NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH Public Access

Author Manuscript

Alcohol. Author manuscript; available in PMC 2013 June 01.

Published in final edited form as:

Alcohol. 2012 June ; 46(4): 377–387. doi:10.1016/j.alcohol.2011.10.003.

Ethanol up-regulates nucleus accumbens neuronal activity dependent pentraxin (Narp): implications for alcohol-induced behavioral plasticity

Alexis W. Ary, B.A.[1], **Debra K. Cozzoli, B.A.**[1,*], **Deborah A. Finn, Ph.D.**[2], **John C. Crabbe, Ph.D.**[2], **Marlin H. Dehoff, M.S.**[3], **Paul F. Worley, M.D.**[3], and **Karen K. Szumlinski, Ph.D.**[1]

[1]Department of Psychology and the Neuroscience Research Institute, University of California, Santa Barbara, Santa Barbara, CA 93106-9660, U.S.A.

[2]Department of Behavioral Neuroscience, Oregon Health & Science University and VA Medical Research, Portland, OR 97239

[3]The Solomon H. Snyder Department of Neuroscience, Johns Hopkins University, School of Medicine, Baltimore, MD 21205, U.S.A.

Abstract

Neuronal activity-dependent pentraxin (Narp) interacts with α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) glutamate receptors to facilitate excitatory synapse formation by aggregating them at established synapses. Alcohol is well-characterized to influence central glutamatergic transmission, including AMPA receptor function. Herein, we examined the influence of injected and ingested alcohol upon Narp protein expression, as well as basal Narp expression in mouse lines selectively bred for high blood alcohol concentrations under limited access conditions. Alcohol up-regulated accumbens Narp levels, concomitant with increases in levels of the GluR1 AMPA receptor subunit. However, accumbens Narp or GluR1 levels did not vary as a function of selectively bred genotype. We next employed a *Narp* knock-out (KO) strategy to begin to understand the behavioral relevance of alcohol-induced changes in protein expression in several assays of alcohol reward. Compared to wild-type mice, *Narp* KO animals: fail to escalate daily intake of high alcohol concentrations under free-access conditions; shift their preference away from high alcohol concentrations with repeated alcohol experience; exhibit a conditioned place-aversion in response to the repeated pairing of 3 g/kg alcohol with a distinct environment and fail to exhibit alcohol-induced locomotor hyperactivity following repeated alcohol treatment. *Narp* deletion did not influence the daily intake of either food or water, nor did it alter any aspect of spontaneous or alcohol-induced motor activity, including the development of tolerance to its motor-impairing effects with repeated treatment. Taken together, these data indicate that Narp induction, and presumably subsequent aggregation of AMPA receptors, may be important for neuroplasticity within limbic subcircuits mediating or maintaining the rewarding properties of alcohol.

Correspondence: Karen K. Szumlinski, Ph.D. University of California, Santa Barbara Psychological and Brain Sciences Department, Building 551 Santa Barbara, CA 93106-9660 szumlinski@psych.ucsb.edu Phone: (805) 893-2984 Fax: (805) 893-4303.

*Present address: Department of Behavioral Neuroscience, Oregon Health & Science University and VA Medical Research, Portland, OR 97239

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

NIH-PA Author Manuscript

Keywords

alcohol; AMPA receptors; conditioning; escalation; Neuronal activity dependent pentraxin (Narp); alcohol intake

Introduction

Alcoholism is a chronic neuropsychiatric disorder, characterized by an unmanageable motivation to consume alcohol. Alcoholism affects approximately 18.2 million people in the United States (Substance Abuse and Mental Health Services Administration 2006); however, despite the wide-spread prevalence of alcoholism and the heavy socioeconomic burden associated with this disease, the precise molecular substrates mediating vulnerability to consume large amounts of alcohol are still not fully understood.

Alcohol targets directly and acutely inhibits certain subtypes of glutamate receptors, including the ionotropic *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptors, as well as the Group1 metabotropic glutamate receptor subtype 5 (mGluR5) (e.g., Läck *et al.* 2007; Lovinger 1996; Minami *et al.* 1998; Weiner *et al.* 1999). Although the AMPA receptor is the primary glutamate receptor mediating fast excitatory neurotransmission in the brain (c.f., Dodd *et al.* 2000), their role in regulating alcohol-induced behavioral and neural plasticity is not yet well-understood. This is owing, in part, to earlier reports suggesting that the sensitivity of AMPA receptors to the depressant effects of alcohol is less than that of NMDA receptors (e.g., Didly-Mayfield *et al.* 1992; Lovinger *et al.* 1989). However, AMPA receptors do exhibit significant acute alcohol sensitivity within brain regions implicated in the rewarding, cognitive- and motor-impairing properties of this drug [e.g., the nucleus accumbens (NAC), ventral tegmental area (VTA), hippocampus and cerebellum] (e.g., Lovinger *et al.* 1996; Martin *et al.* 1995; Möykkynen *et al.* 2003; Nie *et al.* 1994; Wanat *et al.* 2009; Wirkner *et al.* 2000). This being said, drug-induced changes in AMPA function may result indirectly through homeostatic mechanisms that regulate neuronal excitability (e.g., Sanderson and Dell'Acqua, 2011; Wolf and Ferrario 2010). Regardless, prolonged alcohol exposure or repeated bouts of alcohol intake up-regulates indices of AMPA receptor function *in vitro* and *in vivo* (Läck *et al.* 2007; Long *et al.* 2007; Netzeband *et al.* 1999a, 1999b; Stuber *et al.* 2008; Wanat *et al.* 2009) – effects consistent with reports of alcohol- or alcohol withdrawal-induced increases in GluR1 and/or GluR2/3 receptor subunit expression within addiction-relevant brain regions (Brückner *et al.* 1997; Chandler *et al.* 1999; Chen *et al.* 1999; Haugbøl *et al.* 2005; Ortiz *et al.* 1995; Pickering *et al.*, 2007; but see Ferriera *et al.* 2001).

In support of a potential key role for AMPA receptors in the behavioral effects of alcohol, pharmacological inhibition of AMPA function reduces the manifestation of alcohol withdrawal symptoms in both humans and rodents (Krupitsky *et al.* 2007; Palachick *et al.* 2008). However, the behavioral pharmacological and genetic data to date concerning the role for AMPA receptors in regulating alcohol-induced changes in motor and motivated behavior, including alcohol self-administration have been inconsistent at best (e.g., Broadbent *et al.* 2003; Cowen *et al.* 2003; Jones *et al.* 2008; Palachick *et al.* 2008; Rial *et al.* 2008; Sanchis-Segura *et al.* 2006; Stephens and Brown 1999), although some studies have found both selective and non-selective AMPA receptor antagonists or *GluR3* gene deletion to be effective at reducing alcohol self-administration (Nguyen *et al.* 2007; Sanchis-Segura *et al.* 2006), alcohol-induced conditioned place-preference (Zhu *et al.* 2007) and cue-induced reinstatement of alcohol-seeking behavior (Bäckstrom and Hyytiä 2004; Sanchis-Segura *et al.* 2006). These more recent data for alcohol reward/reinforcement measures are consistent with data implicating a role for the AMPA receptor in addiction-related behaviors for other

drugs of abuse (c.f., Gass and Olive 2008; Everitt and Robbins 2005; Wolf and Ferrario 2010) and pose intra- and extracellular regulators of AMPA receptor function as potentially critical for the addiction process (Möykkynen *et al.* 2009; Wolf and Ferrario 2010).

A potential candidate in this regard may be the family of Neuronal Pentraxins, which are enriched within the extracellular matrix and regulate AMPA receptor aggregation, as well as synaptogenesis, at the post-synpatic density (Chang *et al.* 2010; O'Brien *et al.* 1999; 2002; Tsui *et al.* 1996). This family consists of 3 members, NP1 (neuronal pentraxin 1), NPR (neuronal pentraxin receptor) and Narp (Neuronal activity regulated pentraxin or NP2), the last of which is induced in an immediate early gene-like fashion following synaptic activity and acts, in concert with NP1, to enhance AMPA receptor clustering and function (Chang *et al.* 2010; Kirkpatrick *et al.* 2000; O'Brien *et al.* 1999; 2002; Xu *et al.* 2003). Narp expression is elevated during both "natural" and precipitated withdrawal from repeated morphine, nicotine and 9Δ-THC (Reti *et al.* 2008; 2009) and is changed in concert with AMPA receptor subunits during extinction of a lever-press response for cocaine (Self *et al.* 2004). While the role for Narp in regulating alcoholism-relevant behaviors has not been described, *Narp* KO mice exhibit a number of differences from wild-type (WT) animals in behavioral adaptations produced by repeated exposure to other drugs of abuse, including morphine (Crombag *et al.* 2009; Reti *et al.* 2008) and cocaine (Pacchioni *et al.* 2009). Of relevance for drug reward, *Narp* KO mice exhibit greater cocaine-conditioned place-preference, compared to WT animals (Pacchioni *et al.* 2009), but do not differ from WT animals regarding the conditioned rewarding effects of morphine (Crombag *et al.* 2009). This being said, *Narp* KO mice do exhibit greater indices of conditioned aversion to environments paired with morphine withdrawal (Reti et al. 2008), suggesting a potentially important role for Narp in regulating both the positive and negative effective states produced by certain drugs of abuse. As AMPA receptors appear to contribute, at least in part, to certain alcohol-induced changes in behavior and given the current data favoring a role for Narp in drug-induced behavioral plasticity, the present study sought to examine alcohol-induced changes in Narp expression within forebrain structures and to confirm, using a *Narp* KO strategy, a role for the induction of this immediate early gene in alcohol-induced changes in motor and motivated behavior.

