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Metabotropic glutamate 5 receptors regulate sensitivity to ethanol in mice



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Abstract

The metabotropic glutamate receptor 5 (mGlu5) has been implicated in ethanol- and drug-seeking behaviours in rodent studies. Here we examine a number of ethanol-related behavioural assays in mice lacking mGlu5 and wild-type littermates. In a two-bottle free-choice paradigm, mGlu5-deficient mice consumed less ethanol with a reduced preference compared to wild-type mice. Indeed, mGlu5-deficient mice were ethanol-avoiding at both concentrations of ethanol proffered (5% and 10% v/v). However, there was no difference in the rate of hepatic ethanol and acetaldehyde metabolism between genotypes and consumption of saccharin was similar. In a conditioned place preference study, mGlu5-deficient mice displayed a place preference for ethanol when conditioned with a low dose (1 g/kg), a phenomenon not observed in wild-type littermates, suggesting increased sensitivity to the rewarding effects of ethanol in mutant mice. Finally, mGlu5-deficient mice were more sensitive to ethanol-induced hypnosis at a high dose (3.5 g/kg) of ethanol. Thus, while mGlu5-deficient mice consume less ethanol (with a reduced preference) than wild-type mice, this is not apparently related to impaired hepatic metabolism or a lack of reward from ethanol. Rather, we provide evidence that deletion of the mGlu5 receptor increases sensitivity to centrally mediated effects of ethanol.

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Key words: Alcohol consumption, mGlu5 receptor, mice, place preference, reward.

Introduction

Ethanol is one of the most widely used and readily available recreational drugs in the world, with approximately 76.3 million people considered to suffer from an ethanol use disorder (WHO, 2004). Broadly defined, addiction is the loss of control over drug use, including compulsive drug-seeking and drug-taking behaviours that continue despite significant negative consequences and/or reductions in drug efficacy (Hyman and Malenka, 2001). While a desire to elevate mood or alleviate stress may initiate drug use, the development of addiction is more complex than simply being unable to cease drug taking due to the desire to experience acute effects. A key question in addiction research is what factors, be they genetic, neurological

and/or sociological, predispose the individual to progress from casual to compulsive drug use.

L-glutamate elicits physiological effects by activation of both ionotropic (*N*-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and 2-carboxy-3-carboxymethyl-4-isopropenylpyrrolidine (kainate) receptors, and metabotropic (mGlu1–8) receptors (Kew and Kemp, 2005). The metabotropic glutamate receptor family is divided into three groups, based on pharmacology, sequence homology and receptor coupling. Upon activation, group I (mGlu1 and 5) receptors elevate intracellular calcium levels by coupling to G_q/G_{11} proteins, which in turn activate the phospholipase C pathway (Abe et al., 1992). Group II (mGlu2 and 3) and group III (mGlu4, 6, 7 and 8) receptors typically couple through G_i/G_o proteins and reduce cyclic AMP levels via inhibition of adenylate cyclase (Conn and Pin, 1997). Using the alcohol deprivation effect in rodents to model seeking and relapse behaviour, strong links have been established between the glutamatergic

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system and behavioural responses to ethanol. Inhibition of NMDA receptor function in rats by the competitive antagonist CGP37849 was able to significantly reduce ethanol consumption following a deprivation period, a phenomenon that was replicated by both an NMDA channel blocker (neramexane) and specific NR2B subunit antagonist (ifenprodil; Vengeliene et al., 2005). Specific antagonism of AMPA receptors (GYKI 52466) also reduced reinstatement to ethanol-seeking in mice, as did deletion of the AMPA subunit GluR1 (Sanchis-Segura et al., 2006).

Metabotropic glutamate 5 (mGlu5) receptors are located pre-synaptically on the terminals of glutamatergic and dopaminergic neurons as well as post-synaptically on medium spiny neurons in the basal ganglia (Shigemoto et al., 1993). Thus, this receptor represents a potential mechanism for regulation of the corticostriatal synapses, and recent studies have implicated mGlu receptors in the process of addiction to drugs of abuse (Hermans and Challiss, 2001; Kenny and Markou, 2004). Ethanol has a broad range of effects in the mammalian nervous system, including inhibition of mGlu5 signalling via a PKC-dependent mechanism (Minami et al., 1998). A number of studies show that the mGlu5 antagonist, 2-methyl-6-(phenyl-ethynyl)-pyridine (MPEP), reduces the onset and maintenance of ethanol-seeking and attenuates relapse to ethanol-seeking after repeated deprivations in rats, and the motivation to self-administer ethanol (Backstrom et al., 2004; Besheer et al., 2007; Schroeder et al., 2005). Due to a number of shortcomings in MPEP, including off-target activity, further development resulted in the discovery of 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine (MTEP; Cosford et al., 2003). Like MPEP, MTEP is capable of reducing ethanol self-administration in multiple rat strains at doses devoid of anxiolytic or antidepressant activity (Adams et al., 2007; Cowen et al., 2005). Studies in C57BL/6J mice also indicate that MPEP can reduce ethanol self-administration (Hodge et al., 2006) and notably also enhances the sedative and hypnotic properties of ethanol (Sharko and Hodge, 2008), while MTEP is also able to diminish the motivation to consume ethanol (Cowen et al., 2007).

