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Homer2 within the nucleus accumbens core bidirectionally regulates alcohol intake by both P and Wistar rats

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ABSTRACT

In murine models of alcoholism, the glutamate receptor scaffolding protein Homer2 bidirectionally regulates alcohol intake. Although chronic alcohol drinking increases Homer2 expression within the core subregion of the nucleus accumbens (NAc) of alcohol-preferring P rats, the relevance of this neuroadaptation for alcohol intake has yet to be determined in rats. Thus, the present study employed an adeno-associated viral vector (AAV) strategy to over-express and knock down the major rodent isoform Homer2b within the NAc of both P and outbred Wistar rats to examine for changes in alcohol preference and intake (0–30% v/v) under continuous-access procedures. The generalization of AAV effects to non-drug, palatable, sweet solutions was also determined in tests of sucrose (0–5% w/v) and saccharin (0–0.125% w/v) intake/preference. No net-flux *in vivo* microdialysis was conducted for glutamate in the NAc to relate Homer2-dependent changes in alcohol intake to extracellular levels of glutamate. Line differences were noted for sweet solution preference and intake, but these variables were not affected by intra-NAc AAV infusion in either line. In contrast, Homer2b over-expression elevated, while Homer2b knock-down reduced, alcohol intake in both lines, and this effect was greatest at the highest concentration. Strikingly, in P rats there was a direct association between changes in Homer2b expression and NAc extracellular glutamate levels, but this effect was not seen in Wistar rats. These data indicate that NAc Homer2b expression actively regulates alcohol consumption by rats, paralleling this previous observation in mice. Overall, these findings underscore the importance of mesocorticolimbic glutamate activity in alcohol abuse/dependence and suggest that Homer2b and/or its constituents may serve as molecular targets for the treatment of these disorders.

Introduction

The Homer family of post-synaptic scaffolding proteins are encoded by 3 genes (*Homer1, 2, 3*), which give rise to both constitutively expressed and activity-induced genes products (c.f., Shiraishi-Yamaguchi & Furuichi, 2007). All *Homer* gene products contain an Ena/VASP-1 homology domain, which recognizes a proline-rich sequence found on a number of different proteins within the postsynaptic density, including the Group1 metabotropic glutamate receptors (mGluRs), Shank, the IP3 receptor, dynamin-3, Debrin/F-actin, and diacylglycerol lipase-α (c.f., Szumlinski, Ary, & Lominac, 2008; see also Jung et al., 2007; Shiraishi-Yamaguchi et al., 2009). Constitutively expressed *Homer* gene products also contain a coiled-coil domain that enables their multimerization. The capacity to multimerize is critical for the ability of Homer proteins to regulate the functional architecture of excitatory synapses. To this end, constitutively expressed Homer proteins play active roles in regulating dendritic morphology, intracellular signaling through both Group1 mGluRs and NMDA receptors, the integration of intracellular calcium signals, extracellular glutamate, and stimulated glutamate release, as well as the generation of anadamide (e.g., Iasevoli, Tomasetti, & de Bartolomeis, 2013; Jung et al., 2007; Shiraishi-Yamaguchi & Furuichi, 2007; Szumlinski, Ary, & Lominac, 2008).

Alcohol influences both pre- and postsynaptic aspects of glutamate transmission within mesocorticolimbic brain structures implicated in drug reward/reinforcement (c.f., Gass & Olive, 2008), including elevating Homer protein expression (c.f., Cui et al., 2013; Szumlinski, Ary, & Lominac, 2008). Interestingly, the effect of

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voluntary alcohol intake upon Homer protein expression within such structures as the nucleus accumbens (NAc) or the central nucleus of the amygdala (CeA) appears to be selective for the constitutively expressed *Homer2* gene product Homer2a/b, with increases in protein detected during both early and protracted withdrawal in both rats (Obara et al., 2009) and mice (e.g., Cozzoli et al., 2009; Cozzoli et al., 2012, 2014; Szumlinski, Ary, Lominac, Klugmann, & Kippin, 2008). Notably, alcohol-induced increases in mesolimbic Homer2a/b expression likely reflect a pharmacodynamic response to alcohol, because they are observed also upon systemic alcohol injections in inbred mice exhibiting either high or low alcohol-drinking phenotypes (Goulding et al., 2011). Given the role for Homer proteins in regulating synaptic and neurochemical plasticity at glutamatergic synapses (e.g., Hu et al., 2010; Shiraishi-Yamaguchi & Furuichi, 2007; Szumlinski, Ary, & Lominac, 2008), alcohol-induced or idiopathic increases in Homer2a/b expression are theorized to contribute to a hyper-glutamatergic state that promotes alcohol intake (e.g., Cui et al., 2013; Szumlinski, Ary, & Lominac, 2008).

Although withdrawal from voluntary alcohol intake elevates Homer2a/b in the NAc of both rats and mice, the relevance of this neuroadaptation for alcohol-induced changes in brain and behavior has been explored only in the mouse (c.f., Cui et al., 2013; Szumlinski, Ary, & Lominac, 2008). Homer2b is the major Homer2 isoform in rodents (Soloviev, Ciruela, Chan, & McIlhinney, 2000). In the NAc of mice, virus-mediated *Homer2b* gene transfer and knockdown promotes and reduces, respectively, alcohol drinking (Cozzoli et al., 2009, 2012; Szumlinski, Ary, & Lominac, 2008; Szumlinski et al., 2005). Thus, the first goal of this study was to determine the functional relevance of alcohol-induced increases in Homer2b within the core subregion of the NAc (NAcC) reported previously for selectively bred, alcohol-preferring P rats (Obara et al., 2009), to compare with earlier data derived exclusively from mice. Secondly, as the effects of virus-mediated changes in NAc Homer2b expression upon alcohol intake appear to be strain-independent in mice (Goulding et al., 2011), we also determined whether or not the effects of manipulating NAc Homer2b expression in P rats might extend to outbred Wistar rats as well. Finally, to explore the potential relationship between individual differences in basal extracellular glutamate and subsequent alcohol-drinking behavior, we examined the effects of transgenically manipulating Homer2b expression on extracellular glutamate content within the NAcC using no net-flux *in vivo* microdialysis approaches.

Materials and methods

Subjects

Male, selectively bred alcohol-preferring P rats ($n = 40$) were bred in-house at Indiana University School of Medicine (Indianapolis, IN) and shipped to the University of California at Santa Barbara (UCSB), weighing approximately 200 g. P rats were housed in pairs within a quarantine facility in the UCSB main campus vivarium for 6 weeks, at which time they were transported to the vivarium within the Department of Psychological and Brain Sciences and housed singly for 1 week prior to surgery. Male Wistar rats ($n = 30$), weighing 250–275 g, were purchased from Harlan Laboratories (Livermore, CA, USA) and housed singly within the department vivarium upon arrival for a minimum of 1 week prior to surgery. The vivarium room was maintained on a 12-h/12-h light/dark cycle (lights off at 7:00 AM) in a facility fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). Food and water were available in the home cage *ad libitum* throughout the experiment. All research protocols were approved by the Institutional Animal Care and Use Committees of both the Indiana University School of Medicine and the University of California at Santa Barbara and were in accordance with the guidelines of the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (8th edition, 2011).