Materials and Methods

Animals

For immunoblotting studies to assay the effects of injected and ingested alcohol upon Narp expression, male C57BL/6J (B6) mice (25-30 g; ~8 weeks of age) were obtained from Jackson Laboratory (Bar Harbor, ME). For immunoblotting studies to assay the potential relation between genetic predisposition to consume high amounts of alcohol and protein expression, two studies comparing between selectively bred mouse lines were conducted. As described in detail in Cozzoli *et al.* (2009), the first study compared between mouse lines selectively bred for high versus low blood alcohol content (BAC) following alcohol consumption under Scheduled High Alcohol Consumption procedures (SHAC and SLAC, respectively; generated in the laboratory of Dr. D.A. Finn, Oregon Health and Science University, Portland, OR). As in our previous report (Cozzoli *et al.* 2009), we studied tissue from mice from the 4th generation of selective breeding for the SHAC and SLAC phenotype and these two lines exhibited a statistically significant divergence in drinking behavior as assayed by the selection criterion of BAC (SHAC: ~150 mg% vs. SLAC: ~100 mg%; see Cozzoli *et al.* 2009). The second study compared between mice selectively bred for high BACs following alcohol consumption under Drinking-in-the-Dark conditions (HDID-1) and mice from their heterogeneous HS/Npt founder population (generated in the laboratory of Dr. J.C. Crabbe, Oregon Health and Science University, Portland, OR). Immunoblotting was conducted on tissue from the 14th generation of HDID-1 selective breeding and on tissue from the 65th intercross generation of the HS/Npt founder population served as a control. As

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

demonstrated in Crabbe *et al.* (2009), the alcohol drinking behavior of the HDID-1 line was significantly higher than that exhibited by the HS/Npt founder population as assayed by the selection criterion of BAC (HDID-1 > 100 mg%). To confirm a necessary role for Narp induction in mediating alcohol-induced changes in behavior, male *Narp* WT and KO littermate mice were obtained from the laboratory of Dr. P.F. Worley (Johns Hopkins University School of Medicine, Baltimore, MD) from breeding of heterozygous mating pairs on a mixed 129sv/B6 background (see Pacchioni *et al.* 2009) and shipped to the University of California at Santa Barbara at approximately 6 weeks of age. WT/KO mice were allowed 2 weeks to acclimatize to the colony room conditions prior to experimentation.

For all experiments, mice were housed in standard polyethylene cages in a temperature (25°C) and humidity (71%) controlled vivarium, under a 12-hr light cycle. For experiments involving alcohol consumption, mice were housed individually under a reverse light cycle (lights off at 0500 h), while all other animals were housed in groups of 3-5 under a regular light cycle (lights off at 2000 h). With the exception of the Scheduled High Alcohol Consumption study, which involved fluid restriction procedures (see below), food and water was provided *ad libitum*. All experimental protocols were approved by the University of California at Santa Barbara Institutional Animal Care and Use Committee and conducted in accordance with the guidelines set forth in the National Institute of Health (NIH) *Guide for Care and Use of Laboratory Animals* (NIH Publication No. 80–23, revised 1996).

Immunoblotting

Three separate experiments were conducted in B6 mice to assess whether alcohol alters Narp expression in brain (see alcohol delivery procedures below). As conducted previously (e.g., Cozzoli *et al.* 2009; Goulding *et al.* 2011), alcohol-treated and control animals were rapidly decapitated at 24 hr following their treatment, brains extracted, and sectioned along the coronal plane (0.5 mm thick) in an ice-cold brain mold at the level of the prefrontal cortex (PFC), and at the level of the nucleus accumbens (NAC)/dorsal striatum (STR). Over an ice-cold plate, the PFC was dissected out using forceps while the NAC and STR were dissected out using micropunches. Two separate experiments were conducted in selectively bred high alcohol-consuming and control mouse lines (SHAC vs. SLAC and HDID-1 vs. HS/Npt; see above) to determine whether basal NAC Narp and GluR1 expression is a genetic correlate of excessive alcohol consumption. For these experiments, frozen whole brains of adult S_4 SHAC, S_4 SLAC, S_{14} HDID-1 and G_{65}HS/Npt mice (Oregon Health & Science University) were shipped to the University of California Santa Barbara 3 months following alcohol testing. Brains were sectioned along the coronal plane (0.5 mm thick) at the level of the NAC and tissue dissected out over ice using a micropunch. All tissue was stored frozen at −80°C until homogenization.

Sample preparation and immunoblotting procedures for the detection of Narp and the GluR1 subunit of the AMPA receptor were similar to those previously described by our laboratory for other glutamate-related proteins (e.g., Cozzoli *et al.* 2009; Goulding *et al.* 2011). Homogenates were subjected to SDS-polyacrylamide gel electrophoresis on Bis-Tris gradient gels (4–12%) (Invitrogen, Carlsbad, CA, USA), with standard transfer and pre-blocking procedures. Membranes were incubated in a rabbit polyclonal primary anti-Narp antibody (1:1000 dilution) that recognizes an ~ 60 kDa band in a manner consistent with previously published studies (Chang *et al.* 2010; O'Brien *et al.* 1999) and/or in a rabbit polyclonal anti-GluR1 antibody (1:1000 dilution; Upstate Cell Signaling Solutions, Lake Placid, NY, USA) over-night at 4^0C. Membranes were then washed and incubated in a horseradish peroxidase-conjugated goat anti-rabbit secondary anti-body (Upstate, Charlottesville, VA, USA; 1:40,000 dilution) for 90 min, then washed again and immunoreactive bands were detected by enhanced chemiluminescence (ECL Plus; Amersham Biosciences, Inc., Piscataway, NJ, USA). Even protein transfer was determined

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

using a rabbit anti-calnexin polyclonal primary antibody (Stressgen, Victoria, BC, Canada; 1:1000 dilution; e.g., Cozzoli *et al.* 2009; Goulding *et al.* 2011). Immunoreactivity levels were quantified using the Area X Density measures with Image J (NIH, Bethesda, MD, USA) and the data for Narp and GluR1 expression for each animal was normalized to its respective calnexin control. Similar to our previous alcohol studies (e.g., Goulding *et al.* 2011), the protein:calnexin ratios for alcohol-treated and high alcohol-drinking mice were expressed as a percentage of those obtained for their respective control groups for each gel (n = 5–7/gel) and analyzed using one-way ANOVA (injection study) or Student's t-tests (drinking and genetic correlation studies). $\alpha = 0.05$.

Alcohol Injection Study—The first immunoblotting experiment determined whether or not Narp expression is induced by the acute administration of a fixed moderately intoxicating dose of alcohol (e.g., Szumlinski *et al.* 2005) and to determine how Narp expression changes with repeated alcohol treatment. For this, 3 groups of B6 mice were tested. The acute alcohol group received 7 intraperitoneal (IP) injections of saline, followed by 1 injection of 3 g/kg alcohol (vol=0.02 ml/kg). The repeated alcohol group received 8 IP injections of 3 g/kg alcohol – a dosing regimen demonstrated previously to elicit the development of tolerance to the motor-inhibiting effects of alcohol (Szumlinski *et al.* 2005). The control group received 8 IP injections of saline. For all groups, the injections were spaced every 2 days (in accordance to the alcohol regimen employed in the conditioned place-preference/locomotor behavior studies below).

Scheduled High Alcohol Consumption (SHAC) Study—A history of binge alcohol drinking under SHAC procedures sensitizes glutamate release and augments the expression of Group1 metabotropic and NMDA receptors within the NAC (Cozzoli *et al.* 2009; Szumlinski *et al.* 2007). Thus, the second immunoblotting experiment determined whether or not a history of binge alcohol drinking by B6 mice could also affect Narp expression within forebrain structures. For this study, we utilized a variation of the murine SHAC model of binge-like alcohol drinking (e.g., Finn *et al.* 2005), employing procedures identical to those previously described by our group (see Cozzoli *et al.* 2009; Szumlinski *et al.* 2007). These procedures consisted of a 30-min presentation of a 50 ml sipper tube containing a 5% (v/v) alcohol solution, every 3rd day, at 3 hrs into the dark cycle for a total of 6 presentations. On intervening days, mice received a 30-min presentation of water. As described in greater detail in Szumlinski *et al.* (2007), early in the SHAC procedures, animals experienced 10 hrs of fluid restriction daily that was gradually reduced to 4 hrs/day over the course of experimentation. Food was available *ad libitum*. These modified SHAC procedures produce high levels of alcohol consumption (1.3–1.9 g/kg per 30 min), resulting in BACs >1.0 mg/ml (Cozzoli *et al.* 2009; Szumlinski *et al.* 2007). Control animals were subjected to identical drinking bouts and fluid restriction, with tap water presented in a 50 ml sipper tube during all 30-min drinking sessions. Due to limited tissue availability, tissue from alcohol- and water-drinking animals under SHAC procedures was processed only for Narp and calnexin levels.

Drinking-in-the-Dark (DID) Study—The third immunoblotting experiment employed DID procedures to determine whether or not the data derived from the SHAC immunoblotting study generalized to another murine model of binge alcohol drinking, in which animals are not fluid-restricted. To correlate Narp levels with those of the AMPA receptor subunit GluR1, we processed tissue for both proteins, as well as calnexin as the loading control. The DID procedures were similar to those employed by other laboratories (e.g., Crabbe *et al.* 2009; Gupta *et al.* 2008; Moore and Boehm 2009; Rhodes *et al.* 2005) and consisted of daily, 2-hr presentations of a 50 ml sipper tube containing a 20% (v/v) alcohol solution, at 3 hrs into the dark cycle. Food was available *ad libitum* and water was

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

available at all times with the exception of the 2-hr alcohol delivery period. As with the SHAC procedures, these DID procedures produce consistently high levels of alcohol consumption (mean alcohol intake/2-hr session = 4.8 ± 0.43 g/kg), which have been shown previously to result in BACs >1.0 mg/ml (e.g., Crabbe *et al.* 2009; Moore and Boehm 2009; Rhodes *et al.* 2005). Control animals were subjected to an identical procedure with the exception that tap water was presented in a 50 ml sipper tube during the 2-hr drinking period. DID procedures were conducted for 30 consecutive days and animals sacrificed 24 hrs following their last alcohol/water presentation. As the results of *in vitro* studies indicated that Narp induction promotes AMPA receptor aggregation and expression within the post-synaptic density (O'Brien *et al.* 1999; 2002; Tsui *et al.* 1996), Pearson's correlational analyses were conducted to relate levels of Narp and GluR1 expression within the NAC.