There has been extensive investigation of alcohol-related behaviours using genetic models. For an excellent review encompassing the vast majority of alcohol studies in genetic mouse models see Crabbe et al. (2006). Studies on the mGlu5 receptor in this regard are conspicuous by their absence. The aim of the present study was to characterize the ethanol-related phenotype using mGlu5-deficient C57BL/6J mice compared to wild-type littermates. Here we report

that while mGlu5-deficient mice consume less ethanol (with a reduced preference) than wild-type mice, this is not apparently related to impaired hepatic metabolism or a lack of reward from ethanol. Rather, we provide evidence that deletion of the mGlu5 receptor increases sensitivity to centrally mediated effects of ethanol.

Method

Animals

All experiments were performed in accordance with the Prevention of Cruelty to Animals Act, 1986 under the guidelines of the National Health and Medical Research Council (NHMRC) Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia. mGlu5-deficient mice (Lu et al., 1997) on a C57BL/6J background (*Grm5^{tm1Rod}*; stock 003558) were obtained from Jackson Laboratories (Bar Harbor, ME, USA). A heterozygous breeding colony (currently F10) was established by crossing mGlu5^{-/-} mice with C57BL/6J wild-type mice from the Animal Resources Centre (Perth, Australia). This strategy ensured the provision of wild-type littermates as control subjects. The genotype of individual mice was determined by PCR using primers complementary to sequences in the wild-type mGlu5 gene and the inserted mutant sequence (5'-CACGAGACTAGTGAGACGTG-3', 5'-CACATGCCAGGTGACATCAT-3', 5'-CCATGCTGGTTGCAGAGTAA-3'; Geneworks, Hindmarsh, Australia). The absence of mGlu5 protein in mice identified as mGlu5-deficient by PCR was confirmed by immunohistochemistry on brain slices using an anti-mGlu5 primary antibody (Upstate Biotechnology, Lake Placid, NY, USA). This anti-rabbit IgG was a 21-residue synthetic peptide (KSSPKYDTLIIRDYT NSSSSL) corresponding to the C-terminal of mGlu5 with a lysine added to the N-terminal. All mice were given free access to food and water in home cages, and were maintained on a 12 h light-dark cycle (lights on: 07:00 hours). All experiments were conducted using adult male mice aged between 6 and 10 wk, age matched for each experiment.

Two-bottle free-choice drinking

The effect of genotype on drinking behaviour was determined by a two-bottle free-choice paradigm as previously described (Short et al., 2006a,b). Two separate cohorts of mice were used to examine saccharin (data not shown) and ethanol-drinking behaviours, and mice were weighed every 3 d. Bottles were monitored at the same time each day, and bottle location was randomized to eliminate any influence of side

preference. Consumption of test solution (g/kg of bodyweight per day), preference for test solution (compared to water, expressed as a percentage) and total fluid intake (ml/kg of bodyweight per day) were monitored for both saccharin (0.1% w/v) and ethanol (5% and 10% v/v). For the ethanol investigation, mice were exposed to progressively increasing concentrations of ethanol as previously described (Moore et al., 2007). Briefly, the standard protocol for alcohol consumption consisted of mice exposed for at least 14 d at each concentration of ethanol (5% and 10% v/v).

Hepatic metabolism of ethanol and acetaldehyde

Liver preparation

Liver preparation was performed essentially as previously described (Lodge and Lawrence, 2003). Mice were anaesthetized (sodium pentobarbitone, 80 mg/kg i.p.) prior to cervical dislocation. This was performed ~3 h into the dark phase for all subjects, to avoid any circadian influence on enzyme activity. Livers were dissected out and rinsed with ice-cold physiological saline solution [(PSS); composition in mM: NaCl, 118.0; KCl, 4.7; NaH₂PO₄, 1.0; MgCl₂, 1.2; CaCl₂, 1.3; NaHCO₃, 25.0; and ethylene diamine-tetraacetate (EDTA), 0.04] and weighed (wet). Livers were then incubated for 1 h at 37 °C in carbogenated PSS and subsequently manually homogenized in 8 ml ice-cold sucrose buffer (0.25 M sucrose, 5 mM Tris, 0.5 mM EDTA, and 0.5 mM dithiothreitol; pH 7.5). The homogenates were centrifuged (4 °C) at 700 g for 10 min to remove cellular debris, and the resultant supernatant was then centrifuged (4 °C) at 10 000 g for 30 min. The pellet was then resuspended in 0.75 ml pyrophosphate buffer (50 mM sodium pyrophosphate; pH 8.8) and centrifuged (4 °C) at 48 000 g for 1 h. The resultant supernatant was the cytosolic fraction, and the pellet (mitochondrial fraction) was resuspended in 0.75 ml of pyrophosphate buffer. The protein concentration of each sample (mitochondrial and cytosolic) was then determined in duplicate using the recommended protocol of a BCATM Protein Assay kit (Pierce Biotechnology, Rockford, IL, USA). Samples were stored at –80 °C overnight before determination of enzyme activity.