Craniotomy and AAV infusion

Using 1–2% isoflurane anesthesia, microdialysis guide cannulae (20 gauge, 20 mm, Plastics One, Roanoke, VA) were implanted 3 mm above the NAcC using the following coordinates (AP: +1.1 mm; ML: ±20.7 mm; DV: −4.5 mm; 6° angle from vertical) (Paxinos & Watson, 2006). The AP and ML coordinates are relative to bregma and all DV coordinates are relative to the surface of the skull. The guide cannulae were fixed to the skull with 4 stainless-steel skull screws (Small Parts, Roanoke VA) and dental acrylic (i.e., Zayara et al., 2011). Following air curing of the dental acrylic, microinjectors (33 gauge, 23 mm in length, fitted with a 26-gauge adapter for stability) were inserted bilaterally into the guide cannulae. Titer-matched AAV vectors (1×10^{12} vector genomes/mL) carrying cDNA for Homer2b, a small hairpin RNA (shRNA) against Homer2b or a scrambled, nonsense control carrying a cassette encoding green fluorescent protein (GFP) (see Klugmann et al., 2005; Klugmann & Szumlinski, 2008 for specific details) were infused at a rate of 0.05 μL/min for 5 min (total volume = 0.25 μL/side). To generate the cDNA-Homer2b AAV, the PCR product for Homer2b was expressed as an N-terminal fusion protein with a hemagglutinin (HA)-tag in an rAAV backbone containing the 1.1-kb CMV enhancer/chicken-actin (CBA) promoter, 800-bp human interferon scaffold attachment region inserted 5′ of the promoter, the woodchuck post-transcriptional regulatory element (WPRE), and the bovine growth hormone polyA flanked by inverted terminal repeats. AAV pseudotyped vectors (virions containing a 1:1 ratio of AAV1 and AAV2 capsid proteins with AAV2 intertrigeminal regions) were generated. For shRNA-Homer2b, the reporter gene encoding green fluorescent protein (GFP), and the mouse U6 RNA polymerase III (U6) promoter upstream of the CBA promoter was added to our standard expression cassette, allowing for insertion of short hairpin RNA (shRNA) cassettes (see Klugmann & Szumlinski, 2008 for a schematic of these cassettes). The same AAV-CBA-WPRE-bGH backbone was used also in the generation of the GFP-AAV control vector. Following infusion, the microinjectors were left in place for an additional 5 min and then slowly removed. Dummy cannulae (26 gauge, 20 mm in length; Plastics One) were inserted into the guide cannulae to prevent externalization. The rats remained undisturbed (with the exception of post-operative monitoring and routine cage changing) for a period of 3 weeks to allow for maximal AAV transduction prior to testing for alcohol intake, as conducted in previous studies of both rats and mice (e.g., Cozzoli et al., 2009, 2012; Goulding et al., 2011; Klugmann et al., 2005; Szumlinski et al., 2006; Szumlinski, Ary, & Lominac, 2008).

Quantification of basal extracellular glutamate levels

To determine whether or not line differences exist with respect to AAV-mediated changes in extracellular glutamate within the NAcC, we employed a no net-flux *in vivo* microdialysis approach to quantify extracellular glutamate levels in Wistar and P rats infused with AAV-GFP, -cDNA or -shRNA. This session was conducted at a minimum of 3 weeks following AAV infusion and thus, occurred at a time when maximal AAV transduction is typically observed (e.g., Klugmann & Szumlinski, 2008). The microdialysis procedures were identical to those described previously by our group (e.g., Ben-Shahar et al., 2012; Goulding et al., 2011; Szumlinski et al., 2006) and involved the reverse dialysis of increasing concentrations of glutamate (0, 2.5, 5, and 10 μM; 1 h each), and probe removal upon

the completion of testing. Samples were subjected to high-pressure liquid chromatographic (HPLC) analyses of the ensuing 50-μL dialysate samples (including 10 μL of preservative). Linear regression analyses were performed on the plot of the net glutamate-flux (glutamate in − glutamate out) versus the concentration of glutamate infused through the probe to yield the point of no net-flux (x-intercept) and the extraction fraction (E_d; an index of release/reuptake) (e.g., Ben-Shahar et al., 2012; Goulding et al., 2011; see also Shippenberg, Hen, & He, 2000). The data for $y = 0$ and E_d were analyzed using two-tailed, one-way analyses of variance (ANOVAs; $\alpha = 0.05$) across levels of the AAV factor (Control, cDNA, shRNA), separately for P and Wistar rats.

Drinking procedures

Three to four days following neurochemical assessment, drinking procedures commenced in AAV-infused Wistar and P rats. These procedures involved the simultaneous presentation of multiple 50-mL sipper tubes; intake from each sipper tube was determined once a day by changes in bottle weight. Bottles were available 5 days/week (Monday to Friday), fresh solutions were prepared at least once a week, and rats were weighed on a weekly basis in order to accurately determine intake. Bottles were also placed on empty cages to provide an index of spillage due to bottle handling, and this spillage was subtracted daily from the volume consumed by the rats. We first assayed for AAV effects upon the intake and preference for palatable non-alcoholic fluids by presenting rats with 0%, 0.00625%, and 0.0125% saccharin (w/v) (Rodd et al., 2008) for 5 days (Monday to Friday). The following week, rats were given access to 0% vs. 5% sucrose (e.g., Cozzoli et al., 2009) for 5 days. Having confirmed no AAV effects upon fluid intake, we next determined the effects of our AAVs upon alcohol intake and preference by concurrently presenting rats with 0%, 15%, and 30% (v/v) alcohol (e.g., Alhaddad, Das, & Sari, 2014) for 5 days/week for a total of 3 weeks. Due to the 6-week quarantine of the P rats, it was not possible to conduct the study of both lines at the same time, but there were no relative-age differences, and an overlap between the Wistar and P study timelines was present. As such, the intake and preference data for each type of solution were initially analyzed using an AAV (control, cDNA, shRNA) × Fluid Concentration (2 or 3 levels, depending upon the fluid) × Drinking Day ANOVA, with repeated measures on the Drinking Day factor (4 or 12 levels, depending upon the solution), separately for the two lines. As the results of these analyses indicated stable preference/intake across days (i.e., no main effects of, or interactions with, the Drinking Day factor, $p > 0.05$), the data were collapsed across the drinking days to facilitate visualization of AAV effects upon intake and preference.

Verification of neuronal transduction by immunohistochemistry

Upon completion of behavioral testing, rats were euthanized by Euthasol overdose, transcardially perfused with phosphate-buffered saline (PBS), followed by 4% paraformaldehyde. Their brains were removed and stored in PBS, until sectioning along the coronal plane on a vibratome at the level of the NAc (50 μm), according to the atlas of Paxinos and Watson (2006). Sections from AAV-cDNA infused rats were stained with an antibody against the hemagglutinin (HA) tag using a mouse anti-HA primary antibody (1:1000 dilution; Covance, San Diego, CA) using standard immunohistochemical techniques and examined for vector spread under a light microscope, while sections from AAV-GFP and AAV-shRNA infused rats were examined for GFP immunofluorescence under a fluorescent microscope (e.g., Cozzoli et al., 2009; 2012, 2014; Gould et al., 2015; Goulding et al., 2011; Szumlinski et al., 2004; 2006). Only rats whose injector cannulae were located within the boundaries of the NAcC and that exhibited neuronal transduction were included in the statistical analyses of the data. Based on these criteria, two rats were excluded from the AAV-GFP group and three rats were excluded from the AAV-shRNA group.