Alcohol Place-Conditioning and Locomotor Behavior

To begin to understand the functional relevance of alcohol-induced increases in NAC Narp expression (Figures 1 & 2) for alcoholism-relevant behaviors, we employed a KO strategy in which WT and *Narp* KO mice were first compared in an alcohol-conditioned place-preference paradigm. The procedures used to produce an alcohol-conditioned place-preference were similar to those previously described by our group (e.g., Lominac *et al.* 2006; Szumlinski *et al.* 2008) and involved an unbiased procedure, using an apparatus with two visually (wall pattern) and tactilely (floor texture) distinct compartments. Video-tracking was used to record time spent in each of the two compartments, as well as the locomotor activity exhibited by each animal during testing (Stoelting Company, Wood Dale, IL, USA). Animals were allowed a 15-min free-access session to habituate them to the entire apparatus and the next day, a 15-min preconditioning test was conducted to verify that the apparatus was unbiased. Alcohol-conditioned place-preference was induced via 8 repeated pairings of IP injections of alcohol (3 g/kg), on alternating days, with one of the compartments and IP saline injections (0.02 ml/kg) with the other compartment. Given the limited number of animals available for our study (i.e., only sufficient animals to assay 1 alcohol dose with statistical confidence), this injection regimen was selected as (1) it produces significant preference for the alcohol-paired environment in both B6 and B6 hybrid mice (Lominac *et al.* 2006; Szumlinski *et al.* 2005) and (2) is optimal at distinguishing both conditioned reward and locomotor behavior between WT mice and mutant mice with altered glutamate signaling (i.e., *Homer2* KO mice; Szumlinski *et al.* 2005). Moreover, in our hands, 3 g/kg alcohol produces sedation when administered acutely to mice and tolerance develops to this effect with repeated administration (e.g., Lominac *et al.* 2006; Szumlinski *et al.* 2005). Thus, employing this dose enabled comparisons of genotypic differences in initial sensitivity, as well as tolerance, to the sedative-hypnotic properties of alcohol, which is typically inversely related to alcohol's rewarding/reinforcing properties (e.g., Szumlinski *et al.* 2005). On the day following the 8th alcohol pairing, a post-conditioning test was conducted to assess for place-preference, where drug-free animals again had free access to both compartments for 15 min. The magnitude of place-preference was indexed as amount of time spent on the alcohol- vs. saline-paired compartment on the post-conditioning test. Data were analyzed using a univariate ANOVA (α=0.05).

Narp KO mice exhibit deficits in acute cocaine-induced locomotion, as well as in the development of locomotor sensitization in the short-term (Pacchioni *et al.* 2009). Thus, we examined also the total distance traveled and time spent immobile (i.e., no detectable movement for a period of 15 sec) exhibited by our mice during the place-conditioning study as collected by our video-tracking system (e.g., Lominac *et al.* 2006; Szumlinski *et al.* 2008). To assay for genotypic differences in alcohol-induced motor activity, we assayed for genotypic differences in response to the 1st alcohol injection and the change in alcohol-induced locomotion observed across the 8 conditioning sessions. To assay for genotypic

NIH-PA Author Manuscript

differences in spontaneous (novelty-induced) locomotion and habituation of locomotor activity, we compared the activity of WT and KO mice during the habituation session and across the 8 saline sessions, respectively. Finally, to assay for genotypic differences in the expression of alcohol withdrawal-induced motor hyperactivity, we compared the activity of WT and KO mice on the pre- and post-conditioning tests (i.e., under alcohol-free conditions, before and after repeated alcohol injections). Data were analyzed using either univariate ANOVAs or repeated measures ANOVAs, with alcohol/saline injection or test day as the within-subjects factor (α=0.05).

Alcohol Preference and Consumption in a Four-Bottle Choice Task

To extend our place conditioning data to an alcohol drinking paradigm, we next compared *Narp* WT and KO littermate mice on a simple free-access, 4-bottle-choice test, which enabled the simultaneous measurement of water, food and alcohol intake, as well as alcohol preference. One week following the place-conditioning study, mice were subjected to our 4-bottle-choice procedures. Thus, all animals had equivalent non-contingent alcohol exposure and withdrawal prior to assessment of alcohol intake. As described in our earlier studies (Cozzoli *et al.* 2009; Goulding *et al.* 2011; Kapasova and Szumlinski 2008; Lominac *et al.* 2006; Szumlinski *et al.* 2008), mice were presented in the home cage with four identical 50 ml sipper tubes containing 0, 3, 6, and 12% alcohol (v/v). The amount of alcohol consumed from each bottle and the amount of food consumed were determined by weight before and after the 24-hr consumption period. As it is our experience that mice escalate their alcohol intake across days, particularly during the 1st week of drinking (e.g., Goulding *et al.* 2011; Lominac *et al.* 2006; Szumlinski *et al.* 2005), we monitored fluid and food intake for 10 consecutive days to examine for potential genotypic differences in escalation. Data were analyzed using repeated measures ANOVAs with alcohol concentration and/or drinking test day as within-subjects factors (α=0.05).

NIH-PA Author Manuscript

Results

NAC Narp induction sensitizes with repeated alcohol treatment

When assayed at 24 hrs following an acute injection of an intoxicating dose of alcohol (3 g/kg), B6 mice exhibited elevated NAC protein levels of Narp and this effect sensitized with repeated alcohol treatment (Figure 1A) [$F(2,24)=11.0$, $p<0.0001$; LSD post-hoc tests]. In contrast to the NAC, acute alcohol did not affect Narp expression within the more dorsal aspects of the STR, although there was a trend towards a decrease in protein levels in animals treated repeatedly with alcohol (Figure 1B; one-way ANOVA, p=0.06). These data provide the first evidence that the non-contingent administration of a fixed intoxicating dose of alcohol elicits regionally distinct increases in Narp expression within striatal structures that are detectable at 24 hrs post-treatment. Moreover, these data show that, akin to alcohol-induced glutamate release (e.g., Goulding *et al.* 2011; Kapasova and Szumlinski 2008; Melendez *et al.* 2003; Szumlinski *et al.* 2005, 2008), the expression of alcohol-induced increases in NAC Narp expression sensitizes with repeated, intermittent, alcohol exposure.

NIH-PA Author Manuscript

Binge alcohol drinking up-regulates NAC Narp expression

Scheduled High Alcohol Consumption procedures—The alcohol intake exhibited by B6 mice under our SHAC procedures was approximately 1.5 g/kg/30 min, which corresponds to a mean BAC=109 mg% (see Cozzoli *et al.* 2009). Thus, the level of alcohol consumption exhibited by the mice in this experiment meets the NIAAA criterion for binge drinking (NIAAA, 2004). Consistent with the alcohol injection data in Figure 1, SHAC mice exhibited 50% higher NAC Narp levels versus water-drinking controls when assayed 24 hrs following the 6th and last binge alcohol drinking session (Figure 2A) ($t_{18}=2.45$; $p=0.03$). In contrast, group differences in Narp expression were not observed for either the more dorsal

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

aspect of the striatum (SLAC: 100 ± 26 vs. SHAC: 108 ± 14; p=0.77) or the PFC (SLAC: 100 ±22 vs. SHAC: 119 ± 24; p=0.77; p=0.64).. Thus, at least within the confines of this study, repeated bouts of excessive binge-like alcohol consumption produces a region-specific up-regulation of NAC Narp expression that is detectable at 24 hrs following the cessation of drinking.

Drinking-in-the-Dark procedures—The average alcohol intake exhibited by B6 mice under DID procedures was approximately 4.8 g/kg/2 hr. This amount of intake is predicted, based on earlier correlational analyses (Rhodes *et al.* 2005), to result in a mean BAC ~ 100 mg%. Thus, consistent with reports from other laboratories (e.g., Crabbe *et al.* 2009; Gupta *et al.* 2008; Moore and Boehm 2009; Rhodes *et al.* 2005), the level of alcohol consumption exhibited by our B6 mice under DID conditions meets the NIAAA criterion for binge drinking (NIAAA, 2004). As observed in B6 mice following a limited (6-bout) history of binge alcohol drinking under fluid-restricted conditions (Figure 2A), B6 mice with a month-long history of daily binge alcohol drinking in the absence of fluid-restriction also exhibited an approximately 50% increase in NAC Narp levels, which was significantly greater than water-drinking controls (Figure 2B; t_{22}=3.74, p=0.001). Moreover, the alcohol-induced rise in NAC Narp levels was accompanied by an approximately 35% rise in GluR1 levels (Figure 2C; t_{22}=2.47, p=0.02) and correlational analyses of this dataset revealed a significant positive correlation between these two protein measures (r^2=0.37, p=0.04; N=24). These data indicate that a history of binge alcohol drinking, in the absence of fluid restriction, also elevates NAC Narp levels and this effect is correlated with a rise in AMPA receptor expression.

Narp deletion promotes alcohol-conditioned aversion, without influencing motor behavior

To extend the role of alcohol-induced increases in Narp expression (Figures 1 and 2) to measures of alcohol reward and motor activity, we compared the ability of 8 pairings of 3 g/kg ethanol with a distinct environment to elicit a conditioned place-preference in WT and KO mice. As depicted in Figure 3A, repeated alcohol elicited a conditioned place-preference in WT animals, while KO mice exhibited a conditioned place-aversion [t_{17}=2.12, p=0.04]. While only the 3 g/kg alcohol dose was tested in the place-conditioning study, these place-preference data further the notion that Narp induction is necessary for the progressive increase in the rewarding properties of higher doses of alcohol that occurs with alcohol experience.

To test the hypothesis that the alcohol-avoiding phenotype of *Narp* KO mice might relate to greater sensitivity to the sedative/hypnotic properties of the drug, we compared alcohol-induced changes in motor behavior (assayed as both distance traveled in m and time spent immobile in sec) between WT and KO mice during the 15-min conditioning sessions. *Narp* deletion did not influence the acute motor-impairing effects of 3 g/kg alcohol nor did it affect the development of tolerance to these effects with repeated alcohol treatment (Table 1; all p's>0.20). These data indicate that Narp induction is not required for the manifestation of the acute sedative/hypnotic properties of alcohol nor for the neural plasticity that underlies the development of tolerance to these properties with repeated alcohol experience. Thus, it is not likely that the conditioned place-aversion exhibited by *Narp* KO mice following repeated alcohol experience reflects a greater sensitivity to the sedative-hypnotic properties of this drug.