Alcohol dehydrogenase (ADH) assay

The endogenous activity of hepatic ADH was determined in both wild-type and mGlu5-deficient mice by the nicotinamide adenine dinucleotide (NADH)-induced increase in absorbance at 340 nm on a microplate spectrophotometer (Bio-Rad Benchmark Plus;

Bio-Rad Laboratories, Hercules, CA, USA) essentially as previously described (Lodge and Lawrence, 2003). Briefly, the assay was performed in duplicate at 37 °C in a final volume of 1.25 ml glycine buffer (0.1 M glycine; pH 10) containing 2.4 mM of β -NAD⁺ and a volume of cytosolic fraction equivalent to 0.5 mg protein. After 5 min of equilibration, the reaction was initiated by the addition of ethanol (10 μ M–100 mM) and incubated for 5 min before the increase of absorbance was measured relative to a blank reaction (no ethanol added). After construction of a concentration–response curve, the effect of the ADH inhibitor pyrazole (10 mM) was examined on the ethanol concentration that yielded maximal ADH activity.

Aldehyde dehydrogenase (ALDH) assay

The endogenous activity of ALDH was determined in both wild-type and mGlu5-deficient mice by the NADH-induced increase in absorbance at 340 nm on a microplate spectrophotometer (Bio-Rad Benchmark Plus), essentially as previously described (Lodge and Lawrence, 2003). Briefly, the assay was performed in duplicate at 37 °C in a final volume of 1.25 ml pyrophosphate buffer (50 mM sodium pyrophosphate; pH 8.8) containing 1.5 mM β -NAD⁺; pyrazole (0.1 mM) to inhibit ADH activity; rotenone (2 μ M in dimethylsulphoxide: 0.2% final volume) to inhibit mitochondrial NADH oxidase; sodium deoxycholate (0.01% w/v), to release latent activity and to increase clarity of the solution for spectrophotometric analysis; and mitochondrial fraction corresponding to 0.5 mg protein. After 5 min of equilibration, the reaction was initiated by the addition of acetaldehyde (0.1 mM–100 mM) and then incubated in a sealed 24-well plate (to minimize acetaldehyde evaporation) for 15 min before the increase in absorbance was measured relative to a blank reaction (no acetaldehyde added). After construction of a concentration–response curve, the effect of the ALDH inhibitor disulfiram (0.1 mM in ethanol: 0.2% of final volume) was examined on the concentration of acetaldehyde that yielded maximal ALDH activity.

Conditioned place preference

The rewarding effect of ethanol and the ability to associate any reward with an environmental context (Pavlovian conditioning) in wild-type and mGlu5-deficient mice ($n = 12$ per genotype) was assessed using a conditioned place preference paradigm. This was performed in specialized motor monitors (42.5 cm $\times$ 21.5 cm), partitioned into a central neutral zone

(6.5 cm × 21.5 cm) and two adjoining conditioning compartments (18 cm × 21.5 cm), each with distinct visual (wall pattern: circles or lines) and tactile (floor surface: smooth or contoured) cues (Hamilton-Kinder, Poway, CA, USA). All mice were habituated to the chambers during a single 30 min session with access to the entire apparatus. The naive non-preferred side became the ethanol-paired chamber, while the preferred chamber was paired with vehicle. This strategy resulted in five mice of each genotype having ethanol paired with the 'circles' wall pattern and seven with the 'lines' wall pattern, excluding the possibility of a simple effect of chamber type. Six days of conditioning followed, where alternating injections of vehicle (0.9% w/v saline, 10 ml/kg i.p.) and ethanol (1 g/kg in a volume of saline equal to 10 ml/kg i.p.) were paired with the appropriate chamber. These sessions were performed twice per day (once each for ethanol and saline) and were of 15 min duration. Preference for each side chamber was then reassessed by once again allowing the mice complete access to the entire apparatus during a 30 min test session.