Verification of neuronal transduction by immunoblotting

As this study is the first to describe the behavioral and neurochemical effects of our shRNA-Homer2b construct in the rat NAcC, we also employed immunoblotting to quantify the extent to which our AAVs altered Homer2b expression within the NAcC of Wistar rats and measured for non-selective effects on Homer1b/c levels. Wistar rats were employed in this study as they are readily available from a commercial source, are similar to the foundation line for the P rats, as well as other selectively bred high-alcohol consuming rats (Bell, Rodd, Engleman, Toalston, & McBride, 2014), and do not require quarantine procedures. The immunoblotting procedures used to quantify AAV-mediated changes in Homer1/2 expression were identical to those used previously in mouse studies (e.g., Ary et al., 2013; Cozzoli et al., 2009; Goulding et al., 2011) and rats (Gould et al., 2015). In brief, at 8 weeks post-AAV infusion, animals were infused with 0.5 μL of black ink to locate the AAV infusion site. Brains were removed immediately following ink infusion and placed into a chilled rat brain mold (Braintree Scientific, Braintree, MA). A 1.0-mm thick coronal section was made at the level of the NAc, flanking the obvious guide cannula tracts. The section was laid flat on an ice-chilled glass plate and an 18-gauge punch was employed to bilaterally dissect out the tissue stained by the ink. The tissue was then subjected to our standard homogenization and immunoblotting procedures (e.g., Ary et al., 2013; Cozzoli et al., 2009; Gould et al., 2015; Goulding et al., 2011), using rabbit anti-Homer2a/b (Cosmo Bio USA Inc., Carlsbad, CA; 1:1000 dilution) and anti-Homer1b/c (GeneTex Inc., Irvine, CA; 1:1000 dilution). Immunoreactive bands were detected by enhanced chemiluminescence (ECL Plus; GE Healthcare, Piscataway, NJ, USA). To be consistent with prior work in our laboratory and facilitate cross-study comparisons (e.g., Cozzoli et al., 2009, 2012, 2014; Goulding et al., 2011; Obara et al., 2009), data were normalized using a rabbit anti-calnexin polyclonal primary antibody (Stressgen, Ann Arbor, MI, USA; 1:1000 dilution). Immunoreactivity levels were quantified using Image J (NIH, Bethesda, MD, USA). The protein:calnexin ratios for each of the GFP-, cDNA- and shRNA-infused rats were expressed as a percentage of those obtained for the average protein:calnexin ratio of the GFP-infused rats on their respective membrane. The resultant data were analyzed using a one-way ANOVA across the AAV factor for Homer2a/b and Homer1b/c levels ($\alpha = 0.05$).

Results

Verification of AAV transduction

Transduction by our cDNA-Homer2b, shRNA-Homer2b, and GFP constructs was quantified in a subset of animals by immunoblotting, and was localized within the boundaries of the NAcC by immunohistochemistry (Fig. 1). Transduction by all three of our AAVs was observed within both the cell bodies and the processes of NAc neurons. In this study, immunostaining was restricted to the core subregion of the NAc, with little spread (~200–400 μm) along the medial-lateral or dorsal–ventral planes (Fig. 1C–F). There was also very little spread along the anterior–posterior plane, with staining observed only across 2 or 3 consecutive 50-μm sections (i.e., across 100–150 μm), with the majority of rats exhibiting transduction in the tissue immediately adjacent to the anterior commissure (see Fig. 1C–F). Immunoblotting conducted on the

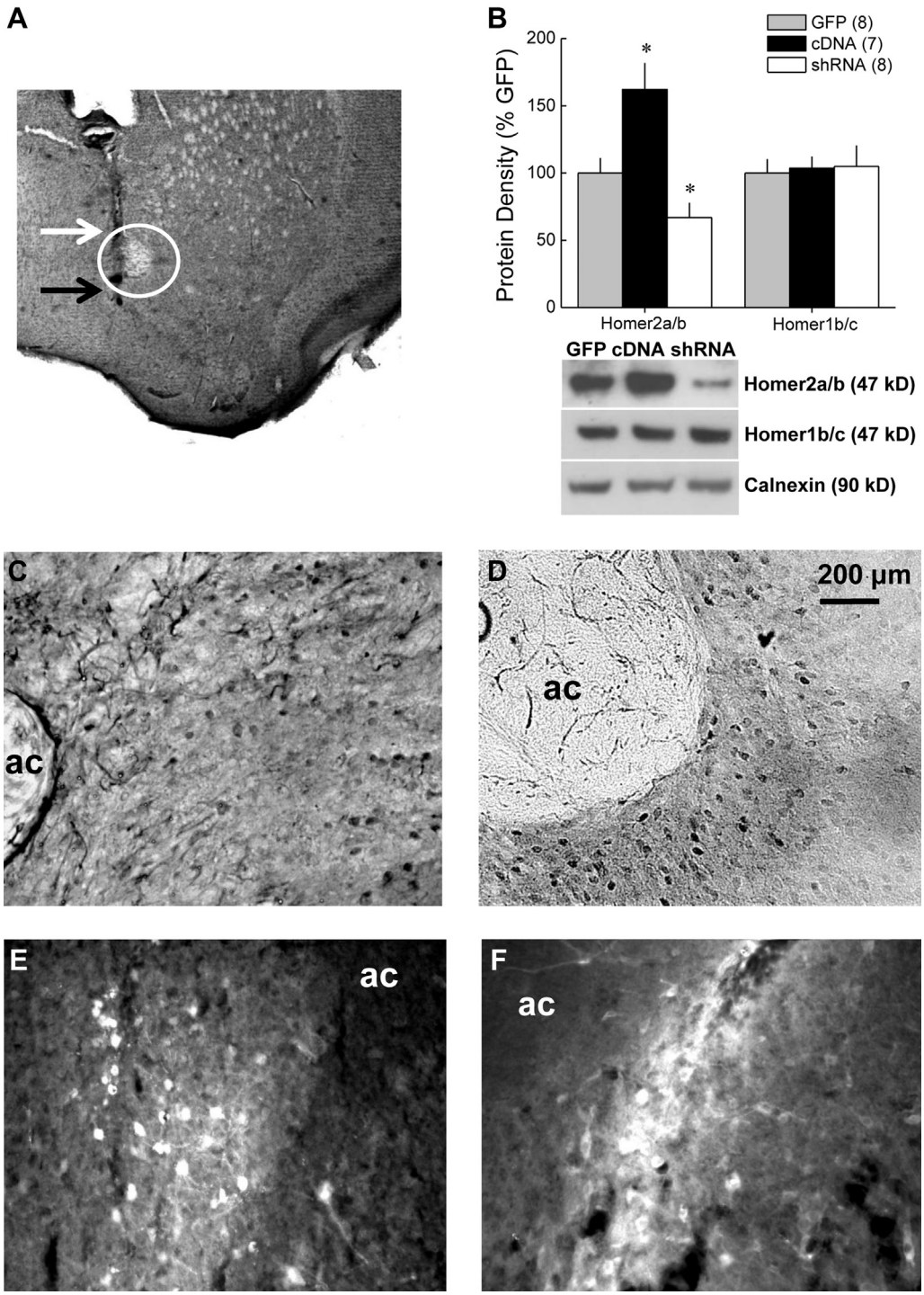

Fig. 1. Verification of neuronal transduction within the NAcC by the local infusion of AAVs carrying GFP, shRNA-Homer2b or cDNA-Homer2b. A) Representative cresyl violet-stained coronal section (50 µm) through the NAc demonstrating the placement of the microdialysis probe within the core subregion. White arrow indicates the start of the active membrane and black arrow indicates the glue plug at the tip of the probe. Circle indicates the size of the tissue micropunch employed in immunoblotting studies in panel B to sample the NAcC. B) Summary of the results of an immunoblotting study conducted on micropunches of the NAcC of Wistar rats to examine the relative protein expression of Homer2a/b (left) and Homer1b/c (right) at 8 weeks following infusion of AAV-GFP (GFP), shRNA-Homer2b (shRNA), or cDNA-Homer2b (cDNA). The data are expressed as a percent of the average protein levels of GFP-infused controls (±SEMs). $^{*}p < 0.05$ vs. GFP (LSD *post hoc* tests). Sample sizes are indicated in parentheses and representative immunoblots for Homer2a/b, Homer1b/c and the calnexin loading/transfer control are provided below. C) Representative picture of a coronal section through the NAc, illustrating immunostaining of the hemagglutinin (HA) tag of our cDNA-Homer2b vector within the core subregion of Wistar rats. D) Image at a comparable plane through the NAcC of a P rat infused with cDNA-Homer2b. Epifluorescent detection of the eGFP reporter to visualize NAcC neurons expressing shRNA-Homer2b in a Wistar rat (E) and a P rat (F) revealed neuronal transduction efficiency and vector spread in both strains as that produced by our cDNA constructs. For panels C–F, ac = anterior commissure and ruler bar in panel D indicates 200 µm.

