Homer2 Is Necessary for EtOH-Induced Neuroplasticity

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Homer proteins are integral to the assembly of proteins regulating glutamate signaling and synaptic plasticity. Constitutive Homer2 gene deletion [knock-out (KO)] and rescue with adeno-associated viral (AAV) transfection of Homer2b was used to demonstrate the importance of Homer proteins in neuroplasticity produced by repeated ethanol (EtOH) administration. Homer2 KO mice avoided drinking high concentrations of EtOH and did not develop place preference or locomotor sensitization after repeated EtOH administration. The deficient behavioral plasticity to EtOH after Homer2 deletion was paralleled by a lack of augmentation in the rise in extracellular dopamine and glutamate elicited by repeated EtOH injections. The genotypic differences in EtOH-induced change in behavior and neurochemistry were essentially reversed by AAV-mediated transfection of Homer2b into accumbens cells including, differences in EtOH preference, locomotor sensitization, and EtOH-induced elevations in extracellular glutamate and dopamine. These data demonstrate a necessary and active role for accumbens Homer2 expression in regulating EtOH-induced behavioral and cellular neuroplasticity.

Key words: Homer proteins; EtOH; NMDA receptor; neuroplasticity; glutamate; dopamine

Introduction

Ethanol (EtOH) is a drug of abuse that inhibits glutamate receptor function and affects neurotransmission within the mesocorticolimbic circuitry implicated in drug and natural reward, including the nucleus accumbens (Koob et al., 1998; Woodward, 2000; Gonzales et al., 2004). Many of the acute behavioral effects of EtOH are related to the inhibition of glutamate receptor signaling (Woodward, 2000), and enduring adaptations in glutamate receptor signaling are implicated in the development of EtOH dependence, tolerance, and addiction (Chandler, 2003; Krystal et al., 2003). Thus, proteins regulating glutamatergic synaptic function are hypothesized to gate the development of EtOH-induced behavioral plasticity (Chandler, 2003).

The Homer family of proteins modulate glutamate signaling in the postsynaptic density (PSD) and are candidate regulators of EtOH-induced plasticity. Homers are encoded by three genes (Homer1–Homer3) that provide constitutive isoforms (Homer1b/c/d, Homer2a/b, and Homer3) and immediate early gene products (Homer1a and ania-3) (Xiao et al., 2000; de Bartolomeis and Iasevoli, 2003). Homer proteins interact via an Ena/ VASP1 (for vasodilator-stimulated phosphoprotein) (EVH1) homology domain with a proline-rich motif located on a number of proteins, including group 1 metabotropic glutamate receptors (mGluR1/5), inositol-1,4,5-triphosphate receptors, TRP (for transient receptor potential) cation channels, and the NMDA receptor-associated scaffolding protein Shank (Xiao et al., 2000; de Bartolomeis and Iasevoli, 2003; Rong et al., 2003; Yuan et al., 2003). In contrast to the truncated immediate early gene isoforms, constitutive Homer isoforms contain a leucine zipper motif and a coiled-coil domain at the N terminus, which enables multimerization and cross-linking between mGluR1/5 and EVH1-bound partners (Tu et al., 1999). Thus, constitutive Homer proteins are integral to synaptic architecture and promote efficient glutamate receptor signaling complexes (Xiao et al., 2000; Sala et al., 2003; Shiraishi et al., 2003).

In vivo evidence indicates a necessary and active role for constitutive Homer protein expression in the nucleus accumbens for the maintenance and regulation of extracellular levels of glutamate relevant to addiction. Mice with null mutations of either Homer1 or Homer2 exhibit abnormalities in accumbens extracellular glutamate that are similar to those observed in animals withdrawn from repeated cocaine treatment (Szumlinski et al., 2004). The glutamatergic abnormalities of Homer mutant mice accompany a “presensitized” behavioral phenotype characterized by heightened sensitivity to cocaine reward and psychomotor activation (Szumlinski et al., 2004). Although Homer1 deletion produced learning deficits that confounded the interpretation of Homer1 role in reward (Szumlinski et al., 2004, 2005), the phe-
notype of Homer2 knock-out (KO) mice appears to be selective for alterations in cocaine reward (Szumlinski et al., 2004).