Ethanol-induced loss of righting reflex (LORR)

A hypnotic dose of ethanol (3.5 g/kg in a volume of saline equal to 10 ml/kg i.p.) was administered to mGlu5-deficient and wild-type mice to induce LORR. Once achieved, mice were orientated supinely and the time required to regain the righting reflex was measured. Restoration of the reflex was considered to have occurred when the mouse could recover from a supine position twice within 1 min. The latency between administration of the ethanol and LORR was also recorded.

Statistical analyses

Saccharin two-bottle free-choice data were assessed using one-way analysis of variance (ANOVA) on raw data for each parameter investigated (consumption, total fluid intake and preference). Ethanol two-bottle free-choice data were assessed using two-way repeated-measures ANOVA with genotype and ethanol concentration as factors (Tukey post-hoc tests). After averaging the absorbance readings of the duplicates for each treatment, both ADH and ALDH assays were assessed using two-way repeated-measures ANOVA with genotype and treatment as factors. The time spent in the ethanol-paired chamber during conditioned place preference was analysed using a two-way repeated-measures ANOVA with genotype and day (habituation and test) as factors (Tukey post-hoc tests). Ethanol-induced LORR was assessed using

a one-way ANOVA on raw time data. In all analyses significance was accepted at $p < 0.05$.

Results

Drinking studies

Saccharin (data not shown)

When saccharin (0.1% w/v) was presented as an alternate fluid to water, mGlu5-deficient mice exhibited a high (~94%) preference for saccharin over water, which was comparable to wild-type littermates (~92%; $n = 16$ wild type, 10 null). These data suggest that deletion of mGlu5 does not impact in a measurable way on taste perception of palatable solutions or the drive to consume a palatable solution.

Ethanol

To determine whether deletion of mGlu5 impacts upon ethanol consumption (as is the case for mGlu5 antagonists), mice were examined under a standard two-bottle free-choice paradigm to assess consumption of ethanol, preference over water and total fluid intake ($n = 20$ wild type, 22 null). Statistical analysis showed a main effect of both genotype ($F_{1,40} = 15.619$, $p < 0.001$) and concentration ($F_{1,40} = 59.954$, $p < 0.001$) on consumption of ethanol, as well as a significant interaction between the aforementioned factors ($F_{1,40} = 5.220$, $p = 0.028$). Tukey post-hoc tests revealed that consumption was significantly reduced in the mGlu5-deficient mice at both concentrations examined (5%: $q = 3.133$, $p = 0.030$; 10%: $q = 6.455$, $p < 0.001$; Figure 1a). Preference for ethanol over water also revealed a main effect of genotype ($F_{1,40} = 20.542$, $p < 0.001$), but did not show an influence of concentration (Figure 1b). This indicates the mGlu5-deficient mice had a lower preference for the test solution compared to wild-type mice regardless of the concentration of ethanol presented to them. Notably, mGlu5-deficient mice expressed an ethanol-aversive phenotype, as preference for ethanol did not reach 50% at either of the concentrations studied. With regard to total fluid intake, statistical analysis revealed a significant effect of concentration ($F_{1,40} = 31.611$, $p < 0.001$) but not of genotype, indicating that both wild-type and mGlu5-deficient mice drank less overall at an ethanol concentration of 10% compared to 5% (Figure 1c).

ADH assay

To examine whether the reason for reduced ethanol consumption and preference in mutant mice was a

