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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