As expected (Pacchioni *et al.* 2009), an examination for genotypic differences for measures of spontaneous motor behavior/anxiety failed to detect WT-KO differences for any variable tested, including, reactivity to initial placement in the novel place-conditioning apparatus and locomotor reactivity to acute or repeated saline injections (Table 1; all p's>0.05).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Despite not affecting measures of spontaneous or alcohol-induced motor behavior (Table 1), *Narp* deletion completely prevented the expression of hyper-locomotion exhibited during the post-conditioning test when animals were tested for a place-preference in a drug-free state (Figure 3B) [t_{17}=3.28, p=0.004]. Conversely, WT animals exhibited a marked reduction in the amount of time spent immobile from the pre- to the post-conditioning test, while the levels of immobility persisted in KO mice (Figure 3C) [t_{17}=2.48, p=0.02]. Together, these data indicate that Narp induction is not required to elicit locomotor hyperactivity in response to mild stressors (novel environment, acute saline injection) or to habituate to the repeated exposure to mild stressors (repeated saline injections). Rather, Narp induction may be important for the development of conditioned motor/emotional responses to drug-paired environments or for the behavioral manifestation of central nervous system hyper-excitability during alcohol withdrawal.

Narp deletion reduces total alcohol intake with repeated testing

To determine whether or not the alcohol-avoiding phenotype of *Narp* KO mice assessed using a place-conditioning procedure (Figure 3A) extended to an assay of alcohol intake, *Narp* KO and WT mice were then assessed for differences in a 4-bottle-choice, free-access, drinking paradigm (0-12% alcohol v/v). While *Narp* KO mice exhibited equivalent total alcohol intake as WT mice upon initial presentation (i.e. Day 1 of the study; see Figure 4A), alcohol intake by KO animals did not escalate upon repeated testing. Consequently, the alcohol intake of KO mice was significantly lower than that of their WT counterparts by the end of the 10-day testing period (Figure 4A) [Genotype X Day: F(9,153)=4.12, p<0.0001; LSD post-hoc tests]. Genotypic differences in total alcohol intake were not paralleled by any effects of gene deletion upon water intake (Figure 4B; all p's>0.05). Thus, the failure of KO mice to exhibit an escalation in their total fluid intake (Figure 4C) [F(9,153)=2.47, p=0.01] reflected genotypic differences in experience-dependent increases in alcohol, rather than water, intake. As observed for water intake (Figure 4B), genotypic differences were not observed regarding food intake (Figure 4D; all p's>0.05), nor were differences apparent for weight change between WT and KO mice across the 10 days of testing (WT: -1.1 ± 1.5 g vs. KO: 1.2 ± 2.3 g; p>0.05). These data indicate that Narp induction is necessary for the escalation of alcohol consumption under free-access conditions, but is not necessary for the normal regulation of food and water intake.

We next determined whether or not the genotypic differences in the escalation of total alcohol intake (Figure 4A) reflected shifts in the alcohol dose-intake function with alcohol experience. While *Narp* deletion did not influence the shape of the acute alcohol dose-intake function (Table 1) [Dose effect: F(3,51)=25.22, p<0.0001; Genotype effect & interaction, p's>0.4], repeated alcohol experience differentially shifted the dose-response function in WT versus KO mice [Genotype X Dose X Day: F(27,459)=2.13, p=0.001]. To determine at which alcohol concentrations genotypic differences were apparent, the significant 3-way interaction was deconstructed along the Dose factor. As presented above (Figure 4B), genotypic differences were not observed regarding the change in 0% alcohol/water intake with repeated testing. While the intake of 3% alcohol was lower overall in KO mice relative to WT animals, the intake of this concentration did not change in either genotype across the 10 days of drinking (Figure 5A) [Genotype effect: F(1,17)=12.12, p=0.003; no Day effect or interaction, p's>0.40]. Moreover, neither genotype nor subsequent alcohol exposure affected the intake of 6% alcohol (Figure 5A; all p's>0.40). However, consistent with the data for total alcohol intake (Figure 4A), WT and KO mice diverged in their intakes of 12% alcohol with repeated testing, with WT mice increasing and KO mice decreasing their intake of this concentration across test days (Figure 5A) [F(9,153)=3.25, p=0.001]. Thus, the genotypic differences in the experience-dependent escalation of total alcohol intake (Figure 4A) reflect

NIH-PA Author Manuscript

primarily effects of *Narp* deletion upon the consumption of the highest alcohol concentration tested and not some overall shift downward in the alcohol dose-intake function.

Narp deletion lowers preference for high alcohol concentrations

To further investigate the effects of *Narp* deletion upon the changes in alcohol sensitivity following repeated alcohol exposure, we also examined for genotypic differences in preference for the 4 different alcohol concentrations. While genotypic differences were not observed for either the initial (Day 1) or average (across the 10-day period) preference for the different alcohol concentrations (Table 1; no Genotype effects or interactions, $p>0.05$), we did observe a modest WT-KO difference regarding the shift in alcohol preference across the 10 days of drinking [Genotype X Dose X Day: $F(27,459)=1.59$, $p=0.05$]. Not surprisingly, deconstruction of the interaction along the Dose factor yielded findings that were more or less in parallel with the results for alcohol intake (Figure 5A), with KO mice exhibiting lower preference for 3% alcohol overall (see Table 2) [Genotype effect: $F(1,17)=20.54$, $p<0.0001$; no Day effect or interaction, p's>0.70] and no effects of either genotype or drinking day upon the preference for 6% alcohol (all p's>0.60). However, WT and KO mice both exhibited experience-dependent shifts in preference for 0% and 12% alcohol, but these shifts were in opposite directions [for 0%, Genotype X Day: $F(9,153)=2.35$, $p=0.02$; for 12%, interaction: $F(9,153)=2.37$, $p=0.02$]. As illustrated in Figure 5B, the preference for 0% alcohol declined across days in WT mice [Day effect: $F(9,90)=2.20$, $p=0.03$], concomitant with a rise in preference for 12% alcohol [$F(9,90)=2.55$, $p=0.009$]. While KO mice exhibited respectively a rise and drop in preference for the 0% and 12% alcohol concentrations, neither of these changes reached statistical significance (all p's>0.10), although the preference for both 0 and 12% alcohol on the last day of drinking was significantly different in KO mice versus their WT counterparts (post-hoc tests). These data indicate that *Narp* deletion prevents the shift in preference for higher alcohol concentrations observed in alcohol-experienced intact WT animals.

Basal NAC Narp/GluR1 expression is not a genetic correlate of binge alcohol drinking behavior

The immunoblotting data above indicates that Narp levels can be up-regulated within the NAC in mice with histories of repeated binge alcohol consumption (Figure 2) and the behavioral data provide support for an important role for Narp induction in regulating alcohol intake and preference, at least under free-access conditions (Figures 4 and 5). As we had available tissue from mice selectively bred for indices of elevated binge alcohol intake and reported divergence in the NAC expression of Group1 metabotropic glutamate receptors between selectively bred SHAC and SLAC mice (Cozzoli *et al.* 2009), we assayed whether or not basal Narp and GluR1 expression within the NAC may also be a genetic correlate of binge alcohol drinking behavior by examining for genotypic differences in protein expression between SHAC and SLAC and between HDID-1 and HS/Npt mice. As summarized in Table 2, no differences in either Narp or GluR1 expression were observed between SHAC and SLAC or between HDID-1 and HS/Npt lines (t-tests, $p>0.05$), although there was a strong trend ($p=0.06$) towards higher basal NAC GluR1 expression in HDID-1 versus HS/Npt mice. Despite the lack of genotypic differences in basal protein expression, significant correlations between Narp and GluR1 levels were observed in both studies (Table 2). These data indicate further a relationship between basal Narp and GluR1 levels *in vivo* but do not support basal NAC Narp or GluR1 expression as a genetic correlate of an excessive alcohol drinking phenotype.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Discussion

Summary of findings

Here, we show that while basal Narp and GluR1 expression is not a genetic correlate of a binge alcohol drinking phenotype, Narp levels are increased selectively within the NAC at 24 hrs following repeated injected or ingested alcohol regimens (Figures 1 and 2) and the alcohol-induced rise in NAC Narp co-occurs with increases in the GluR1 subunit of the AMPA receptor in at least one binge alcohol drinking model. Alcohol-experienced *Narp* KO mice exhibited a high-dose alcohol-avoiding phenotype when assessed by both alcohol drinking and alcohol place-conditioning (Figures 3-5) and this phenotype was not readily accounted for by KO-induced changes in sensitivity to the acute motor-impairing properties of alcohol or to the development of tolerance to alcohol-induced sedation with repeated alcohol administration (Table 1). These data provide novel evidence that the induction of Narp, and presumably subsequent effects upon AMPA receptors, is necessary for the expression of certain forms of alcohol-induced behavioral plasticity, particularly that relevant to the changes in the rewarding properties of alcohol with repeated experience.

Accumbens Narp induction by alcohol

Narp is an immediate early gene member of the Neuronal Pentraxin family that is secreted from presynaptic excitatory terminals upon physiological and supra-physiological neuronal activity, with elevated protein expression detected in brain for up to 48 hrs following cessation of electrical stimulation (Berke *et al.* 1998; Chang *et al.* 2010; Reti and Barban 2000; Tsui *et al.* 1996). Akin to earlier data for acute cocaine effects upon *Narp* mRNA within STR (Berke *et al.* 1998), an acute injection of an intoxicating dose of alcohol [peak BAC ~ 300 mg% at 5 min post-injection; see Szumlinski *et al.* (2005)] increased NAC Narp expression by greater than 300% when assayed 24 hrs later (Figure 1A). However, in contrast to the earlier cocaine study (Berke *et al.* 1998), acute alcohol did not influence STR Narp levels and our effects within NAC were relatively persistent (Figure 1B). Not withstanding a full time-course analysis of protein induction, the fact that NAC Narp levels are elevated at 24 hrs following a single bout of alcohol intoxication poses a potential role for elevated Narp in regulating NAC glutamate hyper-excitability during acute withdrawal.