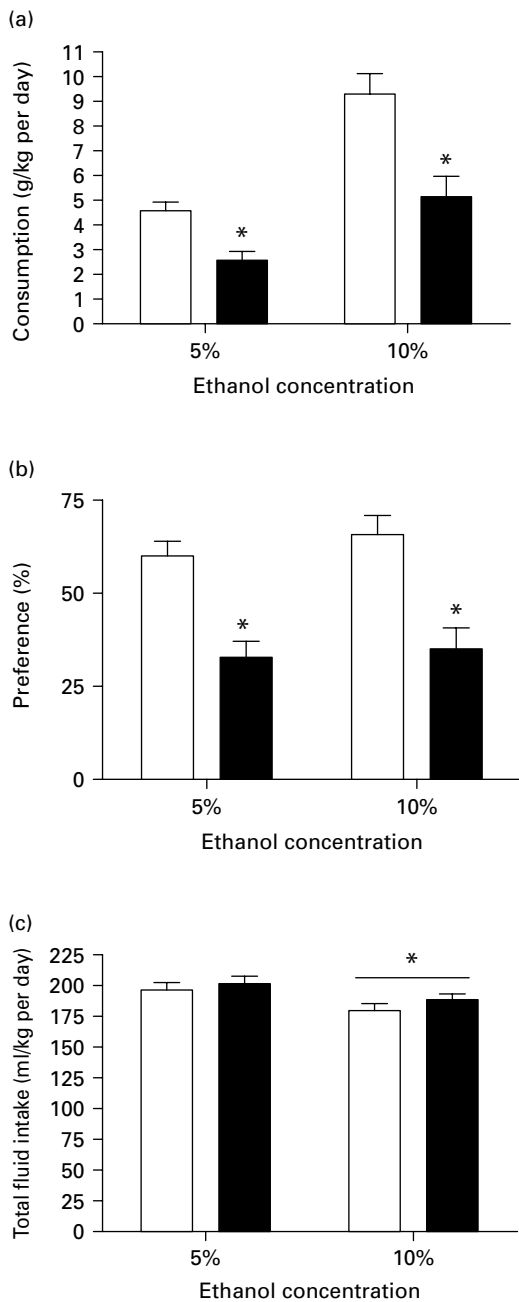


Figure 1. Drinking behaviour over two ethanol concentrations (v/v) in wild-type (□) and mGlu5-deficient (■) C57BL/6J mice as assessed by a two-bottle free-choice paradigm. (a) Consumption of ethanol in g/kg bodyweight per day. (b) Average preference for ethanol solution over water as determined by percentage of total fluid intake. (c) Average total daily fluid intake in ml/kg bodyweight per day. Graphs indicate mean, error bars represent S.E.M. An asterisk (*) over a column represents a significant difference between genotype within concentration, while an asterisk over a line indicates a difference between concentrations within genotypes ($p < 0.05$).

consequence of impaired hepatic metabolism, a separate cohort of mice were used to determine ADH activity. Spectrophotometric analysis of cytosolic ADH revealed that enzyme activity was related to ethanol concentration in both wild-type and mGlu5-deficient liver preparations ($n=4$ wild type, 4 null; $F_{4,24}=53.748$, $p < 0.001$), although there was no significant difference in ADH activity with respect to genotype at any concentration examined (Figure 2a). Selectivity of the assay was confirmed via application of pyrazole (10 mM) on the ethanol concentration that elicited maximal ADH activity (Figure 2b). Statistical analysis revealed a main effect of pyrazole treatment ($F_{2,12}=41.568$, $p < 0.001$), indicating addition of pyrazole significantly reduced the ethanol-induced ADH activity in both wild-type and mGlu5-deficient liver preparations.

ALDH assay

Spectrophotometric analysis of mitochondrial ALDH revealed that, as was the case for ADH, ALDH enzyme activity was related to acetaldehyde concentration in both wild-type and mGlu5-deficient liver preparations ($n=4$ wild type, 4 null; $F_{3,9}=94.893$, $p < 0.001$), although there was no significant difference in ALDH activity with respect to genotype at any concentration examined (Figure 2c). Selectivity of the assay was confirmed by application of disulfiram (0.1 mM) on the acetaldehyde concentration that yielded maximal ALDH activity (Figure 2d). Statistical analyses showed a main effect of disulfiram treatment ($F_{2,12}=39.084$, $p < 0.001$), indicating addition of disulfiram significantly attenuated the acetaldehyde-induced ALDH activity in both wild-type and mGlu5-deficient liver preparations. Collectively, these findings indicate that the mGlu5 receptor does not impair ethanol metabolism.

Conditioned place preference

To determine whether or not the reason for reduced consumption and preference of ethanol in mutant mice is due to a lack of reward from ethanol, the ability of ethanol to instantiate a conditioned place preference was assessed in wild-type and mGlu5-deficient mice. All mice were conditioned with a 1 g/kg dose of ethanol as preliminary experiments indicated that higher doses caused a significant attenuation in locomotor activity in mutant mice ($\sim 26\%$, not shown), an effect that could confound interpretation. Statistical analysis indicated there was a main effect of treatment ($F_{1,22}=12.471$, $p = 0.002$), but not of genotype. However, a possible effect of genotype was diluted due to