Given the effects of Homer gene deletions on glutamate transmission and the fact that EtOH modulates glutamate receptors and transmission (Chandler, 2003; Krystal et al., 2003), the present study investigated the interactions between Homer2 and EtOH. Mice sustaining a constitutive deletion of the Homer2 gene (KO) were administered acute or repeated EtOH, and effects on EtOH-induced behaviors and nucleus accumbens dopamine and glutamate transmission were quantified. In addition, reversal of observed genotypic differences by an adeno-associated viral (AAV) transfection of nucleus accumbens cells was also performed. The results of this study indicate that Homer2 in the accumbens regulates sensitivity to acute EtOH administration and is necessary for EtOH-induced behavioral and neurochemical plasticity.

Materials and Methods

Subjects. Mice with null mutations of Homer2 and their wild-type (WT) mice (F10–F12; C57BL/6/J × 129Xl/SvJ) were generated and maintained by heterozygous mating as described previously (Szumlinski et al., 2004). Because insufficient numbers of male mice were available at the time of study, both adult (8–10 weeks of age at the start of experimentation) male and female mice were used. The numbers of male and female mice within each treatment group were approximately equal ±1. Mice were housed individually under standard conditions as described previously (Szumlinski et al., 2004).

EtOH and saccharin preference. EtOH consumption and preference was assessed using a two-bottle choice procedure similar to that described previously for C57BL/6J (B6) mice (Nocjar et al., 1999). Briefly, mice were presented with two identical 50 ml sipper tubes in the home cage for a period of 24 h. One tube contained tap water and the other tube contained increasing concentrations of EtOH (0, 3, 6, and 12% v/v). Bottles were presented with each EtOH concentration for 4 d, and the volume consumed was calculated daily based on sipper tube weight before and after presentation. The mean volume consumed over the 4 d was used in the statistical analysis. For comparison, saccharin preference was assessed after 4 d presentation of a 0.9% (w/v) saccharin solution versus water. To control for spillage and evaporation, the weights of bottles on two dummy cages were recorded throughout testing, and the average volume lost attributable to bottle handling/evaporation was subtracted from the daily record for each animal.

Place conditioning and motor activity. To assess EtOH conditioned reward and locomotor activity, a biased place conditioning apparatus was used in a manner identical to previous reports for Homer1 and Homer2 KO mice (Szumlinski et al., 2004). The procedures to induce EtOH place conditioning were similar to that described for C57BL/6J mice (Nocjar et al., 1999). In brief, place conditioning to EtOH was produced by eight repeated pairings, on alternating days, of intraperitoneal injections of 0, 1, 2, or 3 g/kg EtOH with the nonpreferred compartment and water vehicle (volume of 0.02 ml/g body weight) with the preferred compartment of the place conditioning apparatus. Control groups received water vehicle in both compartments. A postconditioning test followed the last EtOH/water conditioning session, using identical procedures as those used on the preconditioning test. Both the conditioning and test sessions were a total of 15 min in duration. EtOH-induced changes in locomotor behavior were detected by two photocell beams and recorded on each EtOH conditioning day. The amount of conditioning was expressed as the difference in the amount of time spent in the nonpreferred, EtOH-paired compartment on the preconditioning versus postconditioning tests (occupancy difference in seconds).

EtOH-induced sedation. To assess the motor-impairing effects of higher EtOH doses, Homer2 KO and their WT mice were injected intraperitoneally with 5 g/kg EtOH, and the time to regain their righting reflex was determined. Mice were placed in clear Plexiglas chambers, injected with EtOH, and, once immobile (~2–3 min), placed onto their backs for the duration of the study. The time taken to place all four paws on the floor of the chamber was determined by behavioral observation using a stopwatch.

Blood EtOH concentration. To assess for genotypic differences in EtOH metabolism and the development of metabolic tolerance, WT and Homer2 KO mice received eight intraperitoneal injections of 3.0 g/kg EtOH every other day, as per the place conditioning study. Blood was sampled from the infraorbital sinus at 5, 15, and 30 min after the EtOH injections 1 and 8. Blood EtOH levels (BEL) (milligrams percent) were determined using gas chromatography as described previously for C57BL/6 mice (Middaugh et al., 1992).