Also distinct from earlier reports for other drugs of abuse (Lu *et al.* 2002; Reti *et al.* 2002, 2009), repeated alcohol injections sensitized the alcohol-induced rise in NAC Narp expression observed at 24 hrs following treatment (Figure 1A). This discrepancy across studies may simply relate to the very distinct pharmacology of alcohol, as compared to psychomotor stimulants, nicotine and THC, in that alcohol targets directly and inhibits AMPA receptor function at physiological concentrations (see Introduction), which may instigate a compensatory increase in Narp induction, concomitant with a rise in AMPA receptor levels. Indeed, the hyper-glutamatergia that is observed within mesocorticolimbic structures early (4-6 hrs) during withdrawal in alcohol-dependent animals has an AMPA component (e.g., Brückner *et al.* 1997; Chandler *et al.* 1999; Engberg and Hajos 1992; Haugbøl *et al.* 2005; Netzband *et al.* 1999a, 1999b; Ortiz *et al.* 1995) and is reported to persist, for up to 36 hrs following cessation of alcohol treatment (c.f., Gass and Olive 2008; Hoffman *et al.* 1992; Tsai and Coyle 1998). The presence of glutamatergic anomalies early in alcohol withdrawal contrasts with the time-course of those observed during withdrawal from many other drugs of abuse where glutamate anomalies develop over time, manifesting days or weeks into drug withdrawal (c.f., Gass and Olive 2008; Kalivas 2009; Szumlinski, Ary and Lominac 2008; Wolf and Ferrario 2010). If drug-induced increases in Narp expression contribute in some universal way to drug-induced AMPA receptor/glutamate hyper-sensitivity during abstinence, then the timing of Narp assay might be a critical

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

determinant of the extent to which different drugs of abuse influence the expression of this protein (Self et al. 2004).

Narp deletion produces a high-dose alcohol-avoiding phenotype independent of effects upon alcohol psychomotor reactivity

Narp KO animals exhibit a clear development of high-dose alcohol avoidance when assessed repeatedly for alcohol intake/preference under 24-hr free-access drinking conditions (Figures 4 and 5). This phenotype is not manifest early during alcohol drinking experience and is not paralleled by alterations in food or water intake (Table 1; Figure 4 and 5). Thus, it is unlikely that the alcohol-avoiding phenotype exhibited by alcohol-experienced *Narp* KO mice in the drinking study reflects an innate reactivity to alcohol taste/alcohol aversion, some primary deficiency that influences the capacity at, which animals consume alcohol (e.g., alterations in alcohol metabolism; motor ability, caloric need). Finally, the development of alcohol avoidance also does not appear to reflect genotypic differences in the acute sedative-hypnotic properties of high alcohol doses nor in the development of tolerance to these properties with alcohol experience as we observed no genotypic differences regarding either of these measures when assessed in a place-conditioning paradigm (Table 1). Unfortunately, alcohol metabolism was not directly assayed in WT and KO mice; however, these locomotor findings strongly argue against genotypic differences in alcohol metabolism or changes in alcohol metabolism with alcohol experience as a major contributor to the observed genotypic differences in our measures of alcohol reward. Thus, the emergent alcohol-avoiding phenotype of *Narp* KO mice as manifested under 24-hr access, 4 bottle-choice, conditions does not appear to reflect effects of *Narp* deletion upon a number of factors that critically influence an animal's propensity to drink alcohol.

The high-dose alcohol-avoiding phenotype exhibited by *Narp* KO mice in our assay of alcohol intake extended to a place-conditioning paradigm (Figure 3A). As orosensory cues are completely by-passed in this paradigm and animals are tested for place-conditioning in an alcohol-free state, furthering the notion that the alcohol-avoiding phenotype of alcohol-experienced *Narp* KO mice is unrelated to effects of gene deletion upon orosensory processing or the motor-impairing effects of alcohol. While somewhat consistent with observations that AMPA receptor antagonists can attenuate the rewarding properties of alcohol (Bäckstrom and Hyytiä 2004; Nguyen *et al.* 2007; Sanchis-Segura *et al.* 2006; Zhu *et al.* 2007), the alcohol-avoiding phenotype of alcohol-experienced *Narp* KO mice contrasts with the null alcohol reward phenotype of *GluR1* KO animals (Cowen *et al.* 2003; Sanchis-Segura *et al.* 2006; Stephens and Brown 1999), as well as with data from earlier studies of *Narp* KO mice employing cocaine and morphine place-conditioning paradigms (Crombag *et al.* 2009; Pacchioni *et al.* 2009). Clearly, the results of the place-conditioning studies to date, as well as additional data obtained using operant procedures (Johnson *et al.* 2007), do not support some general role for Narp induction, and presumably its subsequent effects upon AMPA receptors, in either the attribution of salience to reward-related cues/contexts or in the formation/recall of simple Pavlovian associations. Rather, the role for Narp induction in the manifestation of drug reward appears to be drug experience-dependent, which, as discussed above, might relate to distinctions in how different drugs of abuse influence glutamatergic transmission during short- and longer-term withdrawal.

Interestingly, the robust alcohol-conditioned place-aversion exhibited by the *Narp* KOs in the present study is reminiscent of the augmented conditioned place-aversion exhibited by morphine-dependent *Narp* KO mice upon precipitated opiate withdrawal (Reti *et al.* 2009). Not withstanding a full dose-response curve analysis and an explicit study of the effects of *Narp* deletion upon alcohol-induced withdrawal symptoms, the combination of results from the present place-conditioning study and those from Reti *et al.* (2009) pose a potential role also for Narp induction in regulating behavioral sensitivity to drug withdrawal. Arguably,

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

the reduced motor hyperactivity, concomitant with increased immobility, exhibited by alcohol-experienced *Narp* KOs during the alcohol-free conditioning test (Figure 5B and C) may be interpreted as reflecting a withdrawal-induced hyper-anxious state or alternatively, insensitivity to hyper-glutamatergia early during alcohol withdrawal. While the design of the present study cannot discern between these two interpretations, the later is consistent with reports that (1) AMPA receptor blockade attenuates alcohol withdrawal symptoms in both humans and laboratory animals (Krupitsky *et al.* 2007; Palachick *et al.* 2008) and (2) during withdrawal from repeated cocaine, *Narp* KO mice, but not WT mice, are less responsive to the psychomotor-activating effects of intra-NAC infusions of AMPA and exhibit a reduction in the postsynaptic density localization of AMPA receptors in the NAC (Pacchioni *et al.* 2009). Thus, *Narp* WT and KO mice do undergo differential AMPA receptor-related plasticity at least during cocaine withdrawal.

As the mice in the present study were tested for alcohol intake under 24-hr home-cage access conditions, one might argue that some interaction between Narp and alcohol withdrawal severity is not likely to account for the observed genotypic distinctions in alcohol intake/preference. However, even under 24-hr access conditions, mice typically consume the vast majority (> 80%) of their daily food and fluid intake (including alcohol when provided continuously) during the dark phase of their circadian cycle (e.g., Freund 1969; Goldstein and Kakihana 1977; Middaugh *et al.* 1999) and thus, undergo daily bouts of very low to no alcohol intake, particularly during the light phase of their circadian cycle. It follows then that *if Narp* KO mice are more sensitive to the aversive properties of alcohol withdrawal (as they are for opiate-withdrawal; Reti et al. 2009), then such a genotypic difference could certainly impinge upon subsequent alcohol drinking behavior, particularly that of higher alcohol concentrations.

NAC Narp expression is not a genetic correlate of binge alcohol drinking

An examination of basal Narp expression in SHAC versus SLAC or in HDID-1 versus HS/Npt mice failed to indicate genotypic differences (Table 2). Moreover, basal GluR1 expression also did not vary as a function of selective breeding for a binge alcohol drinking phenotype (Table 2). Narp is an inducible protein (Berke *et al.* 1998; Reti and Barban 2000; Tsui *et al.* 1996); thus, it is perhaps not surprising that basal levels did not co-vary with selective breeding for high versus low binge alcohol drinking. While the present findings do not support basal NAC Narp/GluR1 expression as a genetic correlate of a binge alcohol drinking phenotype, negative data in this regard do not necessarily preclude the possibilities that: (1) genetic vulnerability to this form of excessive drinking may relate to the capacity of binge alcohol drinking to induce Narp expression or to the duration of alcohol-induced Narp induction following a bout of binge alcohol intake; (2) Narp plays an important role in the manifestation of binge alcohol drinking; or (3) basal Narp expression in other brain structures may be a genetic correlate of binge alcohol drinking. Given the present indications of a critical role for alcohol-induced Narp expression within NAC in mediating alcoholism-relevant behavioral plasticity, future studies will likely focus on the regulation of Narp expression within the central nucleus of the amygdala as a hypothetical mediator of the aversive properties of this drug and its relation to alcohol intake under both continuous and limited access conditions.

Acknowledgments

Support: This work was funded by NIH grants AA016650 (INIA West) to KKS, AA013478 (INIA West) to DAF, AA13519 (INIA West) and AA10760 to JCC, NS039156 to PFW, and an NRSA fellowship to AWA. This work was also funded by grants from the Department of Veterans Affairs to DAF and JCC. The authors would like to acknowledge Dr. Desheng Xu (JHU) for generating the anti-Narp primary antibody employed in these studies and the technical assistance of Ms. Melissa Wroten (UCSB).

