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Neurobehavioral effects of alcohol in AMPA receptor subunit (GluR1) deficient mice

M.S. Cowen^a, K.-C. Schroff^a, P. Gass^a, R. Sprengel^b, R. Spanagel^{a,*}

^a Departments of Psychopharmacology and Psychiatry, University of Heidelberg, Central Institute of Mental Health (CIMH), J5, 68159 Mannheim, Germany

^b Department of Molecular Neurobiology, Max Planck Institute for Medical Research, 69120 Heidelberg, Germany

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Abstract

Of the ionotropic glutamatergic receptors, the NMDA receptor is clearly implicated in the acute and chronic effects of ethanol; however, the role of the AMPA receptor in mediating the effects of ethanol *in vivo* is as yet unclear. Using mice deficient in the AMPA receptor subunit GluR1 (GluR1^{-/-} mice), we investigated whether the AMPA receptor had a significant role in mediating the effects of ethanol. GluR1^{-/-} mice showed greater locomotor activity in a novel environment, but by the fifth day of repeated testing their activity was the same as that of wild-type mice. In contrast to their enhanced locomotor activity, on an accelerating rotarod GluR1^{-/-} mice performed consistently worse than wild-types. With regard to the effects of ethanol on motor responses, GluR1^{-/-} mice did not differ significantly from wild-type mice in ethanol's sedative or incoordinating effects. However, the GluR1^{-/-} mice were insensitive to the hypothermic effects of a hypnotic dose of ethanol in contrast to wild-types; this effect was dissociable from the hypnotic effects of ethanol. Further, tolerance to ethanol developed equally for GluR1^{-/-} mice versus wild-type mice. In terms of alcohol drinking behavior, compared to wild-types, GluR1^{-/-} mice differed neither in the acquisition of voluntary ethanol consumption nor in stress-induced ethanol drinking, nor in the expression of an alcohol deprivation effect (ADE) which is used as a model of relapse-like drinking behavior. In summary, although the loss of a hypothermic effect of ethanol in GluR1^{-/-} mice indicates a critical role for the AMPA receptors in this effect, the GluR1 subunit of the AMPA receptor does not seem to play a critical role in the etiology of alcohol dependence. However, changes observed in activity patterns may be related to the putative role of AMPA receptors in attention deficit hyperactivity disorder.

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1. Introduction

Glutamate receptors, in particular NMDA receptors, have been implicated in a number of pathophysiologic states associated with alcohol (Tsai and Coyle, 1998; Spanagel and Bienkowski, 2002; Krystal et al., *in press*); including its acute behavioral effects, withdrawal symptoms, relapse behavior and even the development of fetal alcohol syndrome (Ikonomidou et al., 2000). Ethanol has been shown to dose-dependently decrease NMDA-

mediated excitatory post-synaptic currents (Lovinger et al., 1989, 1990) at behaviorally relevant concentrations. The discriminative stimulus properties of ethanol appear to be mediated via the NMDA receptor (Hundt et al., 1998; Krystal et al., *in press*) and in a behavioral model of relapse, the alcohol deprivation effect (ADE), the post-deprivation increase in ethanol consumption has been shown to be prevented by drugs which inhibit NMDA-mediated neurotransmission (and thus partially mimic the effects of ethanol; Höltner et al., 1996, 2000). Although a clear link between ethanol and *in vivo* inhibition of NMDA-mediated neurotransmission has thus been established, an association between ethanol and the other main glutamatergic ionotropic receptor, the AMPA receptor, has been more difficult to determine.

* Corresponding author. Tel.: +49-621-1703-833; fax: +49-621-1703-837.

E-mail address: spanagel@zi-mannheim.de (R. Spanagel).

Different *in vitro* preparations indicate that there are no major differences in the sensitivity of NMDA receptors and AMPA receptors to the acute effects of ethanol; however, in some preparations (depending on receptor subunit composition and other factors) NMDA receptors might be more sensitive to ethanol than AMPA receptors (for reviews see Narahashi et al., 2001; Spanagel and Bienkowski, 2002). Ethanol, at pharmacologically relevant concentrations, suppresses AMPA receptor-mediated currents (Martin et al., 1995; Frye and Fincher, 2000; Akinshola et al., 2001) but following adaptation to chronic ethanol exposure, augmented AMPA receptor-mediated post-synaptic potentials in hippocampal slices from mice have been reported (Molleman and Little, 1995). This enhanced response of AMPA receptors might be mediated by a specific up-regulation of AMPA receptor subunit proteins (Chandler et al., 1999). In summary, there is some indication from several *in vitro* preparations that AMPA receptor-mediated responses are involved in acute and chronic effects of ethanol but the situation *in vivo* is not yet clear.

Recently, Zamanillo et al. (1999) have generated gene-targeted mice lacking the AMPA receptor subunit GluR1 that exhibit normal development. Although the remaining AMPA subunits (GluR2/3/4) appear to be able to form functional AMPA receptors (Zamanillo et al., 1999), differences in GluR1^{-/-} mice compared with wild-type mice were observed in terms of tolerance, development and withdrawal severity to morphine (Vekovischeva et al., 2001). We were therefore interested in using these mice to examine the potential role of the AMPA receptor in the acute effects of ethanol, in tolerance to ethanol and in alcohol drinking behavior. Before acute and chronic effects of ethanol were examined, mice were studied for their motor behavior in activity chambers and on a rotarod.

