirming our previous data, aniracetam (15.0 mg/kg) facilitated the development of tolerance on day 2, since the group pretreated with this drug 30 min before ethanol treatment (SAE) on day 1 showed significant differences in relation to the group treated with saline before ethanol treatment (SSE). ANOVA indicated the effect of aniracetam pretreatment [$F_{(1,72)}=34.30$, $p<0.00001$] and *post hoc* analysis suggested the development of rapid tolerance (Tukey's test).

The groups pretreated with DNQX (5.0 mg/kg) 30 min. before aniracetam administration did not develop rapid tolerance on the second day. ANOVA indicated the effect of DNQX pretreatment [$F_{(1,72)}=16.40$, $p<0.0001$], and there was a significant interaction factor [$F_{(1,72)}=26.65$, $p<0.00001$]. *Post hoc* analysis showed the blockade of rapid tolerance by DNQX in the group DNQX + aniracetam + Ethanol on day 2 (Tukey's test).

4.4. Blood ethanol assay

Blood ethanol concentration was not significantly affected by the treatment with aniracetam 15 mg/kg (120.2 ± 7.0 mg/dl) DNQX 10 mg/kg (120.0 ± 2.9 mg/dl) and the conjunction of both, DNQX 10 mg/kg + Aniracetam 15 mg/kg (123.6 ± 3.8 mg/dl), as compared to the respective control group (126.8 ± 3.7 mg/dl).

5. Discussion

In order to verify whether AMPA-mediated neurotransmission participated in the development of rapid tolerance, we studied the influence of aniracetam and DNQX – an agonist and a competitive antagonist, respectively, at the AMPA/kainate receptor – on ethanol-induced motor impairment. The present results demonstrate for the first time that aniracetam (10, 15 and 22.5 mg/kg) facilitated the development of rapid tolerance. These doses, when given alone, did not affect ethanol-induced motor incoordination on day 1. Aniracetam concentrations appear to modulate the acquisition of rapid tolerance in an inverse U shape response, when the middle dose (15 mg/kg) presented the peak in the facilitation of acquisition of rapid tolerance, while the doses of 10 and 22.5 mg/kg showed diminished facilitation of the same phenomenon. Conversely, DNQX promptly blocked the facilitation of the development of rapid tolerance to ethanol induced by aniracetam. The fact that rapid tolerance was significantly modulated by treatment with aniracetam or DNQX highlights that the AMPA/kainate system is involved in the rapid-tolerance mechanism. The blockade of rapid tolerance by DNQX was obtained with doses that did not influence the behavior of animals treated with either saline or ethanol. Moreover, blood ethanol concentrations of animals treated with aniracetam or DNQX did not show any differences when compared to the respective controls, suggesting that pharmacokinetics interactions between ethanol and aniracetam or DNQX are unlikely.

As cited in the introduction Purkinje neurons are preferable targets of ethanol in CNS. These neurons necessarily receive excitatory inputs via a single climbing fiber. This pathway provides high glutamate release (Schmolesky et al., 2002). AMPA receptors are responsible for triggering the activation of climbing fibers, eliciting an excitatory response. In this perspective, AMPA receptors are in part responsible for the initial response in the modulation of Purkinje neurons. This hypothesis confirms that the pharmacological modulation of AMPA receptors could also modulate the acquisition of rapid tolerance.

There is evidence that learning factors might influence the development of chronic (Chen, 1968; Wenger et al., 1980) and rapid tolerance (Bitran and Kalant, 1991; Kalant, 1996; Khanna et al., 1994; Wazlawik and Morato, 2002). Previous studies have implicated the glutamatergic system, particularly the NMDA receptor, as an important target for acquisition of rapid tolerance (Corso et al., 1998; Khanna et al., 2002; Khanna et al., 1997; Neznanova et al., 2000; Trujillo and Akil, 1995). Several studies also describe the important role of the glutamatergic system in a number of plasticity processes, like long-term potentiation, for example (Bliss and Lomo, 1973; Derkach et al., 2007), a mechanism that is also involved in the rapid tolerance development (Chandler et al., 1998).