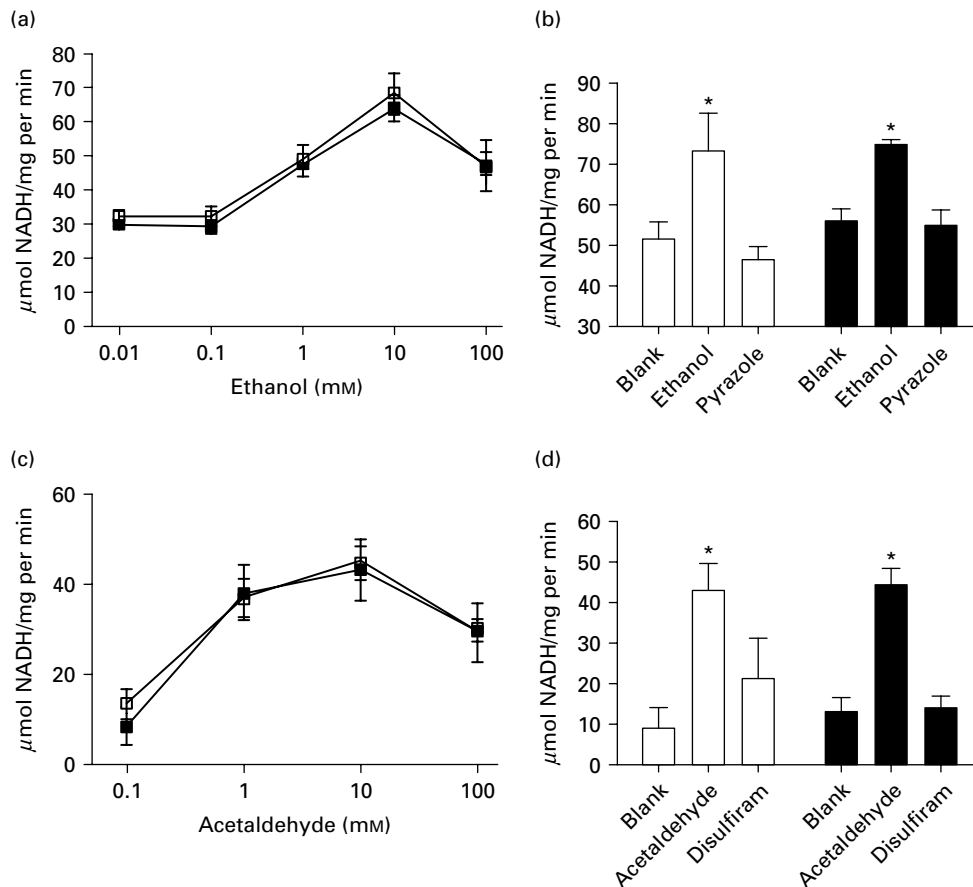


Figure 2. Endogenous liver enzyme activity in both wild-type (open squares/columns, □) and mGlu5-deficient (closed squares/columns, ■) samples as determined by the substrate-dependent increase in absorbance at 340 nm detected by a microplate spectrophotometer. The reduced form of nicotinamide adenine dinucleotide (β -NADH) was produced from the conversion of the oxidized form (β -NAD⁺). (a) The increase in β -NADH as a result of alcohol dehydrogenase (ADH) activity in the cytosolic fraction over a number of substrate (ethanol) concentrations. (b) The effect of the specific ADH inhibitor pyrazole (10 mM) on the increase in β -NADH produced by the addition of 10 mM ethanol. (c) The increase in β -NADH as a result of aldehyde dehydrogenase (ALDH) activity in the mitochondrial fraction over a number of substrate (acetaldehyde) concentrations. (d) The effect of the specific ALDH inhibitor disulfiram (0.1 mM) on the increase in β -NADH by the addition of 10 mM acetaldehyde. Graphs indicate mean, error bars represent S.E.M. * Represents a significant difference in the amount of β -NADH produced compared to blank reaction ($p < 0.05$).

the lack of difference between the genotypes on habituation. When the Tukey post-hoc tests were examined, mGlu5-deficient mice were found to develop a place preference as shown by a significant increase in time spent in the ethanol-paired chamber on the test day compared to the habituation day ($q = 5.185$, $p = 0.002$; Figure 3). Post-hoc analysis also indicated that wild-type mice did not develop a significant preference for the ethanol chamber under the present conditions. This finding would suggest that mGlu5-deficient mice do obtain a rewarding effect from ethanol, and further that they can obtain a place preference to ethanol at a low dose (1 g/kg) that is insufficient to produce a place preference in wild-type mice.

LORR

To further examine the role of the mGlu5 receptor in ethanol sensitivity, another group of mice were used to assess ethanol-induced hypnosis. After the administration of a hypnotic dose of ethanol (3.5 g/kg), the latency to the loss and recovery of the righting reflex was quantified. There was no difference between mGlu5-deficient mice and wild types in latency to LORR from the time of ethanol injection (null 123 ± 7 s, wild type 118 ± 9 s). There was, however, a significant increase in the latency to regain the righting reflex in the mGlu5^{-/-} mice compared to wild types ($n = 12$ wild type, 11 null; $F_{1,19} = 15.772$, $p < 0.001$; Figure 4).

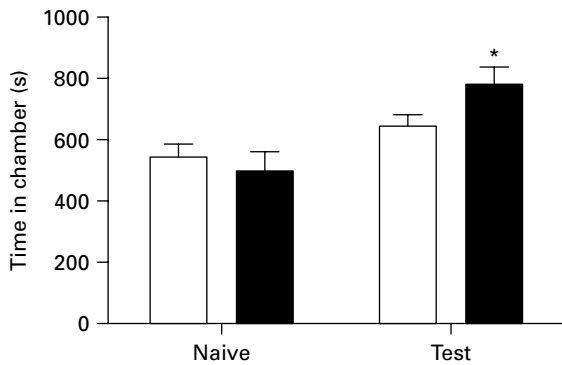


Figure 3. The time spent (s) in the ethanol-paired chamber on both habituation (naive) and after conditioning (test) in wild-type (□) and mGlu5-deficient (■) C57BL/6J mice over a 30 min session. Graph indicates mean, error bars represent S.E.M. * Represents a significant difference in the amount of time spent in the ethanol-paired chamber compared to habituation ($p < 0.05$).