2. Materials and methods

2.1. Animals

A total of 30 wild-type and 29 GluR1^{-/-} male mice were available from the Max Planck Institute for Medical Research in Heidelberg. The genetic background originated from a combination of the 129X1/SvJ and C57BL/6N. Mice were then completely backcrossed to C57BL/6N and then inter-crossed to obtain knockout mice and wild-type littermates. Inter-crosses of heterozygous animals produced 25% GluR1^{-/-} mice (see, for a detailed description, Zamanillo et al., 1999). The mice weighed an average of 35 ± 0.7 g at the beginning of the experiments and there was no significant weight difference between GluR1^{-/-} and wild-type mice. Twenty of these mice were set aside to examine the innate effect of ethanol on rotarod performance; the remainder were

used to examine locomotor activity and the effect of ethanol on locomotor activity, ethanol consumption with deprivation and stress episodes, and acute ethanol tolerance. Mice were housed singly in standard breeding cages (Macrolon Type III) with food (Sniff rodent food pellets, Soest, Germany) and water *ad libitum*. Mice were kept on a 12 h dark/light cycle (lights on between 6.00 a.m. and 6.00 p.m.; light intensity 180 lx) with constant temperature and humidity (temperature: 22 ± 1 °C; humidity: $55 \pm 5\%$) in rooms of the animal facility of the Central Institute of Mental Health, Mannheim, Germany.

The experiments were approved by the Committee on Animal Care and Use of the relevant local government body and carried out following the German Law on the Protection of Animals.

2.2. Locomotor activity

GluR1^{-/-} and wild-type mice were injected with saline (0.9% w/v *i.p.*) for 6 days and placed in TruScan Photobeam Activity Monitors (26 × 26 × 40 cm; Coulburn Instruments, Allentown, PA) for 1 h per day. Locomotor activity, in terms of total distance traveled and total movement time, was determined automatically using the TruScan software. On the seventh day (once levels of locomotor activity were stable across days), GluR1^{-/-} and wild-type mice were injected with ethanol (2 g/kg, 20% v/v *i.p.*) and again placed in the TruScan Photobeam Activity Monitors for 1 h.

2.3. Effect of ethanol on rotarod performance

GluR1^{-/-} and wild-type mice were placed for 3 min on an immobile rotarod cylinder (TSE Systems, Bad Homburg, Germany). The rotarod was then rotated at 10 rpm for 10 min. If the mice fell off during this training period, they were placed back on the cylinder. Three hours later, the first test on an accelerating rotarod was performed. Mice were placed on the immobile cylinder, which was then accelerated for 3 min, from 2.5 to 25 rpm. The time until the mice fell off the rotating cylinder was recorded.

The accelerating test was performed daily until performance by the mice appeared to stabilize. Mice were then injected with ethanol (2 g/kg, 20% v/v *i.p.*; made from 190 proof (95 + % pure) ethanol) and subjected to an accelerating rotarod test as previously, lasting a maximum of 3 min. The mice were re-tested on the accelerating rotarod at 15 min intervals, at $t = 15, 30, 45, 60$ and 75 min after injection with ethanol.

2.4. Ethanol self-administration test

GluR1^{-/-} and wild-type mice were given a free-choice between water and increasing concentrations of

ethanol (2, 4, 8, 12 and 16%) similar to our previously used protocol (Spanagel et al., 2002). Baseline intake of food and water was measured for 4 days. Mice then had access to 2% ethanol for 3 days, 4% ethanol for 3 days, 8% ethanol for 24 days, 12% for 24 days, and 16% for over 24 days. The position of the ethanol solution was changed every 2–3 days to avoid location preferences. Body weight was measured weekly, and water intake (ml/day) and ethanol intake (ml/day) were measured three times per week. From this data, ethanol preference (% of total fluid intake) and ethanol intake (g ethanol/kg body weight/day) were calculated.

2.5. Alcohol deprivation effect

Consumption of 16% ethanol by the GluR1^{-/-} and wild-type mice was allowed to continue until the ethanol consumption had stabilized, and then access to ethanol was withdrawn for 2 weeks. At the end of these 2 weeks, access to a 16% ethanol solution was reinstated (concurrent with access to water). The consumption by the mice of ethanol and water was measured daily for 3 days.

2.6. Forced swim stress and ethanol consumption

Forced swim stress was performed essentially as previously described (Sillaber et al., 2002). Basal ethanol consumption by the GluR1^{-/-} and wild-type mice was measured for 8 days. On two consecutive days, the mice were placed in tall glass cylinders containing enough water to prevent their tails from touching the bottom. The water temperature was 21 °C, and the mice remained in the water for 5 min. The latency to immobility was measured, and the ethanol consumption was measured in both of the subsequent 24-h periods, to examine the effect of swim stress on ethanol consumption. Post-stress ethanol consumption by the mice was then measured for another 8 days.