LTP is the molecular pathway that better represents the acquisition and maintenance of new memories (Bliss and Collingridge, 1993). Several studies have related this plasticity process as a pivotal part of mnemonic events (Izquierdo et al., 1997; Kandel, 2004). The disruption of LTP, by pharmacological blockade or circuitry disturbance, can absolutely abolish the formation of memories (Izquierdo et al., 1993; Quillfeldt et al., 1994; Roesler et al., 2000; Rosen et al., 2006; Rosen et al., 1992; Rosen et al., 1996). In the LTP mechanism, the entire glutamatergic system is involved, and the AMPA/kainate system has been related as the initial trigger for the event (Kakegawa and Yuzaki, 2005; Staubli et al., 1992; Tocco et al., 1992).

As cited above, disturbance of LTP results in non-formation of memories, and that is the mechanism underlying ethanol amnesic effects (Weitemier and Ryabinin, 2003; Wright et al., 2003; Yin et al., 2007). On the other hand, electrophysiological studies have characterized positive properties of ethanol to induce plasticity in the AMPA/kainate system (Frye and Fincher, 2000; Hou et al., 2008; Sager et al., in press; Villareal et al., 2007), but none of the cited studies has related an improvement specifically in LTP-type of plasticity. In fact, recent evidence shows that ethanol induces the reversion of direction in long-term synaptic plasticity (Yin et al., 2007).

Previous studies show that aniracetam facilitates whereas DNQX impairs plasticity processes in vitro (Andras et al., 2007; Blythe et al., 2007; Nicoletti et al., 1992). As mentioned above, the AMPA/kainate receptor system represents the initial trigger for LTP and the increased activity at this site. Thus, it is possible that the administration of an agonist (aniracetam) facilitated plasticity processes, which in turn facilitated the acquisition of tolerance response. Conversely, the inhibition of the AMPA receptor with the antagonist (DNQX) impaired plasticity processes leading to the blockade of tolerance acquisition.

The molecular adaptive changes occurring in the brain after ethanol exposure include fast (phosphorylation) or slow (changes in receptor subunit expression) alterations in different receptor systems such as $GABA_A$ and NMDA (Grobin and Deutch, 1998; Krystal et al., 2003; Loh and Ball, 2000; Ron, 2004). Thus, beyond the direct interaction of aniracetam and DNQX with AMPA/kainate receptor, it is possible that the facilitation of plasticity induced by ethanol could induce neuroadaptative changes and subsequently modulate $GABA_A$ and/or NMDA receptor responses to ethanol, as previously shown in in vivo and in vitro studies (Carpenter-Hyland et al., 2004; Dahchour et al., 2000; Maldve et al., 2002; Pickard et al., 2001; Watt et al., 2004). Therefore, the mechanisms underlying the effects of aniracetam and DNQX on ethanol rapid tolerance could be indirect.

Another possibility to explain how the activation or the blockade of the AMPA/kainate receptor influence rapid tolerance is that pharmacologic interference in the glutamatergic system modulates other neurotransmitters systems such as the cholinergic, GABAergic or noradrenergic system (Akirav, 2007; Hu et al., 2007; Kremin and Hasselmo, 2007; Liang et al., 2007; Ohno et al., 1996; Zheng et al., 2007), which have pivotal role in cognitive processes (Akhavan et al., 2008; Bentley et al., 2008; Bergado et al., 2007; Dumas et al., 2008). In this speculative hypothesis, the influence on the cognitive aspect of the task remains the same, where the disturbance of the cognition modulates the tolerance response.

Nevertheless, it cannot be ignored that the task used to evaluate rapid tolerance (rotarod) contemplates other non-cognitive components that could be responsible for the behavioral alteration as previously reported concerning the modulation of the endocannabinoid (Lemos et al., 2007) or opioid system (Varaschin et al., 2005).

In conclusion, the primary findings of this study suggest that the AMPA/kainate receptor system participates in the development of rapid tolerance to ethanol. In addition, a direct or indirect participation of the AMPA/kainate system in the learning component inherent to the proposed task is likely. Considering that tolerance mechanisms appear to be relevant to the development of ethanol abuse and dependence, as well as representing a predictor of susceptibility to alcoholism (Schuckit, 1986), the present results suggest an additional target to counteract these disorders.