Surgery, in vivo microdialysis, dopamine, and glutamate concentrations. In vivo microdialysis procedures were performed on injection 1 of repeated EtOH administration (3 g/kg, i.p.; volume of 0.02 ml/kg) and then on injection 8 using the contralateral side of the head. The surgical and in vivo microdialysis procedures were similar to those described previously (Szumlinski et al., 2004). Dialysate was collected in 20 min fractions for 1 h before EtOH injection and 3 h thereafter. Consistent with the injection regimen used in the place conditioning study, injections 2–7 were administered every other day to mice in their home cages. Samples were assayed by HPLC with electrochemical detection for dopamine or with fluorescent detection for glutamate as described previously (Szumlinski et al., 2004). After the second microdialysis session, mice were killed, and their brains were removed and stained with cresyl violet for histological examination.

Intra-accumbens NMDA receptor blockade and locomotor activity. Sensitivity to EtOH is associated with sensitivity to the behavioral effects of NMDA receptor antagonists (Krystal et al., 2003). Thus, to assess for genotypic differences in sensitivity to the effects of accumbens NMDA receptor blockade, WT and Homer2 KO mice were fitted bilaterally with indwelling guide cannulas (24 gauge) aimed at the nucleus accumbens. After 7–10 d recovery, a series of locomotor activity tests were conducted using a within-subjects design. For these tests, mice were infused with one of three concentrations of the competitive NMDA receptor antagonist 3-(2-carboxyamidin-4-yl)-propyl-1-phosphonic acid (CPP), and locomotor activity was assessed for 2 h. Testing occurred every 3–4 d, and the order of dosing was counterbalanced within each genotype across the test days.

Behavioral and neurochemical studies after AAV infusion in WT and KO mice. AAVs carrying the Homer2b splice variant or enhanced green fluorescent protein (GFP) were generated and infused as described previously (Szumlinski et al., 2004). Three weeks after AAV infusion, Homer2 WT and KO animals were subjected to either a battery of behavioral tests or in vivo microdialysis procedures. For the behavioral battery, animals were first assessed for EtOH place conditioning and EtOH-induced changes in locomotor activity as described above. Because the greatest genotypic difference in behavior was observed at the 3 g/kg EtOH dose, this dose was selected for study. Four days after place conditioning, animals were assessed for EtOH preference and consumption in a four-bottle choice test (0, 3, 6, vs 12% v/v) under 24 h access conditions in the home cage in a manner similar to that described in rats (Backstrom et al., 2004). Bottles were presented every weekday for a period of 2 weeks, and the average preference for each concentration and EtOH intake during the second week was used in the statistical analysis. For in vivo microdialysis, the procedures were identical to those described above. To verify viral transfection, immunohistochemistry for hemagglutinin (HA)-tagged Homer2 was conducted on 4% paraformaldehyde-fixed accumbens tissue slices (50 μm thick) using standard procedures (primary anti-HA antibody at 1:1,000, and secondary antibody at 1:2,000, followed by light microscopy).

EtOH preference and consumption after AAV infusion in B6 mice. To ascertain whether or not AAV-Homer2b infusion would influence EtOH reward in a mouse strain that exhibits high EtOH preference and consumption, adult male C57BL/6J mice (25–30 g) were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were surgically implanted with guide cannulas and infused intra-accumbens with either AAV-GFP or AAV-Homer2b in a manner identical to that described above. Three weeks after infusion, mice were tested for EtOH preference and consumption using a two-bottle choice procedure in a manner similar to that described for nontransfected WT and KO mice (average across 4 d of
The repeated pairing of EtOH (8 injections at 0, 1, 2, or 3 g/kg, i.p.) with a distinct environment led by EtOH-induced place conditioning. Repeated pairings of EtOH (0–3 g/kg, i.p., for eight injections) with a distinct environment resulted in a place aversion in the time spent in the EtOH-paired compartment in KO mice. In contrast, repeated EtOH administration reduced EtOH intake at 12% EtOH solution (genotype × dose interaction, F_{(3,55)} = 5.52; p = 0.004). The repeated pairing of EtOH (8 injections at 0, 1, 2, or 3 g/kg, i.p.) with a distinct environment induced place conditioning in WT mice but induced place avoidance in KO animals (genotype × dose interaction, F_{(3,55)} = 5.52; p = 0.004). Data are shown as mean ± SEM, and the number of animals used is in parentheses. *p < 0.05 versus 0% or 0 g/kg EtOH; †p < 0.05 versus WT.