2.7. Acute ethanol tolerance

The acute ethanol tolerance test was performed essentially as previously described (Spanagel et al., 2002), 8 weeks after the wild-type and GluR1^{-/-} mice were withdrawn from free-choice ethanol consumption. Mice were injected with ethanol (3.5 g/kg, 20% v/v i.p.). The onset of loss of righting reflex was recorded (inability of the mice to right themselves three times within 1 min, when placed on their backs), as was the duration of the loss of righting reflex. Eight hours after the initial injection of ethanol, the mice were re-injected with ethanol (2 g/kg i.p.). The following day, the mice were then injected again with the larger dose of ethanol (3.5 g/kg i.p.); the onset and duration of the loss of righting reflex were again recorded.

In parallel with the above measurements, the effect of ethanol on body temperature was analyzed in half of the mice. Changes in ventral surface temperature are known to correlate closely with changes in core body temperature (Spanagel et al., 2002); thus the ventral surface temperatures of the GluR1^{-/-} and wild-type mice were measured using an infrared thermometer (Infratherm, Boston, USA) before injection with ethanol on the first day. Thirty minutes after injection of ethanol, the body temperature of the mice was again measured. The following day, the body temperatures of the mice were again measured using an infrared thermometer prior to injection with ethanol, and again 30 min later.

2.8. Statistics

All data are presented as the mean \pm the standard error of the mean (SEM) and a significance level of $p \leq 0.05$ was used throughout. In general, comparisons were by repeated measures analysis of variance (ANOVA). Within factors were either day/treatment, or concentration of ethanol (to examine the effect of increasing concentrations of ethanol on ethanol and water intake). To examine the hypothermic effect of ethanol, both the day of injection and the time relative to injection (pre-injection and post-injection, referred to as the effect of ethanol) were treated as within factors. Genotype (GluR1^{-/-} versus wild-type) was analyzed as a between factor in all of the repeated measure ANOVAs. Analysis of the effect on rotarod performance of genotype was performed using Mann–Whitney *U*-tests, and of training and ethanol using Friedman tests.

3. Results

3.1. Locomotor activity in GluR1^{-/-} mice

On the first four days of saline injections, there was a significant effect of genotype on locomotor activity ($F(1,12) = 10.142$, $p = 0.008$), with the GluR1^{-/-} mice showing a significantly greater locomotor activity compared with wild-type controls (Fig. 1). There was also a significant effect of day ($F(3,36) = 10.332$, $p < 0.001$), as the locomotor activity decreased over days (Fig. 1), with no significant interaction between day and genotype ($F(3,36) = 0.507$, $p = 0.680$). In contrast, on the fifth and sixth days of saline injections, there was neither a significant effect of genotype ($F(1,13) = 1246$, $p = 0.284$) nor of day ($F(1,13) = 3.842$, $p = 0.072$) with again no significant interaction, ($F(1,13) = 0.299$, $p = 0.594$). Ethanol at a dose of 2 g/kg i.p. caused a significant decrease in distance moved ($F(1,12) = 28.000$, $p < 0.001$) by the GluR1^{-/-} and wild-type mice (Fig. 1), relative to the distance moved on the previous day. Again however, there was neither a significant effect of

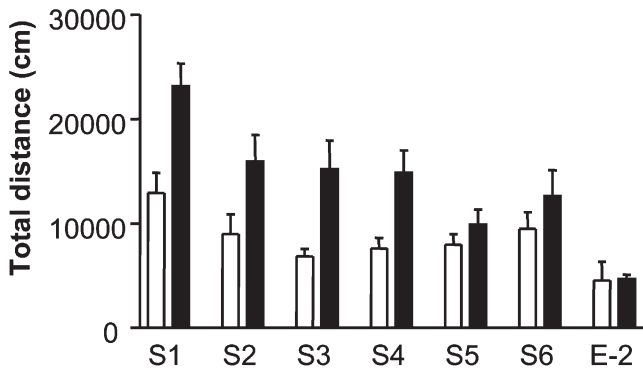


Fig. 1. Locomotor activity of wild-type (open columns) and GluR1^{-/-} mice (closed columns) in 1 h sessions on successive days, in terms of distance moved. S prefix indicates days of saline injections; ethanol (E-2) was injected at the dose of 2 g/kg. Locomotor activity was significantly greater for the GluR1^{-/-} mice compared with the wild-type mice over the first four days ($p = 0.008$); however, beyond this time point there was no significant difference between genotypes ($p > 0.05$). Ethanol caused a significant decrease in distance moved ($p < 0.001$).

genotype ($F(1,12) = 0.590$, $p = 0.457$) nor an interaction between day and genotype ($F(1,12) = 1.572$, $p = 0.234$) at this dose of ethanol.