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References

American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington, DC: Am. Psychiatric Assoc; 1994 (DSM-IV).
Akhavan MM, Emami-Abarghoie M, Safari M, Sadighi-Moghaddam B, Vafaei AA, Bandegi AR, et al. Serotonergic and noradrenergic lesions suppress the enhancing effect of maternal exercise during pregnancy on learning and memory in rat pups. Neuroscience 2008;151:1173–83.
Akirav I. NMDA Partial agonist reverses blocking of extinction of aversive memory by GABA(A) agonist in the amygdala. Neuropsychopharmacology 2007;32:542–50.
Allison C, Pratt JA. Differential effects of two chronic diazepam treatment regimes on withdrawal anxiety and AMPA receptor characteristics. Neuropsychopharmacology 2006;31:602–19.
Altman J, Everitt BJ, Glautier S, Markou A, Nutt D, Oretti R, et al. The biological, social and clinical bases of drug addiction: commentary and debate. Psychopharmacology (Berl) 1996;125:285–345.
Andras IE, Deli MA, Veszelka S, Hayashi K, Hennig B, Toborek M. The NMDA and AMPA/KA receptors are involved in glutamate-induced alterations of occludin expression and phosphorylation in brain endothelial cells. J Cereb Blood Flow Metab 2007;27:1431–43.
Bare DJ, McKinzie JH, McBride WJ. Development of rapid tolerance to ethanol-stimulated serotonin release in the ventral hippocampus. Alcohol Clin Exp Res 1998;22:1272–6.
Bashir ZI, Collingridge GL. Synaptic plasticity: long-term potentiation in the hippocampus. Curr Opin Neurobiol 1992;2:328–35.
Bast T, da Silva BM, Morris RG. Distinct contributions of hippocampal NMDA and AMPA receptors to encoding and retrieval of one-trial place memory. J Neurosci 2005;25:5845–56.
Bentley P, Driver J, Dolan RJ. Cholinesterase inhibition modulates visual and attentional brain responses in Alzheimer's disease and health. Brain 2008;131:409–24.
Bergado JA, Frey S, Lopez J, Almaguer-Melian W, Frey JU. Cholinergic afferents to the locus coeruleus and noradrenergic afferents to the medial septum mediate LTP-reinforcement in the dentate gyrus by stimulation of the amygdala. Neurobiol Learn Mem 2007;88:331–41.
Bitran M, Kalant H. Learning factor in rapid tolerance to ethanol-induced motor impairment. Pharmacol Biochem Behav 1991;39:917–22.
Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 1993;361:31–9.
Bliss TV, Lomo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol 1973;232:331–56.
Blythe SN, Atherton JF, Bevan MD. Synaptic activation of dendritic AMPA and NMDA receptors generates transient high-frequency firing in substantia nigra dopamine neurons in vitro. J Neurophysiol 2007;97:2837–50.
Botta P, Radcliffe RA, Carta M, Mameli M, Daly E, Floyd KL, et al. Modulation of GABAA receptors in cerebellar granule neurons by ethanol: a review of genetic and electrophysiological studies. Alcohol 2007;41:187–99.
Brugger F, Wicki U, Nassenstein-Elton D, Fagg GE, Olpe HR, Pozza MF. Modulation of the NMDA receptor by D-serine in the cortex and the spinal cord, in vitro. Eur J Pharmacol 1990;191:29–38.
Carpenter-Hyland EP, Woodward JJ, Chandler LJ. Chronic ethanol induces synaptic but not extrasynaptic targeting of NMDA receptors. J Neurosci 2004;24:7859–68.
Carta M, Mameli M, Valenzuela CF. Alcohol potently modulates climbing fiber->Purkinje neuron synapses: role of metabotropic glutamate receptors. J Neurosci 2006;26:1906–12.