These data confirm that deletion of mGlu5 increases sensitivity to ethanol-induced behaviours.

Discussion

Here we show that mGlu5-deficient mice drink less ethanol (with a reduced preference) and are more sensitive to ethanol-induced conditioned place preference (low-dose ethanol) and ethanol-induced hypnosis (high-dose ethanol) compared to wild-type littermates. Moreover, this phenotype is not a product of differential ethanol or acetaldehyde metabolism between the genotypes. Apparently, the reason for reduced consumption and preference of ethanol in mGlu5-deficient mice relates to increased sensitivity to the centrally mediated pharmacological effects of ethanol.

Previous research in rodent models suggests that the mGlu5 receptor is involved in a number of aspects of alcohol consumption. The mGlu5 receptor antagonist MPEP decreases the onset and maintenance of ethanol self-administration in mice, and also significantly reduces overall consumption (Hodge et al., 2006; Olive et al., 2005). Similar observations have been made using rat models, whereby MPEP decreases ethanol consumption during maintenance and after repeated deprivations, while another mGlu5 antagonist, MTEP, also decreases ethanol self-administration and seeking (Cowen et al., 2005; Schroeder et al., 2005). In a similar manner, MTEP reduces both appetitive and consummatory components of ethanol self-administration in C57BL/6J mice (Cowen et al., 2007). Thus, pharmacological studies suggest that

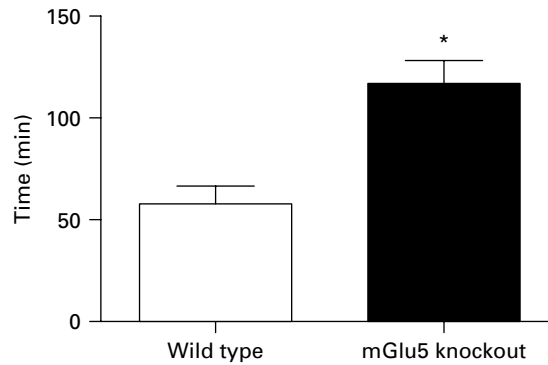


Figure 4. Latency to recovery of the righting reflex in wild-type (□) and mGlu5-deficient knockout (■) C57BL/6J mice administered with a hypnotic (3.5 g/kg, i.p.) dose of ethanol. Graph indicates mean, error bars represent S.E.M. * Represents a significant difference between genotype ($p < 0.05$).

acute antagonism of mGlu5 receptors reduces alcohol-seeking and consumption in rodents via a combination of pre-ingestive (altering the motivational properties of ethanol) and post-ingestive (altering the pharmacological effect of ethanol) mechanisms. The current data show that mGlu5-deficient mice consume less ethanol than wild-type littermates, and have a markedly lower preference to consume ethanol over water. Indeed, mutant mice on the genetic background examined display an alcohol-avoiding phenotype, in that their preference for alcohol remains below 50% at both concentrations examined in this study.

Since the two-bottle free-choice paradigm measures consummatory behaviour, we considered the possibility that this phenotype may be caused by altered metabolism, as there are mGlu5 receptors located in the liver (Storto et al., 2000). However, the current data do not support this theory, as there is no difference in ethanol or acetaldehyde metabolism at any substrate concentration examined. The ethanol-avoiding phenotype of the mutant mice could therefore be the net outcome of a number of divergent potential consequences caused by the mutation. Given the data from pharmacological studies in rodents, one possibility is that mGlu5-deficient mice have decreased motivation to consume a rewarding solution. The fact that mutant mice display a high preference for saccharin indicates that taste discrimination is largely intact and also suggests that mutant mice preferentially consume a 'natural' reward/palatable solution over water.