3.2. Rotarod performance in GluR1^{-/-} mice

With repeated training days, GluR1^{-/-} and wild-type mice improved their performance on the rotarod ($\chi^2_{4|} = 29.638$, $p < 0.001$; Fig. 2A). Unexpectedly however, compared with the wild-type mice, the GluR1^{-/-} mice performed significantly worse over the days of training (Fig. 2A; Day 1, $U(11,7) = 25$, $p = 0.221$; Day 2, $U = 29$, $p = 0.358$; Day 3, $U = 17$, $p = 0.027$; Day 4, $U = 14$, $p = 0.020$; Day 5, $U = 5.5$, $p < 0.001$), such that on the fifth day of training the wild-type mice remained on the rotarod an average of 180 s (the full length of time), whereas the GluR1^{-/-} mice remained on the rotarod an average of 120 s. Because of this significant difference in performance, the effect of ethanol (2 g/kg i.p.) on the sixth day was determined as a ratio, relative for each mouse to time $t = 0$ (i.e. before injection), of performance at all subsequent times. Injection of ethanol caused a significant impairment in rotarod performance ($\chi^2_{5|} = 65.008$, $p < 0.001$; Fig. 2B), such that at $t = 15$ min, wild-types remained on the accelerating rotarod for an average of 27 s and GluR1^{-/-} mice for an average of 22 s. At $t = 75$ min, both groups had returned to pre-injection performance (Fig. 2B). There was, however, no impact of genotype when the data was calculated as a ratio of pre-injection performance (Fig. 2B; $t = 15$ min, $U(11,7) = 34$, $p = 0.683$; $t = 30$ min, $U = 33.5$, $p = 0.650$; $t = 45$ min, $U = 29.5$, $p = 0.406$; $t = 60$ min, $U = 32$, $p = 0.552$; $t = 75$ min, $U = 35$, $p = 0.718$).

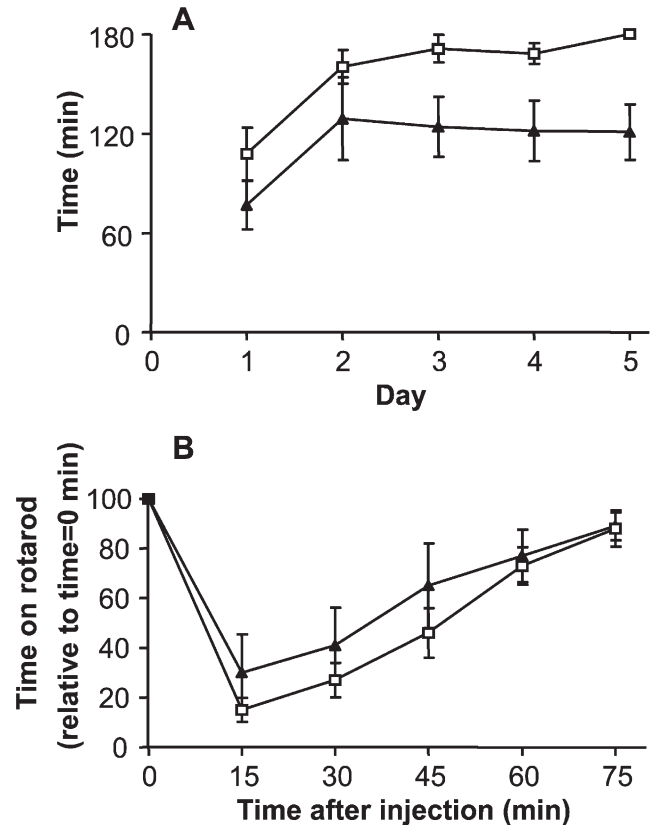


Fig. 2. Rotarod performance by GluR1^{-/-} and wild-type mice. (A) Over successive days, rotarod performance by both GluR1^{-/-} (closed triangles) and wild-type (open squares) mice improved significantly ($p < 0.001$); however, performance by GluR1^{-/-} mice remained significantly worse than that of wild-type mice (Days 3–5, $p < 0.05$). (B) Relative to pre-injection performance ($t = 0$), ethanol (2 g/kg i.p.) caused a significant impairment of rotarod performance by both GluR1^{-/-} and wild-type mice ($p < 0.001$); however, there was no significant difference between genotypes in terms of recovery when adjusted for initial performance ($p > 0.05$).

3.3. Ethanol self-administration and the alcohol deprivation effect (ADE)

The concentration of available ethanol had a significant effect on ethanol consumption ($F(4,140) = 40.599$, $p < 0.001$; Fig. 3A), water consumption ($F(4,140) = 45.591$, $p < 0.001$; Fig. 3B) and ethanol preference ($F(4,140) = 47.888$, $p < 0.001$; Fig. 3C). GluR1^{-/-} and wild-type mice showed no significant difference in ethanol consumption ($F(1,35) = 1.822$, $p = 0.186$; Fig. 3A), water consumption ($F(1,35) = 1.246$, $p = 0.272$; Fig. 3B) or ethanol preference ($F(1,35) = 0.002$, $p = 0.962$; Fig. 3C) at any concentration of ethanol offered, nor was there a significant interaction between concentration of ethanol and genotype for ethanol consumption ($F(4,140) = 1.999$, $p = 0.098$), water consumption ($F(4,140) = 1.890$, $p = 0.116$) and ethanol preference ($F(4,140) = 1.140$, $p = 0.340$).