Chandler LJ, Newsom H, Sumners C, Crews F. Chronic ethanol exposure potentiates NMDA excitotoxicity in cerebral cortical neurons. J Neurochem 1993;60:1578–81.
Chandler LJ, Harris RA, Crews FT. Ethanol tolerance and synaptic plasticity. Trends Pharmacol Sci 1998;19:491–5.
Chen CS. A study of the alcohol-tolerance effect and an introduction of a new behavioural technique. Psychopharmacologia 1968;12:433–40.
Cheng JK, Chiou LC. Mechanisms of the antinociceptive action of gabapentin. J Pharmacol Sci 2006;100:471–86.
Corso TD, Mostafa HM, Collins MA, Neafsey EJ. Brain neuronal degeneration caused by episodic alcohol intoxication in rats: effects of nimodipine, 6,7-dinitro-quinoxaline-2,3-dione, and MK-801. Alcohol Clin Exp Res 1998;22:217–24.
Crabbe JC, Rigter H, Uijlen J, Strijbos C. Rapid development of tolerance to the hypothermic effect of ethanol in mice. J Pharmacol Exp Ther 1979;208:128–33.
da Silva GE, Morato GS, Takahashi RN. Rapid tolerance to Delta(9)-tetrahydrocannabinol and cross-tolerance between ethanol and Delta(9)-tetrahydrocannabinol in mice. Eur J Pharmacol 2001;431:201–7.
Dahchour A, Hoffman A, Deitrich R, de Witte P. Effects of ethanol on extracellular amino acid levels in high-and low-alcohol sensitive rats: a microdialysis study. Alcohol Alcohol 2000;35:548–53.
Del Rio P, Montiel T, Massieu L. Contribution of NMDA and non-NMDA receptors to in vivo glutamate-induced calpain activation in the rat striatum relation to neuronal damage. Neurochem Res in press.
Derkach VA, Oh MC, Guire ES, Soderling TR. Regulatory mechanisms of AMPA receptors in synaptic plasticity. Nat Rev Neurosci 2007;8:101–13.
Dumas JA, Saykin AJ, McDonald BC, McAllister TW, Hynes ML, Newhouse PA. Nicotinic versus muscarinic blockade alters verbal working memory-related brain activity in older women. Am J Geriatr Psychiatry 2008;16:272–82.
Evans SM, Levin FR, Brooks DJ, Garawi F. A pilot double-blind treatment trial of memantine for alcohol dependence. Alcohol Clin Exp Res 2007;31:775–82.
Ferreira MB, Wolfman C, Walz R, Da Silva RC, Zanatta MS, Medina JH, et al. NMDA-receptor-dependent, muscimol-sensitive role of the entorhinal cortex in post-training memory processing. Behav Pharmacol 1992;3:387–91.
Frye GD, Fincher A. Sustained ethanol inhibition of native AMPA receptors on medial septum/diagonal band (MS/DB) neurons. Br J Pharmacol 2000;129:87–94.
Grant KA, Lovinger DM. Cellular and behavioral neurobiology of alcohol: receptor-mediated neuronal processes. Clin Neurosci 1995;3:155–64.
Grobin AC, Deutch AY. Dopaminergic regulation of extracellular gamma-aminobutyric acid levels in the prefrontal cortex of the rat. J Pharmacol Exp Ther 1998;285:350–7.
Hendricson AW, Maldve RE, Salinas AG, Theile JW, Zhang TA, Diaz LM, et al. Aberrant synaptic activation of *N*-methyl-D-aspartate receptors underlies ethanol withdrawal hyperexcitability. J Pharmacol Exp Ther 2007;321:60–72.
Himori N, Mishima K. Amelioration by aniracetam of abnormalities as revealed in choice reaction performance and shuttle behavior. Pharmacol Biochem Behav 1994;47:219–25.
Hou Q, Zhang D, Jarzylo L, Huganir RL, Man HY. Homeostatic regulation of AMPA receptor expression at single hippocampal synapses. Proc Natl Acad Sci U S A 2008;105:775–80.