**Results**

**Homer2 deletion increased the aversive properties of higher EtOH concentrations**

EtOH preference and consumption were examined using a 24 h ad libitum access, two-bottle choice procedure (0 vs 3, 6, or 12% EtOH, v/v) and revealed genotype differences at the highest EtOH concentration tested. Both WT and KO mice showed a biphasic EtOH preference curve with significant preference over water at 6% but not at 3 or 12% EtOH.

However, at 12% EtOH, the Homer2 KO mice drank preferentially from the bottle containing water (Fig. 1A), and their total EtOH intake was less than half that of WT mice (WT, 17.52 ± 4.21 g/kg; KO, 8.06 ± 1.33 g/kg; p > 0.05) (Fig. 1B). No genotype differences were observed for EtOH preference or consumption at lower EtOH concentrations, nor were significant genotype differences observed for the average volume of water consumed during testing (WT, 7.56 ± 1.44 ml; KO, 8.37 ± 1.18 ml; p > 0.05) or for their preference for a palatable 0.9% (w/v) saccharin solution (Fig. 1A, right).

The genotypic differences in EtOH consumption were paralleled by EtOH-induced place conditioning. Repeated pairings of EtOH (0–3 g/kg, i.p., for eight injections) with a distinct environment resulted in a dose-dependent increase in the time spent in the EtOH-paired compartment in WT mice. In contrast, repeated EtOH administration produced a dose-dependent decrease in the time spent in the EtOH-paired compartment in KO animals. In fact, when compared with 0 g/kg controls, KO mice injected repeatedly with 3 g/kg EtOH exhibited place aversion toward the EtOH-paired compartment (Fig. 1C). The genotypic difference in place conditioning was not related to differences in the time spent in the nonpreferred compartment before conditioning, because WT and KO mice spent a similar amount of time in the nonpreferred compartment on the pretest session (WT, 121.9 ± 21.8 s vs KO = 133.7 ± 21.3 s; p > 0.05). Collectively, the EtOH consumption and place conditioning data indicate that Homer2 deletion increases the aversive or reduces the rewarding properties of higher EtOH concentrations, without impairing the ability to discriminate between different EtOH concentrations. The blunted EtOH reward exhibited by Homer2 KO mice does not appear to be related to an impairment in general reward processes because Homer2 KO mice (1) exhibit enhanced, not blunted, cocaine reward (Szumlinski et al., 2004), (2) did not differ from WT mice regarding water intake and saccharin preference in the present study, and (3) do not differ from WT mice regarding food-induced place conditioning or instrumental responding for sucrose (Szumlinski et al., 2004, 2005).

**Homer2 deletion increased sedation and prevented locomotor sensitization by EtOH**

EtOH-induced effects on motor activity were determined by measuring locomotor behavior during the 15 min EtOH conditioning sessions in the conditioned place preference experiment (Fig. 1C). The motor response to vehicle (0 g/kg) controls was equivalent between WT and KO mice and decreased to an equal extent by the eighth injection (Fig. 2A). Doses of acute EtOH <3 g/kg did not alter the locomotor behavior of either WT or KO mice (data not shown), and 3 g/kg EtOH marginally reduced locomotion to a similar extent in both genotypes. However, whereas WT mice showed locomotor sensitization by the eighth injection of 3 g/kg EtOH, the motor response in Homer2 KO mice was unaltered between the first and eighth injection (Fig. 2A). The genotypic distinction in EtOH-induced motor plasticity did not result from differences in EtOH metabolism because the BELs attained at 5, 15, or 30 min after acute or repeated administration of 3 g/kg EtOH did not differ between WT and KO mice, and repeated EtOH administration reduced the maximum BEL attained in either genotype to a similar extent (Fig. 2B). Thus, both EtOH metabolism and the development of pharmacokinetically equivalent were equivalent between WT and KO mice.