Pre-deprivation ethanol consumption averaged 4.7 g/kg/day for GluR1^{-/-} and wild-type mice. On the first

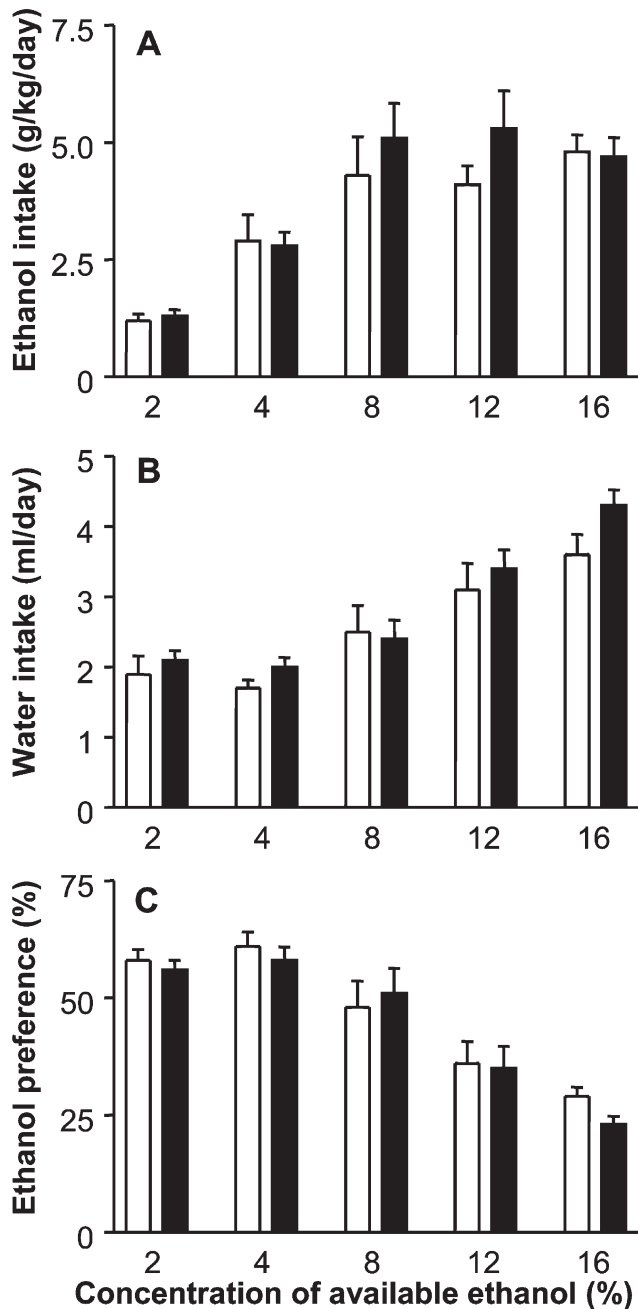


Fig. 3. Ethanol intake (A), water intake (B) and ethanol preference (C) of wild-type (open columns) and GluR1^{-/-} (closed columns) mice with increasing concentrations of ethanol. The concentration of available ethanol had a significant effect on ethanol intake ($p < 0.001$), water intake ($p < 0.001$) and ethanol preference ($p < 0.001$). There was no significant effect of genotype on ethanol or water intake, or preference for ethanol ($p > 0.05$).

post-deprivation day, ethanol consumption averaged 7.9 g/kg/day. Whereas deprivation from ethanol led to a significant increase in ethanol consumption by both GluR1^{-/-} and wild-type mice ($F(1,34) = 7.295$, $p = 0.011$; Fig. 4), ethanol deprivation had no effect on water consumption ($F(1,34) = 0.077$, $p = 0.784$; data not shown). However, there was no significant impact of

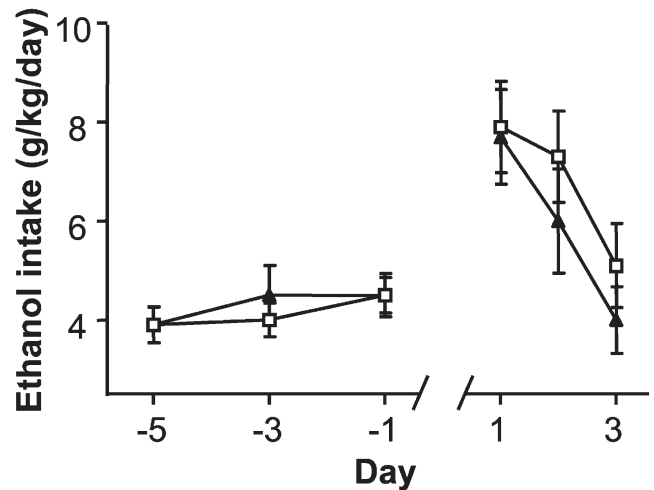


Fig. 4. Effect of deprivation from ethanol on ethanol consumption in g/kg per day by wild-type (open squares) and GluR1^{-/-} (closed triangles) mice. Whereas deprivation caused a significant increase in ethanol consumption ($p = 0.011$), deprivation from ethanol had no significant effect on water consumption (data not shown). There was no significant difference between GluR1^{-/-} and wild-type mice in terms of ethanol during the alcohol deprivation effect (ADE) ($p > 0.05$). X-axis-break indicates a 14-day period of deprivation.

genotype on ethanol consumption ($F(1,34) = 0.017$, $p = 0.897$; Fig. 4) or water consumption ($F(1,34) = 4.108$, $p = 0.051$; data not shown) during the ADE, nor was there an interaction between genotype and deprivation on ethanol consumption ($F(1,34) = 2.063$, $p = 0.160$) or water consumption ($F(1,34) = 0.131$, $p = 0.719$).