Hu H, Real E, Takamiya K, Kang MG, Ledoux J, Huganir RL, et al. Emotion enhances learning via norepinephrine regulation of AMPA-receptor trafficking. Cell 2007;131:160–73.
Iorio KR, Tabakoff B, Hoffman PL. Glutamate-induced neurotoxicity is increased in cerebellar granule cells exposed chronically to ethanol. Eur J Pharmacol 1993;248:209–12.
Izquierdo I, Medina JH, Bianchin M, Walz R, Zanatta MS, Da Silva RC, et al. Memory processing by the limbic system: role of specific neurotransmitter systems. Behav Brain Res 1993;58:91–8.
Izquierdo I, Quillfeldt JA, Zanatta MS, Quevedo J, Schaeffer E, Schmitz PK, et al. Sequential role of hippocampus and amygdala, entorhinal cortex and parietal cortex in formation and retrieval of memory for inhibitory avoidance in rats. Eur J Neurosci 1997;9:786–93.
Jackson A, Brown G, Stephens DN. *N*-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate (AMPA) glutamate-receptor antagonists have different interactions with the discriminative stimuli of abused drugs. Psychopharmacology (Berl) 1996;128:320–7.
Kakegawa W, Yuzaki M. A mechanism underlying AMPA receptor trafficking during cerebellar long-term potentiation. Proc Natl Acad Sci U S A 2005;102:17846–51.
Kalant H. Current state of knowledge about the mechanisms of alcohol tolerance. Addict Biol 1996;1:133–41.
Kalant H. Research on tolerance: what can we learn from history? Alcohol Clin Exp Res 1998;22:67–76.
Kalant H, LeBlanc AE, Gibbins RJ. Tolerance to, and dependence on, some non-opiate psychotropic drugs. Pharmacol Rev 1971;23:135–91.
Kandel ER. The molecular biology of memory storage: a dialog between genes and synapses. Biosci Rep 2004;24:475–522.
Karolewicz B, Johnson L, Szebeni K, Stockmeier CA, Ordway GA. Glutamate signaling proteins and tyrosine hydroxylase in the locus coeruleus of alcoholics. J Psychiatr Res 2008;42:348–55.
Khanna JM, Morato GS, Chau A, Shah G, Kalant H. Effect of NMDA antagonists on rapid and chronic tolerance to ethanol: importance of intoxicated practice. Pharmacol Biochem Behav 1994;48:755–63.
Khanna JM, Shah G, Chau A. Effect of NMDA antagonists on rapid tolerance to ethanol under two different testing paradigms. Pharmacol Biochem Behav 1997;57:693–7.
Khanna JM, Morato GS, Kalant H. Effect of NMDA antagonists, an NMDA agonist, and serotonin depletion on acute tolerance to ethanol. Pharmacol Biochem Behav 2002;72:291–8.
Kolta A, Lynch G, Ambros-Ingerson J. Effects of aniracetam after LTP induction are suggestive of interactions on the kinetics of the AMPA receptor channel. Brain Res 1998;788:269–86.
Kremin T, Hasselmo ME. Cholinergic suppression of glutamatergic synaptic transmission in hippocampal region CA3 exhibits laminar selectivity: Implication for hippocampal network dynamics. Neuroscience 2007;149:760–7.
Krystal JH, Petrakis IL, Krupitsky E, Schutz C, Trevisan L, D'Souza DC. NMDA receptor antagonism and the ethanol intoxication signal: from alcoholism risk to pharmacotherapy. Ann N Y Acad Sci 2003;1003:176–84.
Lemos JI, Takahashi RN, Morato GS. Effects of SR141716 and WIN 55,212-2 on tolerance to ethanol in rats using the acute and rapid procedures. Psychopharmacology (Berl) 2007;194:139–49.
Liang J, Suryanarayanan A, Abriam A, Snyder B, Olsen RW, Spigelman I. Mechanisms of reversible GABAA receptor plasticity after ethanol intoxication. J Neurosci 2007;27:12367–77.