The sedative actions of EtOH were examined by measuring the time to regain a righting reflex after injecting an anesthetic
dose of EtOH (5 g/kg). Homer2 KO mice exhibited a longer latency to regain their righting reflex than did WT mice (Fig. 2C).

**Homer2 deletion prevented EtOH-induced neurochemical adaptation in the accumbens**

Genetic variation in vulnerability to high EtOH consumption is associated with an augmented capacity of either acute or repeated EtOH to increase extracellular levels of dopamine (Smith and Weiss, 1999) and glutamate (Selim and Bradberry, 1996). Thus, the effect of acute and repeated EtOH administration (3 g/kg for eight injections) on accumbens extracellular levels of these neurotransmitters was assessed using in vivo microdialysis. As reported previously (Szumlinski et al., 2004), basal extracellular levels of dopamine did not differ between WT and KO mice, whereas basal extracellular levels of glutamate of KO mice were ~50% that of WT before both injections 1 and 8 of repeated EtOH (Table 1). Compared with injection 1, the capacity of EtOH to elevate extracellular levels of both dopamine and glutamate was enhanced on injection 8 in WT mice (Fig. 3A, C). In contrast, neither dopamine nor glutamate changed between injection 1 and 8 in KO mice (Fig. 3B, D).

**Homer2 deletion enhanced sensitivity to intra-accumbens NMDA receptor blockade**

EtOH modulates NMDA channel opening and trafficking (Chandler, 2003), and genetic vulnerability to alcoholism has been associated with a blunting of the perceptual and dysphoric responses to NMDA receptor antagonists (Krystal et al., 2003). The locomotor response to an intra-accumbens microinjection of the competitive NMDA receptor antagonist CPP was used to determine whether the decrease in EtOH-induced behavioral and neurochemical plasticity in Homer2 KO mice was correspondingly associated with altered responsiveness to NMDA antagonism. CPP elicited a greater increase in locomotor activity in Homer2 KO mice (Fig. 4A). Because Homer2 regulates the synaptic localization of NMDA receptors during development (Shiraishi et al., 2003), the enhanced responsiveness to NMDA antagonism could result from reduced surface expression of NMDA receptors. The amount of the NR2a subunit in the accumbens membrane fraction was significantly reduced in KO compared with WT mice, whereas the NR2b subunit showed a trend toward a reduction (Fig. 4B). In contrast, although mGluR5 binds directly to Homer (Tu et al., 1998), no significant genotypic difference was observed in membrane content of the dimer and mono-

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**Table 1. Comparison of the basal extracellular levels (± SEM) of dopamine and glutamate in the nucleus accumbens of Homer2 WT and KO mice on injections 1 and 8 of repeated EtOH treatment (3 g/kg, i.p.)**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>AAV</th>
<th>Injection 1</th>
<th>Injection 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>None</td>
<td>13.9 ± 0.9 (7)</td>
<td>13.9 ± 1.1 (5)</td>
</tr>
<tr>
<td>KO</td>
<td>None</td>
<td>12.2 ± 0.5 (5)</td>
<td>13.6 ± 0.9 (5)</td>
</tr>
<tr>
<td>GFP</td>
<td>Homer2</td>
<td>10.1 ± 1.7 (9)</td>
<td>12.3 ± 1.1 (8)</td>
</tr>
<tr>
<td>WT</td>
<td>GFP</td>
<td>9.5 ± 3.5 (6)</td>
<td>9.4 ± 1.5 (8)</td>
</tr>
<tr>
<td>KO</td>
<td>Homer2b</td>
<td>9.1 ± 1.2 (9)</td>
<td>12.7 ± 3.6 (7)</td>
</tr>
<tr>
<td>WT</td>
<td>GFP</td>
<td>8.7 ± 1.0 (7)</td>
<td>8.7 ± 0.6 (10)</td>
</tr>
<tr>
<td>KO</td>
<td>Homer2b</td>
<td>8.8 (11)</td>
<td>7.4 (5)</td>
</tr>
</tbody>
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Sample sizes are indicated in parentheses. *p < 0.05 versus WT; †p < 0.05 versus AAV-GFP.