3.4. Stress-induced alcohol drinking

Forced swim stress led to a significant increase in ethanol consumption by the GluR1^{-/-} and wild-type mice in the 24-h periods immediately following the stress episodes, relative both to pre- and post-stress ethanol consumption ($F(2,34) = 82.596$, $p < 0.001$; Fig. 5). There was no significant difference between GluR1^{-/-} and wild-type mice in terms of stress-induced ethanol consumption ($F(1,17) = 0.053$, $p = 0.747$), nor was there an interaction between stress and genotype ($F(2,34) = 0.200$, $p = 0.819$). Forced swim stress also caused an increase in water consumption in the two 24-h periods immediately following the stress episodes ($F(2,34) = 11.006$, $p < 0.001$; data not shown). Throughout the measurement period there was also a small but significant difference between the GluR1^{-/-} and wild-type mice in water consumption ($F(1,17) = 10.262$, $p < 0.001$), with wild-type mice drinking significantly less water than GluR1^{-/-} mice except in the 24-h periods immediately following stress, but there was no interaction between stress and genotype ($F(2,34) = 0.886$, $p = 0.422$). Stress caused an increase in ethanol preference in both GluR1^{-/-} and wild-type mice ($F(2,34) =$

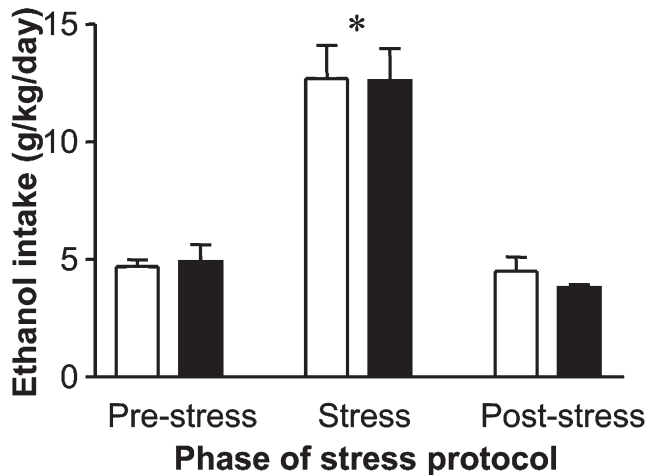


Fig. 5. The effect of forced swim stress on ethanol consumption by wild-type (open columns) and GluR1^{-/-} (closed columns) mice in the 8-day period preceding the days of swim stress (pre-stress), in the two 24-h periods immediately following stress episodes (Stress) and in the 8 days following (post-stress). * Stress caused a significant overall increase in ethanol ($p < 0.001$) and water ($p < 0.001$) consumption (data not shown) in the two 24-h periods immediately following episodes of swim stress, relative to both pre- and post-stress consumption. Genotype had no significant effect on ethanol consumption over the measurement period ($p > 0.05$), but during this time GluR1^{-/-} mice drank significantly greater amounts of water than wild-type mice ($p < 0.001$; data not shown).

50.248, $p < 0.001$) with preference for the ethanol solution averaging 28% prior to the stress episodes, 46% during the stress episodes and 26% immediately subsequent to the forced swim stress; however, there was neither an effect of genotype on ethanol preference ($F(1,17) = 0.210$, $p = 0.653$), nor an interaction between genotype and stress on ethanol preference ($F(2,34) = 0.238$, $p = 0.789$). Latency to immobility on both days of swim stress was not significantly different between the genotypes (GluR1^{-/-} mice, 40 ± 11 s; wild-type mice, 66 ± 15 s; $F(1,17) = 1.812$, $p = 0.196$), however, as expected there was a significant decrease in latency to immobility on the second day (GluR1^{-/-} mice, 9 ± 1 s; wild-type mice, 9 ± 5 s; $F(1,17) = 27.43$, $p < 0.001$) with no interaction between genotype and day ($F(1,17) = 2.601$, $p = 0.125$).

3.5. Loss of righting reflex, ethanol-induced hypothermia and acute ethanol tolerance

No significant difference in onset of loss of righting reflex was observed over days ($F(1,29) = 0.066$; $p = 0.800$; Table 1); there was neither a significant effect of genotype on onset of loss of righting reflex ($F(1,29) = 0.020$, $p = 0.888$; Table 1) nor an interaction between genotype and day ($F(1,29) = 0.036$, $p = 0.850$). However, the duration of loss of righting reflex was significantly lower on the second day compared with the first ($F(1,29) = 23.175$, $p < 0.001$; Table 1), although there

Table 1

Onset and duration of loss of righting reflex by wild-type and GluR1^{-/-} mice following successive hypnotic doses of ethanol (2×3.5 g/kg)

	Onset (s)		Duration (min)	
	Wild-type	GluR1 ^{-/-}	Wild-type	GluR1 ^{-/-}
Day 1	224 ± 32	225 ± 33	104 ± 11	103 ± 12
Day 2	227 ± 76	246 ± 79	71 ± 9	44 ± 9 ^a

Neither genotype nor repeated injection had an effect on the onset of loss of righting reflex due to a hypnotic dose of ethanol (3.5 g/kg, $p > 0.05$).