Littleton JM, John GR, Jones PA, Grieve SJ. The rapid onset of functional tolerance to ethanol—role of different neurotransmitters and synaptosomal membrane lipids. Acta Psychiatr Scand Suppl 1980;286:137–51.
Loh EW, Ball D. Role of the GABA(A)beta2, GABA(A)alpha6, GABA(A)alpha1 and GABA(A) gamma2 receptor subunit genes cluster in drug responses and the development of alcohol dependence. Neurochem Int 2000;37:413–23.
LoTurco JJ, Owens DF, Heath MJ, Davis MB, Kriegstein AR. GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. Neuron 1995;15:1287–98.
Lynch G, Gall CM. Ampakines and the threefold path to cognitive enhancement. Trends Neurosci 2006;29:554–62.
Maldve RE, Zhang TA, Ferrani-Kile K, Schreiber SS, Lippmann MJ, Snyder GL, et al. DARPP-32 and regulation of the ethanol sensitivity of NMDA receptors in the nucleus accumbens. Nat Neurosci 2002;5:641–8.
Mapelli J, D'Angelo E. The spatial organization of long-term synaptic plasticity at the input stage of cerebellum. J Neurosci 2007;27:1285–96.
Martins RA, Linden R, Dyer MA. Glutamate regulates retinal progenitors cells proliferation during development. Eur J Neurosci 2006;24:969–80.
Molz S, Decker H, Dal-Cim T, Cremonez C, Cordova FM, Leal RB, et al. Glutamate-induced toxicity in hippocampal slices involves apoptotic features and p38 MAPK signaling. Neurochem Res 2008;33:27–36.
Netzeband JG, Trotter C, Caguioa JN, Gruol DL. Chronic ethanol exposure enhances AMPA-elicited Ca2+ signals in the somatic and dendritic regions of cerebellar Purkinje neurons. Neurochem Int 1999;35:163–74.
Neznanova ON, Blokhina EA, Sukhotina IA, Bespalov AY. Motor impairment produced by ethanol and site-selective NMDA receptor antagonists in mice: tolerance and cross-tolerance. Alcohol 2000;20:31–6.
Nicoletti F, Casabona G, Genazzani AA, Copani A, Aleppo G, Canonico PL, et al. Excitatory amino acids and neuronal plasticity: modulation of AMPA receptors as a novel substrate for the action of nootropic drugs. Funct Neurol 1992;7:413–22.
Ohno M, Yoshimatsu A, Kobayashi M, Watanabe S. Beta-adrenergic dysfunction exacerbates impairment of working memory induced by hippocampal NMDA receptor blockade in rats. Eur J Pharmacol 1996;307:21–6.
Pastalkova E, Serrano P, Pinkhasova D, Wallace E, Fenton AA, Sacktor TC. Storage of spatial information by the maintenance mechanism of LTP. Science 2006;313:1141–4.
Pickard L, Noel J, Duckworth JK, Fitzjohn SM, Henley JM, Collingridge GL, et al. Transient synaptic activation of NMDA receptors leads to the insertion of native AMPA receptors at hippocampal neuronal plasma membranes. Neuropharmacology 2001;41:700–13.
Pickering C, Avesson L, Lindblom J, Liljequist S, Schioth HB. Identification of neurotransmitter receptor genes involved in alcohol self-administration in the rat prefrontal cortex, hippocampus and amygdala. Prog Neuropsychopharmacol Biol Psychiatry 2007;31:53–64.
Poklis A, Mackell MA. Evaluation of a modified alcohol dehydrogenase assay for the determination of ethanol in blood. Clin Chem 1982;28:2125–7.
Porter BE, Cui XN, Brooks-Kayal AR. Status epilepticus differentially alters AMPA and kainate receptor subunit expression in mature and immature dentate granule neurons. Eur J Neurosci 2006;23:2857–63.
Quillfeldt JA, Schmitz PK, Walz R, Bianchin M, Zanatta MS, Medina JH, et al. CNQX infused into entorhinal cortex blocks memory expression, and AMPA reverses the effect. Pharmacol Biochem Behav 1994;48:437–40.
Roesler R, Vianna MR, De-Paris F, Quevedo J, Walz R, Bianchin M. Infusions of AP5 into the basolateral amygdala impair the formation, but not the expression, of step-down inhibitory avoidance. Braz J Med Biol Res 2000;33:829–34.