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

References

Bäckstrom P, Hyytiä P. Ionotropic glutamate receptor antagonists modulate cue-induced reinstatement of ethanol-seeking behavior. Alcohol. Clin. Exp. Res. 2004; 28:558–565. [PubMed: 15100606]

Berke JD, Paletzki RF, Aronson GJ, Hyman SE, Gerfen CR. A complex program of striatal gene expression induced by dopaminergic stimulation. J. Neurosci. 1998; 18:5301–5310. [PubMed: 9651213]

Broadbent J, Kampmueller KM, Koonse SA. Expression of behavioural sensitization to ethanol in DBA/2J mice: The role of NMDA and non-NMDA glutamate receptors. Psychopharmacology. 2003; 167:225–234. [PubMed: 12669179]

Brückner MK, Rossner S, Arendt T. Differential changes in the expression of AMPA receptors genes in rat brain after chronic exposure to ethanol: an in situ hybridization study. J. Hirnforsch. 1997; 38:369–376. [PubMed: 9350508]

Carpenter-Hyland EP, Chandler LJ. Adaptive plasticity of NMDA receptors and dendritic spines: implications for enhanced vulnerability of the adolescent brain to alcohol addiction. Pharmacol. Biochem. Behav. 2007; 86:200–208. [PubMed: 17291572]

Chandler LJ, Norwood D, Sutton G. Chronic ethanol upregulates NMDA and AMPA, but not kainate receptor subunit proteins in rat primary cortical cultures. Alcohol. Clin. Exp. Res. 1999; 23:363–370. [PubMed: 10069569]

Chang MC, Park JM, Pelkey KA, Grabenstatter HL, Xu D, Linden DJ, Sutula TP, McBain CJ, Worley PF. Narp regulates homeostatic scaling of excitatory synapses on parvalbumin-expressing interneurons. Nat. Neurosci. 2010; 13:1090–1097. [PubMed: 20729843]

Chen F, Jarrott B, Lawrence AJ. Up-regulation of cortical AMPA receptor binding in the fawn-hooded rat following ethanol withdrawal. Eur. J. Pharmacol. 1999; 384:139–146. [PubMed: 10611433]

Cowen MS, Schroff KC, Gass P, Sprengel R, Spanagel R. Neurobehavioral effects of alcohol in AMPA receptor subunit (GluR1) deficient mice. Neuropharmacology. 2003; 45:325–333. [PubMed: 12871650]

Cozzoli DK, Goulding SP, Zhang PW, Xiao B, Hu JH, Ary AW, Obara I, Rahn A, Abou-Ziab H, Tyrrel B, Marini C, Yoneyama N, Metten P, Snelling C, Dehoff MH, Crabbe JC, Finn DA, Klugmann M, Worley PF, Szumlinski KK. Binge drinking upregulates accumbens mGluR5-Homer2-PI3K signaling: functional implications for alcoholism. J. Neurosci. 2009; 29:8655–8668. [PubMed: 19587272]

Crabbe JC, Metten P, Rhodes JS, Yu CH, Brown LL, Phillips TJ, Finn DA. A line of mice selected for high blood ethanol concentrations shows drinking in the dark to intoxication. Biol. Psychiatry. 2009; 65:662–670. [PubMed: 19095222]

Crombag HS, Dickson M, Dinenna M, Johnson AW, Perin MS, Holland PC, Baraban JM, Reti IM. Narp deletion blocks extinction of morphine place preference conditioning. Neuropsychopharmacology. 2009; 34:857–866. [PubMed: 18536700]

Dildy-Mayfield JE, Harris RA. Comparison of ethanol sensitivity of rat brain kainite, DL-alpha-amino-3-hydroxy-5-methyl-4-isoxalone proprionic acid and N-methyl-Daspartate receptors expressed in Xenopus oocytes. J. Pharmacol. Exp. Ther. 1992; 262:487–494. [PubMed: 1380078]

Dodd PR, Beckmann AM, Davidson MS, Wilce PA. Glutamate-mediated transmission, alcohol, and alcoholism, Neurochem. Int. 2000; 37:509–533.

Engberg G, Hajos M. Alcohol withdrawal reaction as a result of adaptive changes of excitatory amino acid receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 1992; 346:437–441.

Everitt BJ, Robbins TW. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat. Neurosci. 2005; 8:1481–1489. [PubMed: 16251991]

Ferreira VM, Frausto S, Browning MD, Savage DD, Morato GS, Valenzuela CF. Ionotropic glutamate receptor subunit expression in the rat hippocampus: lack of an effect of a long-term ethanol exposure paradigm. Alcohol. Clin. Exp. Res. 2001; 25:1536–1541. [PubMed: 11696675]

Finn DA, Belknap JK, Cronise K, Yoneyama N, Murillo A, Crabbe JC. A procedure to produce high alcohol intake in mice. Psychopharmacology. 2005; 178:471–80. [PubMed: 15765261]

Freund G. Alcohol withdrawal syndrome in mice. Arch. Neurol. 1969; 21:315–320. [PubMed: 5816696]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Gass JT, Olive MF. Glutamatergic substrates of drug addiction and alcoholism. Biochem. Pharmacol. 2008; 75:218–265. [PubMed: 17706608]

Goldstein DB, Kakihana R. Circadian rhythms of ethanol consumption by mice: a simple computer analysis for chronopharmacology. Psychopharmacology (Berl). 1977; 52:41–45. [PubMed: 403556]

Goulding SP, Obara I, Lominac KD, Gould AT, Miller BW, Klugmann M, Szumlinski KK. Accumbens Homer2-mediated signaling: a factor contributing to mouse strain differences in alcohol drinking? Genes Brain Behav. 2011

Gupta T, Syed YM, Revis AA, Miller SA, Martinez M, Cohn KA, Demeyer MR, Patel KY, Brzezinska WJ, Rhodes JS. Acute effects of acamprosate and MPEP on ethanol Drinking-in-the-Dark in male C57BL/6J mice. Alcohol. Clin. Exp. Res. 2008; 32:1992–1998. [PubMed: 18782337]

Haugbøl SR, Ebert B, Ulrichsen J. Upregulation of glutamate receptor subtypes during alcohol withdrawal in rats. Alcohol Alcohol. 2005; 40:89–95. [PubMed: 15569719]

Hoffman PL, Grant KA, Snell LD, Reinlib L, Iorio K, Tabakoff B. NMDA receptors: role in ethanol withdrawal seizures. Ann. N. Y. Acad. Sci. 1992; 654:52–60. [PubMed: 1321581]

Hoffman PL, Rabe CS, Moses F, Tabakoff B. *N*-methyl-Daspartate receptors and ethanol: inhibition of calcium flux and cyclic GMP production. J. Neurochem. 1989; 52:1937–1940. [PubMed: 2542453]

Johnson AW, Crombag HS, Takamiya K, Baraban JM, Holland PC, Huganir RL, Reti IM. A selective role for neuronal activity regulated pentraxin in the processing of sensory-specific incentive value. J. Neurosci. 2007; 27:13430–13435. [PubMed: 18057201]

Jones N, Messenger MJ, O'Neill MJ, Oldershaw A, Gilmour G, Simmons RM, Iyengar S, Libri V, Tricklebank M, Williams SC. AMPA receptor potentiation can prevent ethanol-induced intoxication. Neuropsychopharmacology. 2008; 33:1713–1723. [PubMed: 17851540]

Kalivas PW. The glutamate homeostasis hypothesis of addiction. Nat. Rev. Neurosci. 2009; 10:561–572. [PubMed: 19571793]

Kapasova Z, Szumlinski KK. Strain differences in alcohol-induced neurochemical plasticity: a role for accumbens glutamate in alcohol intake. Alcohol. Clin. Exp. Res. 2008; 32:617–31. [PubMed: 18341649]

Kirkpatrick LL, Matzuk MM, Dodds DC, Perin MD. Biochemical interactions of the neuronal pentraxins: neuronal pentraxin (NP) receptor binds to taipoxin and taipoxin-associated calcium-binding protein 49 via NP1 and NP2. J. Biol. Chem. 2000; 275:17786–17792. [PubMed: 10748068]

Krupitsky EM, Rudenko AA, Burakov AM, Slavina TY, Grinenko AA, Pittman B, Gueorguieva R, Petrakis IL, Zvartau EE, Krystal JH. Antiglutamatergic strategies for ethanol detoxification: comparison with placebo and diazepam. Alcohol. Clin. Exp. Res. 2007; 31:604–611. [PubMed: 17374039]

Läck AK, Diaz MR, Chappell A, DuBois DW, McCool BA. Chronic ethanol and withdrawal differentially modulate pre- and postsynaptic function at glutamatergic synapses in rat basolateral amygdala. J. Neurophysiol. 2007; 98:3185–3196. [PubMed: 17898152]

Lominac KD, Kapasova Z, Hannun RA, Patterson C, Middaugh LD, Szumlinski KK. Behavioral and neurochemical interactions between Group 1 mGluR antagonists and ethanol: potential insight into their anti-addictive properties. Drug Alcohol Depend. 2006; 85:142–156. [PubMed: 16697125]

Long C, Yang L, Faingold CL, Evans M. Steven. Excitatory amino acid receptor-mediated responses in periaqueductal gray neurons are increased during ethanol withdrawal. Neuropharmacology. 2007; 52:802–811. [PubMed: 17123553]

Lovinger DM. Interactions between ethanol and agents that act on the NMDA-type glutamate receptor. Alcohol. Clin. Exp. Res. 1996; 20(Suppl):187A–191A.

Lovinger DM, White G, Weight FF. Ethanol inhibits NMDA-activated ion current in hippocampal neurons. Science. 1989; 243:1721–1724. [PubMed: 2467382]

Lu W, Marinelli M, Xu D, Worley PF, Wolf ME. Amphetamine and cocaine do not increase Narp expression in rat ventral tegmental area, nucleus accumbens or prefrontal cortex, but Narp may

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

contribute to individual differences in responding to a novel environment. Eur. J. Neurosci. 2002; 15:2027–2036. [PubMed: 12099908]

Martin D, Tayyeb MI, Swartzwelder HS. Ethanol inhibition of AMPA and kainate receptor-mediated depolarizations of hippocampal area CA1. Alcohol. Clin. Exp. Res. 1995; 19:1312–1316. [PubMed: 8561307]

Middaugh LD, Kelley BM, Bandy AL, McGroarty KK. Ethanol consumption by C57BL/6 mice: influence of gender and procedural variables. Alcohol. 1999; 17:175–183. [PubMed: 10231165]

Minami K, Gereau RW 4th, Minami M, Heinemann SF, Harris RA. Effects of ethanol and anesthetics on type 1 and 5 metabotropic glutamate receptors expressed in Xenopus oocytes. Mol. Pharmacol. 1998; 53:148–156. [PubMed: 9443943]

Moore EM, Boehm SL 2nd. Site-specific microinjection of baclofen into the anterior ventral tegmental area reduces binge-like ethanol intake in male C57BL/6J mice. Behav. Neurosci. 2009; 123:555–563. [PubMed: 19485562]

Möykkynen TP, Coleman SK, Keinänen K, Lovinger DM, Korpi ER. Ethanol increases desensitization of recombinant GluR-D AMPA receptor and TARP combinations. Alcohol. 2009; 43(4):277–284. [PubMed: 19560629]

Möykkynen TP, Korpi ER, Lovinger DM. Ethanol inhibits alpha-amino-3-hydyroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor function in central nervous system neurons by stabilizing desensitization. J. Pharmacol. Exp. Ther. 2003; 306:546–555. [PubMed: 12734392]

National Institute on Alcohol Abuse and Alcoholism. NIAAA Newsletter. Vol. Vol. 3. National Institute on Alcohol Abuse and Alcoholism; Bethesda, MD: 2004.