tissue surrounding the microinjector tips within the NAcC (see circle in Fig. 1A) revealed changes in Homer2a/b expression appropriate for the infused construct with AAV-cDNA and AAV-shRNA infusion elevating and reducing, respectively, protein expression by approximately 50% and 40%, relative to GFP-infused controls (Fig. 1B) ($F[2,22] = 8.78$, $p = 0.002$; LSD *post hoc* tests,

$p < 0.05$). Moreover, the effects of our AAVs were selective for Homer2 isoforms because no changes in Homer1b/c expression were observed in these same animals (Fig. 1B; one-way ANOVA: $p = 0.96$).

Basal extracellular glutamate within the NAc core

Standard histological approaches were employed to localize the active membranes of the *in vivo* microdialysis probes within the NAc, and only data from rats in which the probes were appropriately positioned within the core subregion (e.g., Fig. 1A) were included in the statistical analyses of the results. Linear regression analyses were conducted on the plots of the net flux of glutamate versus the glutamate infused through the microdialysis probe (Fig. 2A and B) to assay for group differences in basal extracellular glutamate content (y = 0; Fig. 2C) and glutamate clearance (extraction fraction or E_d; slope; Fig. 2D). Line differences existed regarding basal extracellular glutamate content within the NAcC, as well as regarding the effects of AAV infusion upon basal extracellular glutamate content (Fig. 2C) (AAV × Line: $F[2,48] = 7.33$, $p = 0.002$). Deconstruction of this significant interaction along the Line factor failed to indicate significant AAV effects in Wistar rats (one-way ANOVA, $p = 0.52$). However, significant AAV differences were apparent in P rats ($F[2,23] = 15.78$, $p < 0.0001$), which reflected higher glutamate content in cDNA- vs. GFP-infused controls (LSD *post hoc* test: $p < 0.0001$). Inspection of Fig. 2C suggested a reduction in glutamate content in shRNA-infused rats, although the group difference was not significant (LSD *post hoc* test: $p = 0.35$). P rats infused with shRNA-*Homer2b* exhibited elevated glutamate clearance (Fig. 2D). However, analyses of the slopes of the linear regressions (E_d) did not indicate significant group differences (AAV × Line ANOVA, all *p*'s > 0.12). Thus, the observed AAV × Line interaction vis-à-vis basal extracellular glutamate content was not readily explained by changes in glutamate clearance/reuptake.

Saccharin consumption/preference

Fig. 3 illustrates the influence of an intra-NAcC infusion of our AAV vectors on the intake of, and preference for, palatable concentrations of saccharin versus water exhibited by Wistar and P rats. A line difference was noted with respect to both water intake and the effect of intra-NAcC AAV infusion upon water intake during the saccharin phase of testing (Line effect: $F[1,65] = 20.04$, $p < 0.0001$; AAV effect: $p = 0.49$; AAV × Line: $F[2,65] = 3.67$, $p = 0.03$). As illustrated in Fig. 3A, despite consuming more water than P rats, water intake by Wistar rats was not influenced by AAV infusion (one-way ANOVA, $p > 0.35$). In contrast, cDNA-Homer2b infusion elevated water intake significantly above GFP controls in P rats ($F[2,38] = 12.45$, $p < 0.0001$; LSD *post hoc* tests: GFP vs. cDNA $p = 0.003$). Opposite to water intake, P rats exhibited higher saccharin intake than Wistar rats (Fig. 3B) (Line effect: $F[1,65] = 41.94$, $p < 0.0001$). However, saccharin intake was not influenced by AAV infusion in either line (AAV effect and Line × AAV interaction, *p*'s > 0.10). Likewise, the preference for the saccharin solutions, particularly the 0.0125% concentration, was higher in P versus Wistar rats (Concentration: $F[2,120] = 123.20$, $p < 0.0001$; Line × Concentration: $F[2,120] = 12.22$, $p < 0.0001$). However, AAV infusion did not influence saccharin preference in either line (Fig. 3C and D; no AAV effects or interactions, *p*'s > 0.15). These data fail to indicate that NAcC manipulations of Homer2b expression influence saccharin intake or preference in rats.

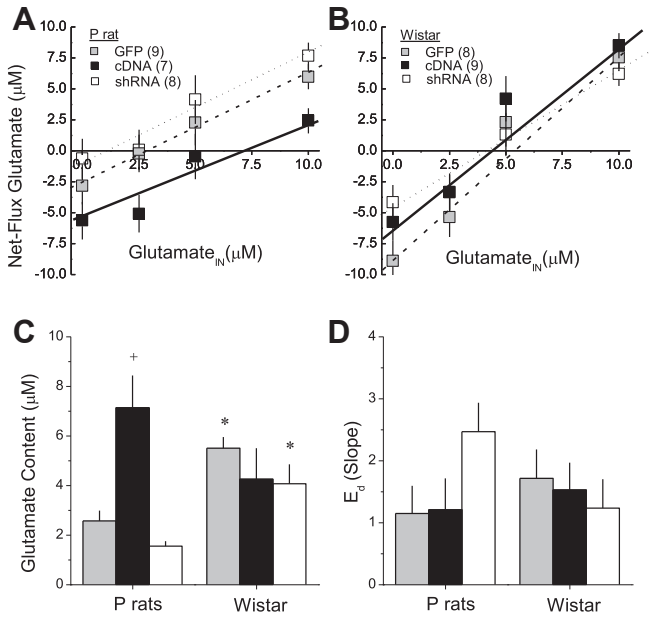

Fig. 2. Summary of the effects of Homer2b manipulations within the NAcC upon extracellular glutamate using quantitative *in vivo* microdialysis approaches. Plots of the relation between the net flux of glutamate (glutamate infused via the probe minus glutamate measured in the dialysate) versus the concentration of glutamate infused through the microdialysis probe for (A) P rats and (B) Wistar rats injected intra-NAcC with AAVs carrying GFP (GFP), cDNA-Homer2b (cDNA), or shRNA-Homer2b (shRNA). C) Summary of the effects of AAV infusion upon basal extracellular glutamate content as determined from the x-intercept (y = 0) from the plots in panels A and B. D) Summary of the effects of AAV infusion upon the extraction fraction (E_d) as determined from the slopes of the plots in panels A and B. Sample sizes are indicated in panel A (P rats) and panel B (Wistar rats). $^*p < 0.05$ vs. P rats; $+p < 0.05$ vs. GFP (LSD *post hoc* tests).