Ron D. Signaling cascades regulating NMDA receptor sensitivity to ethanol. Neuroscientist 2004;10:325–36.
Rosen GD, Press DM, Sherman GF, Galaburda AM. The development of induced cerebrocortical microgyria in the rat. J Neuropathol Exp Neurol 1992;51:601–11.
Rosen GD, Sherman GF, Galaburda AM. Birthdates of neurons in induced microgyria. Brain Res 1996;727:71–8.
Rosen GD, Mesples B, Hendriks M, Galaburda AM. Histometric changes and cell death in the thalamus after neonatal neocortical injury in the rat. Neuroscience 2006;141:875–88.
Sager C, Tapken D, Kott S, Hollmann M. Functional modulation of AMPA receptors by transmembrane AMPA receptor regulatory proteins. Neuroscience in press.
Schmolesky MT, Weber JT, De Zeeuw CI, Hansel C. The making of a complex spike: ionic composition and plasticity. Ann N Y Acad Sci 2002;978:359–90.
Schuckit MA. Genetic aspects of alcoholism. Ann Emerg Med 1986;15:991–6.
Schuckit MA, Smith TL. Assessing the risk for alcoholism among sons of alcoholics. J Stud Alcohol 1997;58:141–5.
Staubli U, Ambros-Ingerson J, Lynch G. Receptor changes and LTP: an analysis using aniracetam, a drug that reversibly modifies glutamate (AMPA) receptors. Hippocampus 1992;2:49–57.
Tocco G, Maren S, Shors TJ, Baudry M, Thompson RF. Long-term potentiation is associated with increased [3H]AMPA binding in rat hippocampus. Brain Res 1992;573:228–34.
Trujillo KA, Akil H. Excitatory amino acids and drugs of abuse: a role for N-methyl-D-aspartate receptors in drug tolerance, sensitization and physical dependence. Drug Alcohol Depend 1995;38:139–54.
Vaglenova J, Pandiella N, Wijayawardhane N, Vaithianathan T, Birru S, Breese C, et al. Aniracetam reversed learning and memory deficits following prenatal ethanol exposure by modulating functions of synaptic AMPA receptors. Neuropsychopharmacology 2008;33:1071–83.
Varaschin RK, Wazlawik E, Morato GS. Systemic and intra-accumbens microinjections of naltrexone interfere with tolerance to ethanol in rats. Psychopharmacology 2005;182:366–74.
Villareal G, Li Q, Cai D, Glanzman DL. The role of rapid, local, postsynaptic protein synthesis in learning-related synaptic facilitation in aplysia. Curr Biol 2007;17:2073–80.
Waller MB, McBride WJ, Lumeng L, Li TK. Initial sensitivity and acute tolerance to ethanol in the P and NP lines of rats. Pharmacol Biochem Behav 1983;19:683–6.
Watt AJ, Sjostrom PJ, Hausser M, Nelson SB, Turrigiano GG. A proportional but slower NMDA potentiation follows AMPA potentiation in LTP. Nat Neurosci 2004;7:518–24.
Wazlawik E, Morato GS. Effects of intracerebroventricular administration of 7-nitroindazole on tolerance to ethanol. Brain Res Bull 2002;57:165–70.
Weitemier AZ, Ryabinin AE. Alcohol-induced memory impairment in trace fear conditioning: a hippocampus-specific effect. Hippocampus 2003;13:305–15.
Wenger JR, Berlin V, Woods SC. Learned tolerance to the behaviorally disruptive effects of ethanol. Behav Neural Biol 1980;28:418–30.
Wright JW, Kramar EA, Myers ED, Davis CJ, Harding JW. Ethanol-induced suppression of LTP can be attenuated with an angiotensin IV analog. Regul Pept 2003;113:49–56.
Xu F, Cai R, Xu J, Zhang J, Sun X. Early music exposure modifies GluR2 protein expression in rat auditory cortex and anterior cingulate cortex. Neurosci Lett 2007;420:179–83.
Yin HH, Park BS, Adermark L, Lovinger DM. Ethanol reverses the direction of long-term synaptic plasticity in the dorsomedial striatum. Eur J Neurosci 2007;25:3226–32.
Zheng G, Zhang X, Chen Y, Zhang Y, Luo W, Chen J. Evidence for a role of GABAA receptor in the acute restraint stress-induced enhancement of spatial memory. Brain Res 2007;1181:61–73.