Netzeband JG, Trotter C, Caguioa JN, Gruol DL. Chronic ethanol exposure enhances AMPA-elicited Ca2+ signals in the somatic and dendritic regions of cerebellar Purkinje neurons. Neurochem. Int. 1999a; 35:163–174. [PubMed: 10406000]

Netzeband JG, Trotter C, Parsons KL, Gruol DL. Chronic ethanol treatment alters AMPA-induced calcium signals in developing Purkinje neurons. Brain Res. 1999b; 826:270–280. [PubMed: 10224304]

Nguyen SA, Malcolm R, Middaugh LD. Topiramate reduces ethanol consumption by C57BL/6 mice. Synapse. 2007; 61:150–156. [PubMed: 17146766]

Nie Z, Madamba SG, Siggins GR. Ethanol inhibits glutamatergic neurotransmission in nucleus accumbens neurons by multiple mechanisms. J. Pharmacol. Exp. Ther. 1994; 271:1566–1573. [PubMed: 7527857]

O'Brien R, Xu D, Mi R, Tang X, Hopf C, Worley P. Synaptically targeted narp plays an essential role in the aggregation of AMPA receptors at excitatory synapses in cultured spinal neurons. J. Neurosci. 2002; 22:4487–4498. [PubMed: 12040056]

O'Brien RJ, Xu D, Petralia RS, Steward O, Huganir RL, Worley P. Synaptic clustering of AMPA receptors by the extracellular immediate-early gene product Narp. Neuron. 1999; 23:309–323. [PubMed: 10399937]

Ortiz J, Fitzgerald LW, Charlton M, Lane S, Trevisan L, Guitart X, Shoemaker W, Duman RS, Nestler EJ. Biochemical actions of chronic ethanol exposure in the mesolimbic dopamine system. Synapse. 1995; 21:289–298. [PubMed: 8869159]

Pacchioni AM, Vallone J, Worley PF, Kalivas PW. Neuronal pentraxins modulate cocaine-induced neuroadaptations. J. Pharmacol. Exp. Ther. 2009; 328:183–192. [PubMed: 18840757]

Palachick B, Chen YC, Enoch AJ, Karlsson RM, Mishina M, Holmes A. Role of major NMDA or AMPA receptor subunits in MK-801 potentiation of ethanol intoxication. Alcohol. Clin. Exp. Res. 2008; 32:1479–1492. [PubMed: 18565157]

Pickering C, Avesson L, Lindblom J, Liljequist S, Schiöth HB. Identification of neurotransmitter receptor genes involved in alcohol self-administration in the rat prefrontal cortex, hippocampus and amygdala. Prog. Neuro-Psychopharmacol. Biol. Psychiat. 2007; 31:53–64.

Reti IM, Baraban JM. Sustained increase in Narp protein expression following repeated electroconvulsive seizure. Neuropsychopharmacology. 2000; 23:439–443. [PubMed: 10989271]

Reti IM, Crombag HS, Takamiya K, Sutton JM, Guo N, Dinenna ML, Huganir RL, Holland PC, Baraban JM. Narp regulates long-term aversive effects of morphine withdrawal. Behav. Neurosci. 2008; 122:760–768. [PubMed: 18729628]

Reti IM, Han S, Miskimon M, Rosen JB, Baraban JM. Nicotine and Delta(9)-tetrahydrocannabinol withdrawal induce Narp in the central nucleus of the amygdala. Synapse. 2009; 63:252–255. [PubMed: 19084905]

Reti IM, Reddy R, Worley PF, Baraban JM. Prominent Narp expression in projection pathways and terminal fields. J. Neurochem. 2002; 82:935–944. [PubMed: 12358799]

Rhodes JS, Best K, Belknap JK, Finn DA, Crabbe JC. Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. Physiol. Behav. 2005; 84:53–63. [PubMed: 15642607]

Rial D, Takahashi RN, Morato GS. Aniracetam and DNQX affect the acquisition of rapid tolerance to ethanol in mice. Pharmacol. Biochem. Behav. 2008; 92:32–38. [PubMed: 18992274]

Sanchis-Segura C, Borchardt T, Vengeliene V, Zghoul T, Bachteler D, Gass P, Sprengel R, Spanagel R. Involvement of the AMPA receptor GluR-C subunit in alcohol-seeking behavior and relapse. J. Neurosci. 2006; 26:1231–1238. [PubMed: 16436610]

Sanderson JL, Dell'Acqua ML. AKAP signaling complexes in regulation of excitatory synaptic plasticity. Neuroscientist. 2011; 17:321–336. [PubMed: 21498812]

Self DW, Choi KH, Simmons D, Walker JR, Smagula CS. Extinction training regulates neuroadaptive responses to withdrawal from chronic cocaine self-administration. Learn. Mem. 2004; 11:648–657. [PubMed: 15466321]

Stephens DN, Brown G. Disruption of operant oral self-administration of ethanol, sucrose, and saccharin by the AMPA/kainate antagonist, NBQX, but not the AMPA antagonist, GYKI 52466. Alcohol. Clin. Exp. Res. 1999; 23:1914–1920. [PubMed: 10630610]

Stuber GD, Hopf FW, Hahn J, Cho SL, Guillory A, Bonci A. Voluntary ethanol intake enhances excitatory synaptic strength in the ventral tegmental area. Alcohol. Clin. Exp. Res. 2008; 32:1714–1720. [PubMed: 18627359]

Substance Abuse and Mental Health Services Administration. The National Survey on Drug Use and Health Report: Ethanol Dependence or Abuse: 2002, 2003, and 2004. 2006. http://www.oas.samhsa.gov/2k6/AlcDepend/AlcDepend.htm

Szumlinski KK, Ary AW, Lominac KD. Homers regulate drug-induced neuroplasticity: implications for addiction. Biochem. Pharmacol. 2008; 75:112–133. [PubMed: 17765204]

Szumlinski KK, Ary AW, Lominac KD, Klugmann M, Kippin TE. Accumbens Homer2 overexpression facilitates alcohol-induced neuroplasticity in C57BL/6J mice. Neuropsychopharmacology. 2008; 33:1365–1378. [PubMed: 17568396]

Szumlinski KK, Diab ME, Friedman R, Henze LM, Lominac KD, Bowers MS. Accumbens neurochemical adaptations produced by binge-like alcohol consumption. Psychopharmacology. 2007; 190:415–431. [PubMed: 17225170]

Szumlinski KK, Lominac KD, Oleson EB, Walker JK, Mason A, Dehoff MH, Klugmann M, Cagle S, Welt K, During M, Worley PF, Middaugh LD, Kalivas PW. Homer2 is necessary for EtOH-induced neuroplasticity. J. Neurosci. 2005; 25:7054–7061. [PubMed: 16049182]

Tsai G, Coyle JT. The role of glutamatergic neurotransmission in the pathophysiology of alcoholism. Annu. Rev. Med. 1998; 49:173–184. [PubMed: 9509257]

Tsui CC, Copeland NG, Gilbert DJ, Jenkins NA, Barnes C, Worley PF. Narp, a novel member of the pentraxin family, promotes neurite outgrowth and is dynamically regulated by neuronal activity. J. Neurosci. 1996; 16:2463–2478. [PubMed: 8786423]

Wanat MJ, Sparta DR, Hopf FW, Bowers MS, Melis M, Bonci A. Strain specific synaptic modifications on ventral tegmental area dopamine neurons after ethanol exposure. Biol. Psychiatry. 2009; 65:646–653. [PubMed: 19118821]

Weiner JL, Dunwiddie TV, Valenzuela CF. Ethanol inhibition of synaptically evoked kainate responses in rat hippocampal CA3 pyramidal neurons. Mol. Pharmacol. 1999; 56:85–90. [PubMed: 10385687]

Wirkner K, Eberts C, Poelchen W, Allgaier C, Illes P. Mechanism of inhibition by ethanol of NMDA and AMPA receptor channel functions in cultured rat cortical neurons. Naunyn Schmiedebergs Arch. Pharmacol. 2000; 362:568–576. [PubMed: 11138850]

Wolf ME, Ferrario CR. AMPA receptor plasticity in the nucleus accumbens after repeated exposure to cocaine. Neurosci. Biobehav. Rev. 2010; 35:185–211. [PubMed: 20109488]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Xu D, Hopf C, Reddy R, Cho RW, Guo L, Lanahan A, Petralia RS, Wenthold RJ, O'Brien RJ, Worley P. Narp and NP1 form heterocomplexes that function in developmental and activity-dependent synaptic plasticity. Neuron. 2003; 39:513–528. [PubMed: 12895424]

Zhu W, Bie B, Pan ZZ. Involvement of non-NMDA glutamate receptors in central amygdala in synaptic actions of ethanol and ethanol-induced reward behavior. J. Neurosci. 2007; 27:289–298. [PubMed: 17215388]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

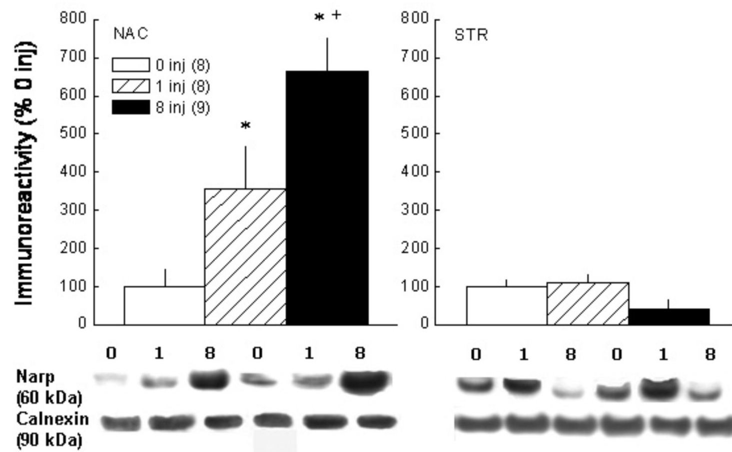

Figure 1. Repeated alcohol injections sensitize NAC Narp expression

When compared to mice injected repeatedly with saline (0 inj), the acute administration of 3 g/kg alcohol (1 inj) elevated the total protein expression of Narp within the nucleus accumbens (NAC; left), but not in the more dorsal aspect of the striatum (STR;right). The alcohol-induced rise in NAC Narp levels was sensitized in animals treated repeatedly with 3 g/kg alcohol (8 inj). The data are expressed as a percent saline control tissue (0 inj) and represent the mean ± SEM of the number of animals indicated in parentheses. *$p<0.05$ vs. 0 inj; + indicates $p<0.05$ as compared to 1 inj.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