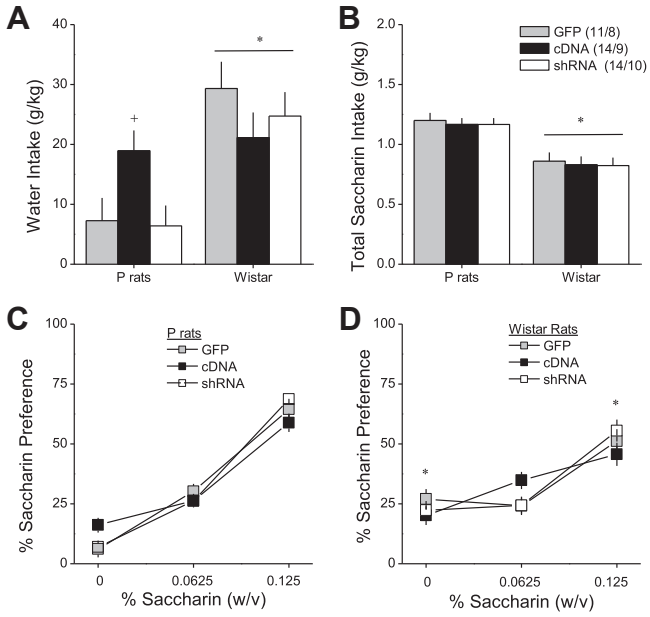

Fig. 3. Summary of the effects of Homer2b manipulations within the NAcC upon indices of saccharin reward exhibited by P and Wistar rats. (A) Overall, Wistar rats consumed more water than P rats. An intra-NAcC infusion of cDNA-Homer2b (cDNA) elevated water intake by P rats, while neither AAV-GFP (GFP) or shRNA-Homer2b (shRNA) influenced water intake in either strain. (B) P rats consumed more saccharin than Wistar rats; however, none of the AAVs influenced the average total saccharin intake in either strain. (C & D) Although P rats exhibited a lower preference for water (0% saccharin) and a higher preference for the 0.0125% saccharin concentration than did Wistars, AAV infusion failed to alter the dose–response function for saccharin preference in either strain. Sample sizes are indicated in parentheses in Panel B (P rats/Wistars). $^*p < 0.05$ vs. P rats; $+p < 0.05$ vs. GFP.

Sucrose consumption/preference

Fig. 4 illustrates the influence of an intra-NAcC infusion of our AAV vectors upon the intake of, and preference for, 5% sucrose versus water exhibited by Wistar and P rats during the week following saccharin testing. Again, the line difference in water intake was also apparent the following week (Wistar > P rat) (Line effect: $F[1,65] = 128.39$, $p < 0.0001$), but the effect of AAV infusion upon water intake by P rats was no longer apparent (Fig. 4A; AAV effect and interaction, p's > 0.15). Opposite the line difference in saccharin intake (Fig. 3B), Wistar rats consumed more sucrose than P rats (Fig. 4B) (Line effect: $F[1,65] = 24.37$, $p < 0.0001$). However, Wistar rats exhibited lower sucrose preference than did their P rat counterparts (Fig. 4C) (Line effect: $F[1,65] = 48.97$, $p < 0.0001$). Yet, AAV infusion failed to influence either variable in either line (for both sucrose intake and sucrose preference, no AAV effects or interactions, p's > 0.14). Thus, manipulations of Homer2b expression within the NAcC do not influence the voluntary intake of, or the preference for, sucrose nor do they consistently influence water intake in rats.

Alcohol consumption and preference

An analysis of the daily intakes of 15 and 30% alcohol across the 3 weeks of alcohol access revealed significant line differences in drinking across days (data not shown) (Line × Day: $F[11,660] = 6.45$, $p < 0.0001$; Line × Concentration × Day: $F[11,660] = 2.48$, $p = 0.005$). This interaction reflected a progressive decline in 30% alcohol intake by Wistar rats over the first few days of drinking, which then stabilized for the remainder of testing (Day effect: $F[11,264] = 2.39$, $p = 0.008$; Concentration × Day: $p = 0.06$). In contrast, P rats maintained their initial level of alcohol intake throughout the 3-week testing period (no main Day effect or Concentration × Day interaction, p's > 0.05). Interestingly, when the entire time course of drinking over the 3-week period was considered, the effects of an intra-NAcC AAV infusion upon alcohol intake was found to be line-dependent (AAV × Concentration: $F[2,60] = 9.61$, $p < 0.0001$; AAV × Line: $F[2,60] = 19.18$, $p < 0.0001$; AAV × Concentration × Line, $p = 0.54$), but the AAV effects did not vary by drinking day (data not shown; no interactions between the AAV and Day factors, p's > 0.06). As such, for clarity, the data for both alcohol and water intake, as well as for the preference for each solution, were averaged across the second and third weeks of drinking and the results are summarized in Fig. 5.

As observed in the saccharin and sucrose phases of the study (Figs. 3 and 4), Wistar rats consumed more water on average than P rats over the 3 weeks of alcohol availability, but the average water intake was not affected by AAV infusion in either line (Fig. 5A) (Line effect: $F[1,65] = 96.91$, $p < 0.0001$; AAV effect and interaction, p's > 0.60). In contrast to the line difference in water intake, the average intake of 15 and 30% alcohol was nearly identical between P and Wistar rats (Fig. 5B and C) (Line effect: $p = 0.98$ and Line × Concentration: $p = 0.99$). Intra-NAcC AAV infusion altered the concentration-response function for the average alcohol intake, and this effect did not vary significantly with line (AAV effect: $F[2,60] = 37.18$, $p < 0.0001$; AAV × Concentration: $F[2,60] = 9.43$, $p < 0.0001$; AAV × Line × Concentration: $p = 0.06$). As illustrated in Fig. 5B, cDNA-Homer2b infusion elevated the average intake of 15% alcohol in a line-independent manner (AAV effect: $F[1,65] = 11.77$, $p < 0.0001$; Line effect and interaction, p's > 0.09; LSD *post hoc* tests for GFP vs. cDNA, $p = 0.002$). Although shRNA-Homer2b did not lower the average intake of 15% alcohol (Fig. 5B), both AAVs significantly affected the average intake of 30% alcohol, but the pattern of AAV effects did not vary with line (Fig. 5C) (AAV effect: $F[2,65] = 30.21$, $p < 0.0001$; no Line effect or interaction, p's > 0.45; LDS *post hoc* tests: GFP vs. cDNA, $p < 0.0001$; GFP vs. shRNA, $p = 0.02$). When the total alcohol consumption was averaged across the 3 weeks of drinking, again we detected significant, bidirectional effects of manipulating Homer2b expression within the NAcC that were indistinguishable between P and Wistar rats (Table 1) (AAV effect: $F[2,66] = 37.18$, $p < 0.0001$; Line effect and interaction, p's > 0.85; LSD *post hoc* tests: GFP vs. cDNA, $p < 0.0001$; GFP vs. shRNA, $p = 0.03$).