^a Repeated injection of ethanol caused a significant decrease in the duration of loss of righting reflex ($p < 0.05$), whereas genotype had no significant effect on the duration of loss of righting reflex ($p > 0.05$).

was neither a significant effect of genotype ($F(1,29) = 1.625$; $p = 0.213$) nor an interaction between genotype and day ($F(1,29) = 1.967$, $p = 0.171$) indicating the development of acute tolerance in both genotypes.

There was a significant hypothermic effect of ethanol on body temperature ($F(1,11) = 6.992$, $p = 0.023$) and a significant interaction between the effect of ethanol and genotype ($F(1,11) = 9.115$, $p = 0.012$) as indicated in Fig. 6: ethanol caused a reduction in body temperature in wild-type but not GluR1^{-/-} mice. Not surprisingly, there was a significant effect of genotype on body temperature ($F(1,11) = 10.690$, $p = 0.007$). This effect of ethanol occurred regardless of the day of injection ($F(1,11) = 0.277$, $p = 0.609$); there was no interaction

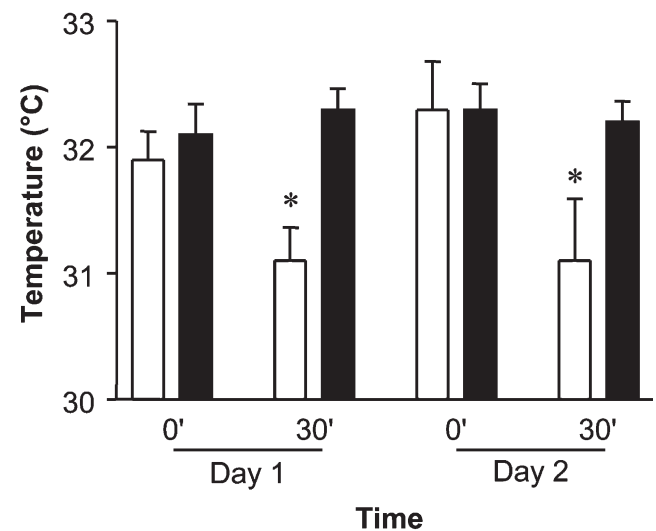


Fig. 6. Effect of ethanol (3.5 g/kg i.p.) on successive days on body temperature in wild-type (open columns) and GluR1^{-/-} (closed columns) mice. * Ethanol caused a significant decrease in body temperature ($p = 0.023$) in wild-type but not GluR1^{-/-} mice. Repeated injection of ethanol had no significant impact ($p > 0.05$) on the hypothermic effect of ethanol.

between the day of injection and the effect of ethanol ($F(1,11) = 1.065$, $p = 0.324$). Finally, there was neither an interaction between genotype and day in this effect ($F(1,11) = 0.073$, $p = 0.792$) nor an interaction between the effect of ethanol, genotype and day ($F(1,11) = 0.081$, $p = 0.781$).

4. Discussion

Neurobehavioral effects of alcohol were studied in mice with a deletion of the GluR1 subunit of the AMPA receptor. Before acute and chronic effects of ethanol were examined, mice were studied for their motor activity and coordination. GluR1 $^{-/-}$ mice demonstrated greater locomotor activity in a novel environment compared with wild-type littermate controls, as previously demonstrated (Vekovischeva et al., 2001); however, by the fifth day of repeated testing, their locomotor responses to the environment were the same as the locomotor responses of wild-type mice. In contrast to their enhanced locomotor activity, on the rotarod the GluR1 $^{-/-}$ mice performed consistently worse than their wild-type counterparts. With regard to the effects of acute ethanol treatment, the GluR1 $^{-/-}$ mice did not differ significantly from wild-type mice in terms of ethanol's sedative effects (locomotor activity), incoordinating effects (on the rotarod), or hypnotic effects (loss of righting reflex). Further, the GluR1 $^{-/-}$ mice did not differ significantly from wild-type mice in the acquisition of voluntary alcohol drinking. Following both stress and deprivation, enhanced alcohol intake was observed in all mice but there was no significant effect of genotype, indicating that AMPA receptors are not involved in stress-induced alcohol drinking or relapse-like drinking behavior (ADE). However, the GluR1 $^{-/-}$ mice were insensitive to the hypothermic effects of ethanol in contrast to wild-type mice; this effect was dissociable from the hypnotic effects of the same dose of ethanol. Acute tolerance to ethanol developed equally in the GluR1 $^{-/-}$ mice versus the wild-type mice, which is in contrast to the diminished development of morphine tolerance in GluR1 $^{-/-}$ mice demonstrated previously (Vekovischeva et al., 2001).

The greater locomotor activity of the GluR1 $^{-/-}$ mice observed in this and the previous study (Vekovischeva et al., 2001) appears to be a general hyperactivity mediated via altered AMPA-mediated currents. The poorer performance in the accelerating rotarod test may then be paradoxically related to this enhanced activity: hyperactivity precluding perseverance in the task. Interestingly, the AMPA modulator CX-516 is now in phase II trials for attention deficit hyperactivity disorder (ADHD; Danysz, 2002) and altered AMPA-mediated neurotransmission has been demonstrated in genetic models of ADHD (Russell, 2001).