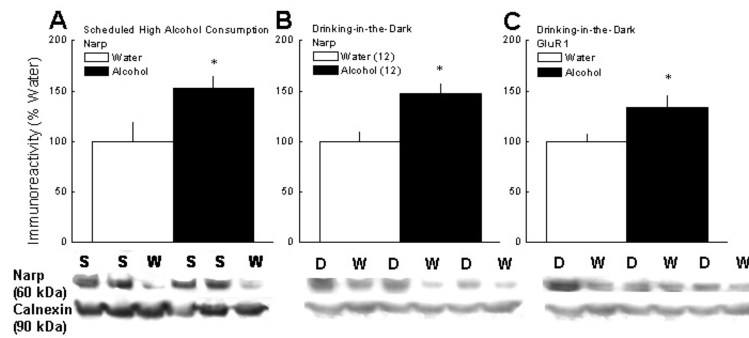

Figure 2. Binge alcohol drinking up-regulates NAC Narp expression

When assessed 24 hrs following the last binge alcohol drinking session under 6 bouts of Scheduled High Alcohol Consumption procedures (A) or 30 days of Drinking-in-the-Dark procedures (B), alcohol drinking mice (Alcohol) exhibited elevations in NAC Narp expression, compared to water drinking controls (Water). (C) Animals consuming alcohol under Drinking-in-the-Dark procedures also exhibited a rise in NAC levels of the GluR1 subunit of the AMPA receptor. The data are expressed as a percent of water controls and represent the mean ± SEM of the number of animals indicated in parentheses. *$p<0.05$ vs. Water.

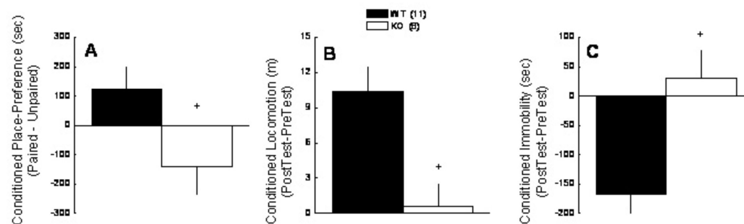

Figure 3. Narp deletion promotes a conditioned place-aversion and reduces motor hyperactivity during withdrawal

When assayed in a place-conditioning paradigm (8 pairings of 3 g/kg alcohol, IP), KO mice exhibited a conditioned place-aversion when tested in an alcohol-free state, while WT mice exhibited a significant place-preference (A). When the locomotor activity exhibited by WT and KO mice under alcohol-free conditions was compared between the 15-min pre- and post-conditioning tests, KO mice exhibited very little change in locomotor activity, while WT mice exhibited elevated locomotion on the post-test (B). Conversely, WT mice spent significantly less time being immobile (no movement detected for 15 sec) on the post-versus pre-conditioning test, while an increase in immobility was observed in KO animals (C). Data present the mean ± SEM of the number of animals indicated in parentheses. $+p<0.05$ vs. WT.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

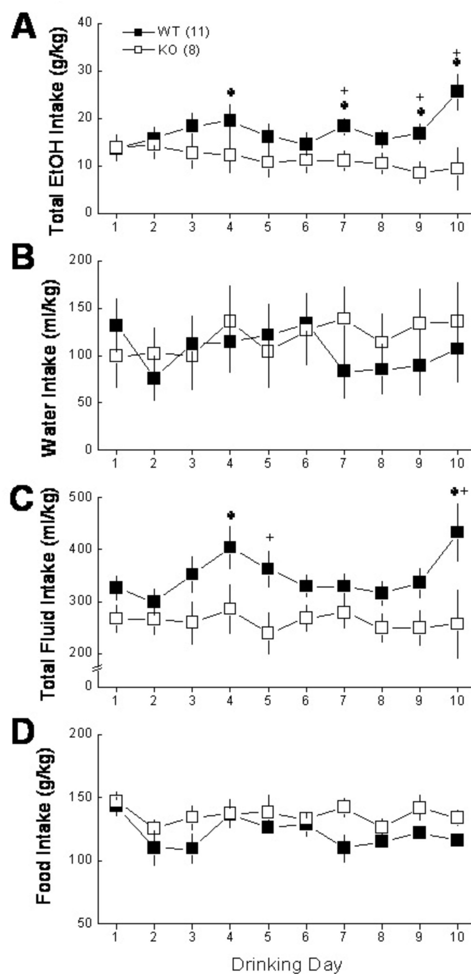

Figure 4. Narp deletion prevents the escalation of total alcohol intake across subsequent drinking sessions

When assessed in a 24-hr 4-bottle-choice procedure (0, 3, 6, and 12% alcohol v/v), *Narp* KO mice failed to exhibit a rise in total daily alcohol intake (A) with subsequent experience, while daily water intake was similar to that of WT mice (B). Consequently, genotypic differences were observed for total daily fluid intake (C). No genotypic differences in daily food intake were noted (D). The data represent the means ± SEMs of the number of animals indicated in parentheses in panel A. *p<0.05 vs. Day 1; +p<0.05 vs. WT.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

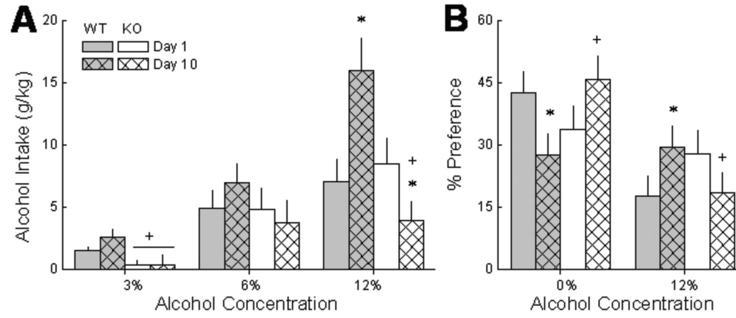

Figure 5. Narp deletion shifts alcohol intake and preference away from high alcohol concentrations

An examination of the change in the dose-response functions for alcohol intake (A) assessed under 24-hr 4-bottle-choice procedures (0, 3, 6 and 12% alcohol v/v) observed across subsequent drinking days revealed lower intake of 3% alcohol overall, no genotypic differences in the intake of 6% alcohol and opposite effects of *Narp* deletion upon the intake of 12% alcohol, with WT and KO mice exhibiting respectively an experience-dependent increase and decrease in the consumption of this concentration. When preference for each concentration was examined (B), WT mice exhibited a shift from 0% to 12% alcohol from Day 1 (open bars) to Day 10 (grey bars) of alcohol drinking experience, while KO mice exhibited an opposite shift in preference. Data present the means ± SEMs of the number of animals indicated in panel A. *p<0.05 vs. Day 1; +p<0.05 vs. WT.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table 1

List of variables for which statistical comparisons of the means ± SEMs obtained from *Narp* WT (n=11) and KO (n=8) mice failed to indicate significant genotypic differences in behavior (i.e., Genotype effects or interactions, p>0.05).

Variable	WT	KO
Day 1 Alcohol Dose-Intake Function (g/kg)		
0%	131.8 ± 28.1	99.4 ± 33.0
3%	1.57 ± 0.3	0.42 ± 0.4
6%	5.0 ± 1.4	4.9 ± 1.6
12%	7.1 ±1.7	8.6 ± 2.0
Day 1 Alcohol Preference-Intake Function (% total fluid intake)		
0%	42.6 ± 5.1%	33.9 ± 5.7%
3%	15.4 ± 2.3%	5.2 ± 2.7%
6%	24.2 ± 6.9%	32.8 ± 8.1%
12%	17.8 ± 4.8%	28.1 ± 5.6%
Mean Alcohol Preference-Intake Function Across Drinking Days (% total fluid intake)		
0%	32.9 ± 8.4%	39.9 ± 9.8%
3%	17.7 ± 1.8%	5.4 ± 2.1%
6%	26.1 ± 6.5%	30.94 ± 7.6%
12%	23.6 ± 4.1%	23.7 ± 4.8%
Spontaneous Motor Behavior		
Novel Environment: Distance (m)	19.2 ± 2.0	17.3 ± 2.0
Novel Environment: Immobility (sec)	17.2 ± 2.7	15.1 ± 5.8
PreTest: Distance (m)	16.5 ± 1.7	11.8 ± 2.4
PreTest: Immobility (sec)	296.4 ± 39.5	348.9 ± 78.1
Saline Injection 1: Distance (m)	8.1 ± 1.1	6.1 ± 1.2
Δ Locomotion across saline inj's (m)	−3.1 ± 1.7	−4.0 ± 1.0
Alcohol-induced Motor Behavior Injection 1: Distance (m)	3.5 ± 1.3	5.6 ± 1.3
Injection 1: Immobility (sec)	787.8 ± 41.0	679.6 ± 63.05
Δ Locomotion across alcohol inj's (m)	9.0 ± 2.7	7.9 ± 3.9
Δ Immobility across alcohol inj's (sec)	−291.5 ± 94.7	−269.7 ± 118.3

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 2

Results of immunoblotting for Narp and GluR1 within the NAC of mouse lines selectively bred for high versus low BACs under Scheduled High Alcohol Consumption procedures (SHAC versus SLAC, respectively) or a line of mice selectively bred for high BACs under Drinking-in-the-Dark procedures (HDID-1) versus mice from their HS/Npt founder population. While genotypic differences in protein expression were not observed for either study (t-tests, $p>0.05$), significant correlations between the basal levels of Narp and GluR1 were observed (* indicates $p<0.05$). The data are presented as the means ± SEM and the sample sizes employed in the statistical analyses of the data are indicated in parentheses.

	SLAC (7)	SHAC (6)	HS/Npt (14)	HDID-1 (13)
Narp	100 ± 7.7	94.8 ± 15.22	100 ± 13.5	75.09 ± 11.3
GluR1	100 ± 13.6	99.1 ± 10.6	100 ± 10.5	145.5 ± 23.5
R^2	0.53*		0.32*	