**Figure 3.** Homer2 KO mice do not exhibit neurochemical adaptation with repeated EtOH administration. A, The capacity of 3 g/kg EtOH to elevate extracellular levels of dopamine in the nucleus accumbens was facilitated by eight injections of EtOH in WT mice. B, Repeated EtOH did not induce a change in extracellular dopamine in KO animals (genotype × injection × time, \( F_{(11,225)} = 2.31; p = 0.007 \)). C, Repeated EtOH augmented the effect of EtOH on extracellular glutamate in WT mice. D, No such neurochemical adaptation was observed in KO animals (genotype × injection × time, \( F_{(11,297)} = 1.59; p = 0.06 \)). Data represent the mean ± SEM extracellular levels of neurotransmitter per 20 min fraction, and the number of animals is in parentheses. *p < 0.05 versus injection 1; †p < 0.05 versus WT on injection 8 (LSD post hoc tests).

**Figure 4.** Homer2 KO mice exhibit abnormalities in accumbens NMDA receptor function and plasma membrane expression. A, Homer2 KO mice exhibited increased sensitivity to the locomotor-stimulant effects of an intra-accumbens infusion of the competitive NMDA receptor antagonist CPP (genotype effect, \( F_{(1,44)} = 2.87, p = 0.04 \); genotype × dose interaction, \( F_{(2,44)} = 2.21, p = 0.07 \)). B, Reduced membrane-bound NMDA receptor subtypes NR2a (\( k_{i} = 2.27 \mathrm{~T} \); \( p = 0.04 \)) and NR2b (\( k_{i} = 1.987 \mathrm{~T} \); \( p = 0.07 \)) in accumbens of KO mice. Protein data were normalized to percentage change from WT, and all data are shown as mean ± SEM. The number of animals is indicated in parentheses or in the bars. *p < 0.05 versus 0 nmol CPP; †p < 0.05 versus WT (LSD post hoc tests or t tests).
mer of mGluR5 (dimer WT, 100 ± 6.6, n = 8; KO, 90.2 ± 4.9, n = 7; monomer WT, 100 ± 7.5, n = 8; KO, 104.2 ± 11.6, n = 8).

Intra-accumbens AAV-Homer2b infusion eliminated the genotypic differences in EtOH-induced neuroplasticity

To confirm an active role for accumbens Homer2 expression in the regulation of EtOH-induced neuroplasticity, an AAV carrying either Homer2b or GFP control cDNA was infused into the accumbens of WT and Homer2 KO mice. Homer2b was HA tagged, and AAV transfection was verified by immunostaining (Fig. 5a). Cellular transfection was restricted to an area of ~1 mm around the injection site in the nucleus accumbens, and protein expression was localized to both processes and soma (Fig. 5a’,a”). Starting 3 weeks after AAV infusion, mice were assessed for EtOH-induced changes in accumbens neurotransmission (3 g/kg for eight injections) or tested for EtOH-induced place conditioning (3 g/kg for eight injections) and then evaluated for EtOH preference and consumption in a 24 h ad libitum access four-bottle choice procedure (0, 3, 6, and 12% EtOH). Intra-accumbens AAV-Homer2b infusion enhanced EtOH preference in both WT and KO mice, as revealed by an upward shift in the EtOH dose-preference curve at low EtOH concentrations (Fig. 5b) and a reduction in the cumulative preference for water (Fig. 5c). Although AAV-Homer2b infusion did not influence total EtOH intake by WT mice, it reversed the genotypic difference over the 24 h test period (Fig. 5d). AAV-Homer2b infusion also reversed the genotypic difference in the expression of EtOH-induced place conditioning, as revealed by a significant difference in the expression of place conditioning between AAV-GFP WT and KO mice but not between AAV-Homer2b WT and KO mice (Fig. 5e). A similar effect of AAV-Homer2b infusion was observed for the change in locomotion produced by repeated EtOH administration between the first and eighth EtOH conditioning session (Fig. 5f).