An analysis of the average preference for each alcohol solution indicated independent effects of both line and AAV on the concentration-preference function (Line × Concentration: $F[2,120] = 11.89$; AAV × Concentration: $F[4,120] = 9.91$, $p < 0.0001$); however, the effect of AAV infusion upon the concentration-preference function did not vary significantly between the two lines (AAV × Line × Concentration: $p = 0.08$). As illustrated in Fig. 5D, Wistar rats exhibited a higher preference for water (0% alcohol) than did P rats (Line effect: $F[1,60] = 20.29$, $p < 0.0001$). Despite this line difference, cDNA-Homer2b infusion lowered, while shRNA-Homer2b did not significantly affect, water preference in both lines (AAV effect: $F[2,60] = 16.02$, $p < 0.0001$; AAV × Line: $p = 0.70$; LSD *post hoc* tests: GFP vs. cDNA $p = 0.001$; GFP vs. shRNA, $p = 0.09$). When 15% alcohol was considered (Fig. 5B), we did observe a significant AAV × Line interaction for alcohol preference ($F[2,65] = 3.20$, $p = 0.04$). Deconstruction of this interaction along the Line factor indicated a significant AAV effect in Wistar rats only (for Wistars: $F[2,26] = 8.06$, $p = 0.002$; for P rats: $p = 0.36$). While the preference for 15% alcohol was greater in Wistar rats infused with cDNA vs. those infused with shRNA ($p = 0.001$), LSD *post hoc* comparisons did not detect significant group differences between GFP-infused control rats and Wistar rats infused with either cDNA ($p = 0.08$) or shRNA ($p = 0.06$). An

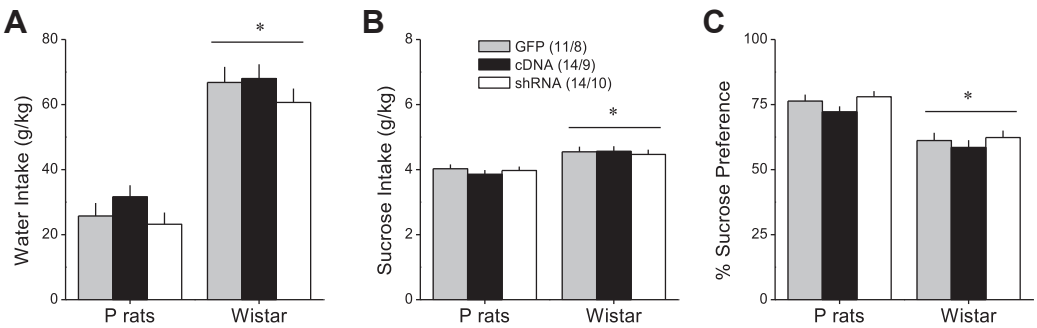

Fig. 4. Summary of the effects of Homer2b manipulations within the NAcC upon indices of sucrose reward exhibited by P and Wistar rats. Overall, Wistar rats exhibited higher intake of both water (A) and a 5% sucrose solution (B), compared to P rats. However, an intra-NAcC infusion of AAV-GFP (GFP), cDNA-Homer2b (cDNA), and shRNA-Homer2b (shRNA) did not influence the intake of either solution in either strain. (C) AAV infusion also failed to affect the average sucrose preference exhibited by either strain under these procedures. Sample sizes are indicated in parentheses in Panel B (P rats/Wistars). *$p < 0.05$ vs. P rats.

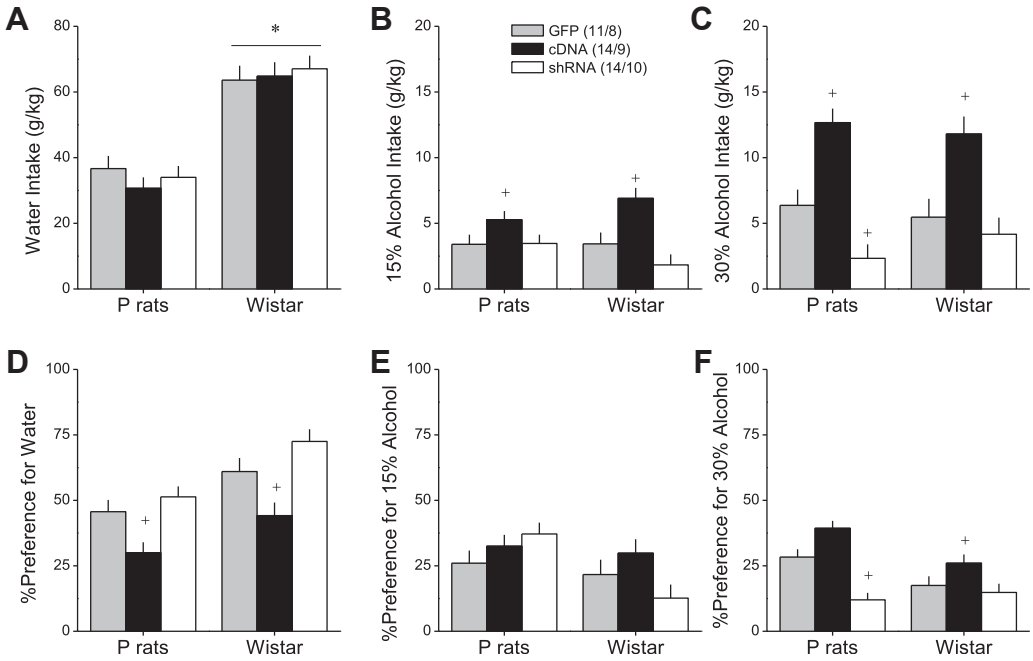

Fig. 5. Summary of the effects of Homer2b manipulations within the NAcC upon indices of alcohol reward exhibited by P and Wistar rats. When assessed for 14 consecutive days, an intra-NAcC infusion of AAV-GFP (GFP), cDNA-Homer2b (cDNA), and shRNA-Homer2b (shRNA) did not influence the average daily intake of water exhibited by either P rats (left) or Wistar rats (right) (A). However, AAV infusion did alter the dose–response function for alcohol intake with cDNA infusion elevating significantly the intake of 15% alcohol in both P and Wistar rats (B). cDNA infusion also elevated the intake of 30% alcohol in both rat strains, while shRNA infusion lowered the intake of 30% alcohol only in P rats (C). With respect to preference for each solution, cDNA infusion lowered water preference in both strains (D). shRNA infusion did not significantly influence the preference for 15% alcohol in either line, although a trend towards a decrease was apparent in Wistar rats (E). Preference for 30% alcohol was bidirectionally influenced by AAV infusion in P rats, but only cDNA infusion elevated the preference for this concentration in Wistar rats (F). Sample sizes are indicated in parentheses in Panel B (P rats/Wistars). $^{*}p < 0.05$ vs. P rats; $+p < 0.05$ vs. GFP (LSD *post hoc* tests).

examination of preference for 30% alcohol also indicated a significant AAV × Line interaction ($F[2,65] = 3.51$, $p = 0.04$). However, at this concentration, AAV effects were apparent in both P rats ($F[2,38] = 15.38$, $p < 0.0001$) and Wistars ($F[2,26] = 9.04$, $p = 0.001$). In the case of P rats, shRNA infusion significantly reduced ($p = 0.002$), while cDNA infusion tended to elevate ($p = 0.07$) preference for 30% alcohol. In Wistar rats, only cDNA infusion affected preference (GFP vs. cDNA, $p = 0.006$; GFP vs. shRNA, $p = 0.37$).