Another potential explanation for the ethanol phenotype of the mutant animals was that they attributed a reduced reward 'value' to ethanol compared

to their wild-type littermates. To assess this theory, mGlu5-deficient mice and wild-type littermates were tested for the development of a conditioned place preference to ethanol. A number of researchers have reported that a conditioning regime using a 2 g/kg dose of ethanol will induce a place preference in C57BL/6J mice (Boyce-Rustay et al., 2006; Gremel et al., 2006). In the present study, 2 g/kg was deemed too high a dose for mGlu5-deficient mice, as there was clear attenuation in locomotor activity at this dose. Studies with MPEP concur with our finding of increased sensitivity to the sedating properties of ethanol in mGlu5-deficient mice (Sharko and Hodge, 2008). Thus, we conditioned mice at a lower dose (1 g/kg). At this dose, mGlu5-deficient mice developed a place preference for the ethanol-paired chamber. There was also a notable lack of a place preference in wild-type mice conditioned at this dose (being presumably too low under our experimental conditions). However, the ability of mGlu5-deficient mice to develop a place preference to ethanol clearly demonstrates that deletion of the mGlu5 receptor does not impair Pavlovian conditioning to a rewarding dose of ethanol. Moreover, these data suggest that deletion of the mGlu5 receptor increases behavioural sensitivity to ethanol rather than altering the reward 'value' of ethanol.

An explanation that would be consistent with both the sedation at 2 g/kg and the place preference developed at 1 g/kg is that mutant mice are more sensitive to the centrally mediated effects of ethanol. This hypothesis would also be consistent with the reduced ethanol consumption in the mGlu5-deficient mice in the two-bottle free-choice study, in that being more sensitive to ethanol, the mGlu5-deficient mice required less than wild types to attain a similar hedonic effect. It is noteworthy that consumption of ethanol in mutant mice does increase as the concentration of ethanol offered is increased and consequently mGlu5-deficient mice can imbibe considerable amounts of ethanol. The notion that mutant mice are indeed more sensitive to ethanol was further explored using an ethanol-induced LORR paradigm. This experiment indicated that the same (hypnotic) dose of ethanol enhanced the length of ethanol-induced sleeping time in mGlu5-deficient mice compared to wild-type littermates. Furthermore, as we established previously, this phenomenon does not reflect altered metabolism between the genotypes. It is, however, cogent to note that altered absorption or distribution of ethanol between genotypes could contribute to the observed phenotype. Importantly, however, studies with MPEP support our notion that functional blockade of mGlu5

receptors enhances both the hypnotic and sedative properties of ethanol (Sharko and Hodge, 2008).

mGlu5 receptors are located pre-synaptically on the terminals of glutamatergic and dopaminergic neurons as well as post-synaptically on medium spiny neurons in the nucleus accumbens (Shigemoto et al., 1993). The alcohol-related phenotype of mGlu5-deficient mice bears some similarity to dopamine D₂ receptor knockout mice, in that while saccharin preference remains unchanged in mutant animals, ethanol consumption and preference is markedly reduced (Phillips et al., 1998). However, the possibility of an interaction between mGlu5 and D₂ receptors can be discounted (at least with regards to ethanol), as D₂ receptor-deficient mice fail to develop a place preference to ethanol, even when conditioned with higher doses of ethanol (2 g/kg; Cunningham et al., 2000). Similarly, deletion of dopamine D₁ receptors in mice also lowers ethanol consumption and preference compared to both wild-type and heterozygote littermates (El-Ghundi et al., 1998). In contrast, however, mice lacking D₁ receptors display reduced consumption (but not preference) for saccharin (Short et al., 2006a) while consumption of saccharin in mGlu5-deficient mice is similar to wild types.

More commonality can be found between the phenotype of mGlu5-deficient mice and that of mice lacking protein kinase C epsilon (PKC $\epsilon^{-/-}$). PKC $\epsilon^{-/-}$ mice drink less ethanol and are more sensitive to ethanol-induced hypnosis, while conditional rescue of PKC $\epsilon^{-/-}$ mice can restore these behaviours (Choi et al., 2002; Hodge et al., 1999). Moreover, studies with MPEP in wild-type mice suggest that mGlu5 antagonism reduces ethanol consumption via a PKC ϵ regulated pathway (Olive et al., 2005).

The present study sheds considerable light on the reduction in ethanol-seeking and consumption observed after antagonism of mGlu5 receptors (Cowen et al., 2007). Importantly, given an appropriate dose, the rewarding properties of ethanol are present despite the removal of mGlu5 receptors. We present evidence that deletion of mGlu5 receptors conveys an increased sensitivity to the centrally mediated effects of ethanol, possibly related to interactions between mGlu5 and GABA_A receptors (Besheer and Hodge, 2005). It is likely that due to their increased sensitivity, mGlu5-deficient mice require less ethanol compared to wild-type littermates to attain a similar pharmacological effect, and accordingly they consume less. The overall phenotype of alcohol-avoiding therefore does not necessarily indicate a lack of ability to integrate the value of a reward. Indeed, recent pharmacological studies indicate a probable role for mGlu5 receptors in

the motivation to self-administer alcohol in mice (Cowen et al., 2007) and rats (Besheer et al., 2007). Currently, there are no published studies of polymorphisms in the human mGlu5 gene. It is tempting to speculate that an mGlu5 gene variant that results in a hypofunctioning receptor could be protective against alcoholism.

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Statement of Interest

None.

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