The EtOH preference data for WT mice indicated a significant but modest shift to the left in EtOH preference by AAV-Homer2b infusion (Fig. 5b). To ascertain whether or not a similar effect of AAV-Homer2b infusion would be observed in mice with a genetic propensity to consume high amounts of EtOH, we examined the effects of AAV infusion on EtOH preference and intake in B6 mice. As illustrated in Figure 6A, AAV-Homer2b infusion into the accumbens of B6 mice produced a marked shift to the left in the EtOH preference function, as well as an enhancement in the preference for 18% EtOH. In a manner akin to that observed for WT mice (Fig. 5d), AAV-Homer2b infusion did not influence the intake of any of the EtOH concentrations tested (Fig. 6B). Thus, AAV-Homer2b infusion increases preference for EtOH in both moderate and high consuming mice, without altering total EtOH intake.

Intra-accumbens AAV-Homer2b infusion elevated accumbens glutamate content in both WT and KO mice but did not influence basal extracellular levels of dopamine (Table 1). Similar to nontransfected WT mice (Fig. 3), repeated EtOH injections increased accumbens extracellular levels of glutamate (Fig. 7A).
and dopamine (Fig. 7E) in AAV-GFP WT mice. Also akin to the data in Figure 3, AAV-GFP-transfected KO mice showed no attenuation in glutamate or dopamine after repeated EtOH (Fig. 7C,G). Although AAV-Homer2b transfection of WT mice did not alter the sensitization of glutamate produced by repeated EtOH (Fig. 7B), the sensitized increase in extracellular dopamine was augmented by AAV-Homer2b transfection (Fig. 7F). Restoring Homer2b with intra-accumbens AAV-Homer2b enabled the enhanced dopamine and glutamate responses to EtOH after repeated treatment in KO mice (Fig. 7D,H).

Discussion
The present report provides in vivo validation of the involvement of Homer proteins in neuroplasticity by showing that Homer2 gene deletion impairs the development of behavioral and neurochemical plasticity to repeated EtOH administration. This complements previous in vitro anatomical and electrophysiological studies showing that Homer proteins regulate synaptic plasticity and remodeling (Xiao et al., 2000; de Bartolomeis and Iasevoli, 2003). Indeed, in vitro studies show that Homer proteins cluster EVH1-bound proteins in the PSD, including NMDA receptor–Shank scaffolding complexes (Tu et al., 1999), and the present study demonstrated that Homer2 deletion decreased the plasma membrane content of NMDA receptor subunits in the accumbens. The nucleus accumbens is a critical site for neuroplasticity in dopamine and glutamate transmission underlying the development of addiction (Nestler, 2001; Chandler, 2003; Kalivas et al., 2005). The fact that Homer2 deletion induced parallel deficits in the capacity of repeated EtOH to induce place preference and motor sensitization and to elicit sensitization in accumbens glutamate and dopamine transmission supports the link between these behaviors and accumbens neurotransmission. Moreover, Homer proteins in the accumbens were shown to be critical in mediating this linkage because intra-accumbens AAV-Homer2b infusion reversed the genotypic differences in EtOH-induced behavioral and neurochemical plasticity.