Discussion

Homer2 isoforms are increased within mesolimbic structures in alcohol-experienced rats and mice (c.f., Cui et al., 2013; Cozzoli et al., 2014; see also Obara et al., 2009). This neuroadaptation is proposed to contribute to the hyper-glutamatergic state that promotes addiction/alcoholism-related behaviors (e.g., Cui et al., 2013; Gass & Olive, 2008) and genetic predisposition to alcoholism-related behaviors is associated with elevated indices of Homer2/glutamate signaling within the NAc, as well as other mesolimbic structures (Cozzoli et al., 2009, 2012, 2014; Goulding et al., 2011; Szumlinski et al., 2005; Szumlinski, Ary, Lominac, et al., 2008). Nearly a decade of transgenic research supports an active role for Homer2b, particularly within the NAc, in bidirectionally regulating behavior in various animal models of alcoholism/alcohol-induced neuroplasticity, including behavioral sensitization (Szumlinski et al., 2005; Szumlinski, Ary, Lominac, et al., 2008), stress-alcohol cross-sensitization (Quadir et al., 2015), place-preference (Szumlinski et al., 2005; Szumlinski, Ary, Lominac, et al., 2008), and alcohol intake under both operant (Szumlinski et al., 2005) and non-operant procedures (Cozzoli et al., 2009, 2012; Goulding et al., 2011; Szumlinski et al., 2005; Szumlinski, Ary, Lominac, et al., 2008). However, in our view, one major shortcoming of this collection of prior behavioral work relates to the exclusive use of mice as the experimental subjects. Arguably, if our understanding of the role for Homer2 in regulating alcoholism-related behaviors extended across mammalian species, this would increase the relevance of our findings as it pertains to the neurobiology of the human condition. Indeed, prior cocaine research indicated that the effects of repeated cocaine upon mesocorticolimbic Homer/glutamate receptor expression (e.g., Ary & Szumlinski, 2007; Ghasemzadeh, Mueller, & Vasudevan, 2009; Ghasemzadeh, Vasudevan, Mueller, Seubert, & Mantsch, 2009; Swanson, Baker, Carson, Worley, & Kalivas, 2001; but see Knackstedt et al., 2010), the interactions between NAc Homer expression and behavioral sensitization (Ghasemzadeh, Permenter, Lake, Worley, & Kalivas, 2003; Szumlinski et al., 2004, 2006), as well as drug self-administration or relapse to drug-seeking (Gould et al., 2015; Szumlinski et al., 2004; but see Knackstedt et al., 2010), generalized across rodent species. However, despite our prior knowledge that a history of alcohol intake elevates Homer/glutamate receptors

Table 1
Summary of the effects of intra-NAcC infusion of AAVs carrying GFP (GFP), cDNA-*Homer2b* (cDNA) or shRNA-*Homer2b* (shRNA) upon the average daily total alcohol intake (g/kg) exhibited by P and Wistar rats across a 3-week drinking period, during which rats were presented simultaneously with 0, 15, and 30% alcohol (v/v) for 5 days/week.

	GFP	cDNA	shRNA
P rats	9.76 ± 1.11	17.93 ± 1.99*	5.83 ± 0.78*
Wistar rats	8.90 ± 1.03	18.70 ± 2.25*	6.03 ± 1.00*

Data represent the means ± SEMs of the number of rats indicated in parentheses in Fig. 2. $^{*}p < 0.005$ vs. GFP (LSD *post hoc* tests).

within P rats, in a manner similar (but not identical) to C57BL/6J mice (e.g., Obara et al., 2009 versus Szumlinski, Ary, Lominac, et al., 2008), we have been remiss in the extension of findings for alcoholism-related behavior in mice to another mammalian species.

NAc Homer2 bidirectionally regulates alcohol intake in both P and Wistar rats

Herein, we show that cDNA-*Homer2b* infusion into the NAcC is sufficient to elevate alcohol intake in both selectively bred high alcohol-preferring P rats, as well as in outbred Wistar rats (Fig. 5). The increase in alcohol intake by NAcC Homer2 over-expression was equivalent across the two rat lines and most robust at the higher (30%) alcohol concentration. Conversely, NAcC shRNA-Homer2b infusion lowered alcohol intake in both rat lines, and this effect was only apparent at the higher alcohol concentration. These findings are consistent with prior AAV studies of C57BL/6J mice, and mice on a mixed C57BL/6J-129Xi/SvJ genetic background, indicating that intracranial manipulations of Homer2 influence most robustly the intake of higher, rather than lower, alcohol concentrations (e.g., Cozzoli et al., 2014; Goulding et al., 2011; Szumlinski, Ary, Lominac, et al., 2008; Szumlinski et al., 2005). Also consistent with prior work in mice (e.g., Goulding et al., 2011; Szumlinski, Ary, Lominac, et al., 2008; Szumlinski et al., 2005), the bidirectional regulation of alcohol intake by our AAVs was not perfectly paralleled by changes in alcohol preference (Fig. 5, top vs. bottom rows). However, it should be noted that NAcC Homer2b over-expression shifted the preference of both lines toward high-concentration alcohol over water (an effect slightly more pronounced in Wistar rats), while NAcC Homer2b knock-down shifted rats' preference away from high-concentration alcohol (an effect slightly more pronounced in P rats), and these shifts in preference were consistent with AAV effects upon intake. These data provide novel evidence that NAcC Homer2b levels bidirectionally regulate alcohol intake by rats, thereby extending to another mammalian species published work in mice (Cozzoli et al., 2009, 2012; Goulding et al., 2011; Szumlinski, Ary, Lominac, et al., 2008; Szumlinski et al., 2005), and supporting the hypothesis that NAc Homer2 expression actively regulates excessive alcohol consumption leading to alcohol abuse/dependence (Cui et al., 2013; Szumlinski, Ary, Lominac, et al., 2008).

There is limited evidence that the magnitude of the effect of transgenic manipulations of NAc Homer2b expression upon alcohol intake can be strain-dependent in mice. AAV-Homer2b infused inbred C57BL/6J mice and mice on a mixed C57BL/6J × 129SvJ/Xi background both exhibit bidirectional changes in alcohol intake/preference akin to those observed in the P and Wistar rats studied herein (Fig. 5 vs. Cozzoli et al., 2009, 2012; Goulding et al., 2011; Szumlinski, Ary, Lominac, et al., 2008; Szumlinski et al., 2005). In contrast, inbred DBA2/J mice are completely insensitive to the effects of NAc Homer2b over- and under-expression upon alcohol preference/intake, at least when assessed under similar continuous-access, multi-bottle choice procedures as those employed in the present study. We attributed the AAV insensitivity of DBA2/J mice to a combination of anomalous alcohol-induced glutamate release and the relatively low glutamate receptor expression exhibited by this strain (see Goulding et al., 2011). The fact that NAc Homer2b over-expression is incapable of enticing greater alcohol intake in DBA2/J mice (Goulding et al., 2011), coupled with the results of our prior work in mice demonstrating no effect of our AAVs upon sucrose or saccharin preference in mice (e.g., Cozzoli et al., 2009; Szumlinski, Ary, Lominac, et al., 2008), argues against changes in taste sensitivity as underpinning the potentiating effects of NAc Homer2b over-expression upon alcohol intake. Herein, we employed a multi-bottle choice procedure (0, 15, and 30% alcohol) in order to assay for potential rat line differences in shifts in the concentration-response function for alcohol preference and intake by intra-NAcC AAV infusion. As is clear from the data in Fig. 5, the alcohol intake/preference, as well as the effects of our AAVs upon alcohol intake/preference, were nearly identical across the two rat lines. Perhaps the lack of line differences in alcohol intake or in the behavioral effects of intra-NAcC AAV infusion should not be surprising, as alcohol-preferring P rats were derived from a closed colony of Wistar rats, based exclusively upon their preference for a 10% alcohol solution over water (e.g., McBride, Rodd, Bell, Lumeng, & Li, 2014). Herein, rats were presented simultaneously with 0, 15, and 30% alcohol over a 24-h period. This multi-bottle procedure was employed herein not only to gauge for group differences in sensitivity to alcohol (via shifts in the dose–response function), but also to engender higher overall intake than that achieved using more conventional 2-bottle choice or single-bottle procedures (see Bell et al., 2014; Cozzoli et al., 2014). Thus, the most obvious explanation to account for our failure to detect lines differences in intake relates to the particular multiple-choice paradigm employed. We do not yet know whether or not line differences exist in the rat regarding the basal expression of Homer2 or its associated glutamate receptors/downstream effectors that might correlate with a high-alcohol drinking/preferring phenotype in this species. However, based on the present results from rats (Fig. 5) and extant data from mice (Cozzoli et al., 2009, 2012; Goulding et al., 2011; Szumlinski, Ary, Lominac, et al., 2008; Szumlinski et al., 2005), we propose that a positive relation exists between idiopathic differences in NAc Homer2 expression and the propensity to consume high-concentration alcohol, which requires further exploration in other mammalian species, most notably humans.