With respect to ethanol-induced sedation in GluR1 $^{-/-}$ and wild-type mice it is important to note that different *in vitro* preparations indicate no major differences in the sensitivity of NMDA receptors and AMPA receptors to the acute effects of ethanol. However, in some preparations, depending on receptor subunit composition and other factors, NMDA receptors might be more sensitive to ethanol than AMPA receptors (Narahashi et al., 2001; Spanagel and Bienkowski, 2002). Thus in their initial study, Lovinger et al. (1989) have made the point that whereas NMDA receptors appear to be inhibited at concentrations of ethanol that lead to sedation and mental impairment, AMPA receptors were inhibited at concentrations of ethanol that lead to stupor and coma. Therefore, it was perhaps not surprising that GluR1 $^{-/-}$ mice showed no significant difference to wild-type controls when the effects of relatively moderate doses of ethanol (up to 2 g/kg) were tested in the locomotor activity and accelerating rotarod tests. However, of interest was that the hypothermic effect of a hypnotic dose of ethanol was prevented in GluR1 $^{-/-}$ mice, all the more significant because other AMPA subunits can compensate partially for the loss of the GluR1 subunit. Interestingly, the AMPA/kainate antagonist NBQX has been shown to cause a significant decrease in body temperature (Nurse and Corbett, 1996), whereas AMPA itself has been shown to cause a significant increase in body temperature (Yanase et al., 1998; Grundy et al., 1999); however, a connection between ethanol-induced hypothermia and the AMPA receptor had not previously been established. Although ethanol-induced hypothermia was prevented in GluR1 $^{-/-}$ mice, these animals showed a similar onset and duration in the loss of righting reflex compared to wild-type animals. Furthermore, a second injection of a hypnotic dose of ethanol resulted in a significantly lower duration of the loss of righting reflex compared to the first injection indicating the development of acute tolerance. Since there was no difference in the duration in the loss of righting reflex between genotypes, we have to assume that AMPA receptors are not involved in tolerance phenomena to ethanol. This finding is in line with other *in vivo* pharmacological data: the AMPA/kainate receptor blocking agents, NBQX and LY326325, had no effect on the development of tolerance to the hypnotic effects of ethanol in C57/BL6 mice (Karcz-Kubicha and Liljequist, 1995). Interestingly, the fact that the GluR1 $^{-/-}$ mice did not differ in the development of tolerance to ethanol highlights the functional dissociation between the mechanism of tolerance to morphine and ethanol, as tolerance to the effects of morphine were clearly impaired in GluR1 $^{-/-}$ mice (Vekovischeva et al., 2001).

Drugs that antagonize NMDA receptor-mediated neurotransmission have been shown to significantly decrease alcohol drinking and relapse-like behavior measured

using the ADE (Hölter et al., 1996, 2000) and one of these compounds is now being tested in a clinical setting (neramexane). In contrast, the present study indicates that the role of the AMPA receptor in the acquisition of voluntary alcohol drinking and subsequent relapse-like drinking behavior is likely to be minimal. Our findings on alcohol drinking and relapse behavior in GluR1 knockout mice are supported by other findings: Stephens and Brown (1999) showed in rats that operant oral self-administration of ethanol was not disrupted by the selective AMPA receptor antagonist GYKI 52466. In addition, the ADE measured under operant conditions in rats was also not affected by GYKI 52466 (Spanagel, 2001). Finally, GYKI 52466 cannot substitute for ethanol in a discrimination task (Hundt et al., 1998) which is usually a common feature of non-competitive NMDA receptor antagonists and which may underlie the anti-relapse properties of these compounds.

Since stress-induced changes in mRNA levels of GluR1 subunits have been reported (Bartanusz et al., 1995), we anticipated an altered stress-responsiveness in GluR1^{-/-} mice. However, neither latency to immobility nor other behavioral parameters in the swim stress test were different to wild-type animals. Although both genotypes reacted to swim stress with enhanced alcohol consumption, there was no difference in the magnitude of the response between genotypes, demonstrating that AMPA receptors play no critical role in mediating stress responses, especially stress-induced alcohol drinking.

In summary, mice lacking the GluR1 subunit of the AMPA receptor do not differ from wild-type mice in their responses to acute and chronic ethanol treatment with one exception: ethanol-induced hypothermia does not occur in these knockout animals. However, ethanol-induced sedation and incoordination, ethanol tolerance as well as ethanol drinking behavior (including relapse-like drinking behavior and stress-induced drinking behavior) are not altered in these mice suggesting that the AMPA receptor does not play a critical role in the etiology of alcoholism. However, such a conclusion is limited by the fact that compensatory changes of other AMPA receptor subunits or glutamate receptors in GluR1^{-/-} mice might occur that will mask the effects of the specific gene deletion on alcohol-related behaviors. Thus it has been observed that in the hippocampus of GluR1^{-/-} mice a redistribution of GluR2 subunits occurs (Zamanillo et al., 1999) and GluR2/3 up-regulation ensues upon GluR1 deletion (Mead and Stephens, 2003). Certainly, genetic deletions of other AMPA receptor subunits have to be studied to get a complete understanding of the role of AMPA receptors in mediating neurobehavioral effects of ethanol. Nevertheless, the observed hyperactivity and incoordination on a rotarod test in GluR1^{-/-} mice gives support to the hypothesis that this glutamate receptor is involved in attention deficit hyperactivity disorder.

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