Homer2 regulates EtOH effects on dopamine and glutamate transmission
Imaging studies in EtOH-dependent humans show an association between the propensity to consume large amounts of EtOH and EtOH-induced increases in metabolic activity in the accumbens and mesocortical structures supplying glutamatergic innervation of the accumbens (Mosley et al., 2001; Meyerhoff et al., 2004). EtOH elevates accumbens extracellular levels of glutamate in EtOH-drinking Lewis rats but not in low EtOH-drinking Fischer 344 rats (Selim and Bradberry, 1996) and facilitates evoked glutamate release in cortical slices in EtOH-prefering, but not in EtOH-nonpreferring, rats (McBridge et al., 1986). Moreover, low-EtOH sensitive rats exhibit an increase in accumbens levels of extracellular glutamate compared with high-EtOH sensitive rats (Dauchour et al., 2000), and EtOH-tolerant rats display less of an EtOH-induced inhibition of accumbens extracellular glutamate compared with EtOH-nontolerant rats (Piepponen et al., 2002). The hyporesponsiveness of accumbens glutamate in rats selectively bred for low EtOH preference and high sensitivity to the motor-impairing effects of EtOH parallels the behavioral and glutamatergic phenotype of Homer2 KO mice. Although a role for Homer2 in the rat strain distinctions remains to be determined, the restoration of EtOH-induced glutamatergic and behavioral plasticity by AAV-Homer2b-mediated overexpression in the accumbens implicates Homer2 regulation of extracellular glutamate in sensitivity to the psychomotor-impairing effects of EtOH, a major predictor of subsequent EtOH consumption (Schuckit and Smith, 2000).
Microdialysis studies in rats selectively bred for high versus low EtOH preference/consumption show an increased capacity of acute or repeated EtOH to elevate accumbens extracellular levels of dopamine in high consuming lines (McBride and Li, 1998). Given the role for accumbens dopamine transmission in mediating the rewarding effects of EtOH (Koob et al., 1998; Gonzales et al., 2004), the EtOH-avoiding phenotype of Homer2 KO mice may result from a failure of repeated EtOH to induce dopaminergic adaptations. The lack of EtOH-induced dopamine plasticity in the accumbens of Homer2 KO mice is not likely a direct effect of gene deletion on basal dopamine transmission or dopamine transporter function because basal extracellular levels of dopamine and the rise in dopamine produced by blocking the dopamine transporter is similar between WT and KO mice (Szumlinski et al., 2004). Interestingly, Homer2b transfection not only restored the sensitization of extracellular dopamine by repeated EtOH administration in Homer2 KO mice but also accentuated the EtOH-induced increase in dopamine in WT mice. Because Homer proteins are traditionally studied in relation to glutamate transmission, mechanisms underlying the augmentation in extracellular dopamine after AAV-Homer2b transfection are unclear. It is possible that AAV-Homer2b transfection of GABAergic accumbens spiny cells projecting to the ventral tegmental area may have reduced inhibitory feedback regulation of dopamine neurons. Supporting this possibility, EtOH-induced increases in accumbens dopamine arise in part from actions on GABA transmission in the ventral tegmental area (Galglego et al., 1999; McBride et al., 1999; Melis et al., 2002).

Homer, NMDA receptors, and EtOH sensitivity

Homers regulate the anchoring and synapnic localization of NMDA receptors by interacting with the Shank–PSD–95–GKAP (for guanylate kinase domain–associated protein) complex of PSD proteins (Naisbitt et al., 1999; Shiraishi et al., 2003). The NR2 subunits of the NMDA receptor contain PDZ (for PSD-95/PSD proteins) (Naisbitt et al., 1999; Shiraishi et al., 2003). The Shank–PSD–95–GKAP complex (Naisbitt et al., 1999). Although Homer2 deletion does not alter the total protein content of NR2a or NR2b in the accumbens (Szumlinski et al., 2004), the plasma membrane localization of NR2a was reduced. Reduced plasma membrane-bound NR2a in the accumbens of Homer2 KO mice is consistent with decreased in vitro cell surface NMDA receptors after either mutagenesis of Homer isoforms or overexpression of the dominant-negative immediate early gene product Homer1a (Sala et al., 2001, 2003).

EtOH antagonizes NMDA channel function, and the NR2 subunit is critical for this action (Lovinger et al., 1989; Woodward, 2000). Inhibition of NMDA by EtOH contributes to the intoxicating and subjective effects of higher EtOH doses (Chandler, 2003; Krystal et al., 2003). Moreover, blunted responsiveness to NMDA antagonists is associated with vulnerability to EtOH addiction (Krystal et al., 2003). Consistent with this clinical association, the reduced EtOH preference in Homer2 KO mice was accompanied by increased sensitivity to the sedative and aversive properties of EtOH and to the psychomotor-activating effects of NMDA receptor blockade. Although more experiments are necessary, reduced surface expression of NR2a to the membrane in Homer2 KO mice is consistent with greater sensitivity to NMDA receptor antagonists. Thus, Homer-mediated anchoring of NMDA receptors may gate behavioral sensitivity to the acute effects of EtOH, which is negatively associated with subsequent EtOH consumption (Schuckit and Smith, 2000; Krystal et al., 2003). Similarly, Homer regulation of NMDA receptor anchor-