NAc Homer2 does not influence sweet solution intake by rats

Despite observing marked line differences in water intake (Wistar > P), transgenic manipulations of NAc Homer2 expression did not consistently influence the water intake by either rat line (Figs. 3–5). One exception was the increased water intake exhibited by cDNA-infused P rats during the initial saccharin phase of the drinking study (Fig. 3A). Notably, the increased water consumption exhibited by P rats had dissipated by the sucrose phase of the study that was conducted the following week (Fig. 4A) and did not re-manifest during the 3-week alcohol-drinking phase of the experiment (Fig. 5A). Thus, if NAcC Homer2b over-expression activated neurocircuitry underpinning thirst or osmoregulation in P rats, this effect was transient and could not readily account for: (1) the effects of Homer2b over-expression upon alcohol intake observed in both rat lines; (2) the lack of any AAV effects upon the intake of either saccharin (Fig. 3B) or sucrose (Fig. 4B); or (3) the relative preference that rats express for these palatable, sweet solutions (Figs. 3 and 4). The negative findings regarding the effects of intra-NAc AAV-Homer2b infusion upon the intake of, and preference for, sweet solutions in rats are consistent with nearly a decade of evidence from studies of mice indicating that Homer2, or its associated mGluRs, do not contribute in any significant manner to regulating the rewarding/reinforcing properties of sweet solutions (e.g., Cozzoli et al., 2009, 2012; Lum, Campbell, Rostock, & Szumlinski, 2014; Szumlinski, Ary, Lominac, et al., 2008; Szumlinski et al., 2005). As such, the present drinking data provide the first cross-species evidence that NAc Homer2 actively regulates alcohol intake, without influencing the neural processes underpinning fluid consumption or the hedonic value of non-drug, highly palatable, positive reinforcers.

Line differences in NAc glutamate and its regulation by Homer2b

Interestingly, the results of our quantitative *in vivo* microdialysis study indicated that the basal extracellular glutamate content within the NAcC of alcohol-naïve P rats was approximately 50% of that exhibited by outbred Wistar rats (Fig. 2C). To the best of our knowledge, this study is the first to directly compare basal extracellular glutamate content within any brain region between alcohol-naïve P rats and rats of another line, but the average extracellular glutamate content obtained for the P rats in this study (2.6 μM; Fig. 2C) is consistent with levels reported previously in the literature (e.g., Ding et al., 2013). While not statistically significant due to high variability in the data, P rats also exhibited a reduced E_d versus Wistar rats (Fig. 2D), suggesting that their lower glutamate content might reflect less basal glutamate release. While it is tempting to propose that lower NAcC extracellular glutamate content may be a biochemical correlate of selection for an alcohol-preferring phenotype in the P rat line, such an interpretation should be cautionary given that the Wistar rats employed in the present study were derived from a commercial vendor (i.e., not from the original colony employed to generate the P rat line). Nevertheless, it is clear from the present data that basal extracellular glutamate content within the NAcC of alcohol-naïve P or Wistar rats does not relate in any obvious manner to subsequent alcohol intake as, despite the marked differences in NAcC glutamate content, the alcohol intake exhibited by the two lines was equivalent (Fig. 5B and C). These latter findings are consistent with earlier indications from studies of alcohol-naïve mice that basal extracellular glutamate content within the NAc is not a reliable predictor of subsequent alcohol-drinking behavior (Goulding et al., 2011; Kapasova & Szumlinski, 2008; Szumlinski, Ary, Lominac, et al., 2008; Szumlinski et al., 2005).

This being said, it is interesting to note that the alcohol intake of AAV-infused P rats, particularly that at 30% alcohol, paralleled the effects of our AAV manipulations upon extracellular glutamate in this line (Fig. 2C vs. Fig. 5C). In stark contrast, our AAVs did not influence extracellular glutamate within the NAcC of Wistar rats (Fig. 2C), despite evidence for neuronal transduction and altered Homer2b protein expression (Fig. 1). Although the possibility exists that the prior exposure to sweet solutions may have altered extracellular glutamate within the NAcC, the dissociation between the effects of our AAVs upon NAcC glutamate vs. alcohol drinking observed in Wistar-associated rat lines furthers the argument that NAc basal extracellular glutamate levels are a poor predictor of alcohol intake/preference. The rat line differences in the sensitivity of NAcC extracellular glutamate to regulation by manipulations of Homer2b levels is very intriguing, as the results of a comparable study conducted between inbred mouse strains failed to detect any effect of intra-NAc cDNA- or shRNA-Homer2b infusion upon basal extracellular glutamate content in either strain, while strain-dependent differences in the effects of intra-NAc AAV infusion upon subsequent alcohol intake were observed (Goulding et al., 2011). While it remains to be determined how and why P rats are sensitive to AAV-mediated changes in extracellular glutamate content, the present rat data, coupled with our early mouse work (Goulding et al., 2011), support the notion that basal NAcC extracellular glutamate content expressed by alcohol-naïve animals can be dissociated from subsequent alcohol intake, at least under continuous-access, multi-bottle choice, conditions.

In mice, the effects of intra-NAc cDNA- and shRNA-Homer2b infusions upon alcohol intake consistently reflect parallel changes in the capacity of repeated alcohol to sensitize glutamate release within the NAc (Goulding et al., 2011; Szumlinski, Ary, Lominac, et al., 2008; Szumlinski et al., 2005). Although the neurochemical outcomes of the present study argue against a major role for Homer2 regulation of *basal* NAc glutamate content as underpinning alcohol consumption, they do not negate the possibility that our AAVs exerted their effects upon drinking via influences upon alcohol-induced glutamate release within this region. As support for this mechanism, acute or repeated alcohol elicits a greater increase in NAc extracellular glutamate in mouse strains that voluntarily consume alcohol versus low-drinking strains (Goulding et al., 2011; Kapasova & Szumlinski, 2008; Selim & Bradberry, 1996), and intra-NAc infusions of cDNA-Homer2b can facilitate, while infusions of shRNA-Homer2b blunt, the capacity of repeated alcohol to sensitize drug-stimulated glutamate release in mice (Goulding et al., 2011; Szumlinski, Ary, Lominac, et al., 2008; Szumlinski et al., 2005). Although the mechanism(s) mediating Homer2's ability to regulate presynaptic aspects of glutamate transmission remain yet unknown, it would be important in future research to determine whether or not the positive relation between NAc Homer2b levels, alcohol-induced glutamate release, and drinking behavior observed in mice holds true across other species and other models of alcoholism.

Conclusions

Homer2 expression within the glutamatergic interconnections of the mesocorticolimbic system is highly implicated in the neurobiological underpinnings of excessive alcohol intake. Complementing prior findings from murine models of alcohol abuse and alcoholism, the present data for P and Wistar rats indicate an active role for NAc Homer2b expression in regulating high-concentration alcohol intake/preference and argue against Homer2 regulation of basal extracellular glutamate content within the NAc as a predictor of excessive alcohol intake. The cross-species generalization of the effects of Homer2 manipulations within the NAc upon alcohol intake support the hypothesis that Homer2 and interrelated signaling molecules contribute to the etiology of alcohol abuse and alcoholism.

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