Extrasynaptic $GABA_A$ receptors in the nucleus accumbens are necessary for alcohol drinking

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Despite the fact that alcohol is one of the most widely used and abused of all psychoactive drugs, there is surprising lack of consensus on the molecular mechanisms of action. One particularly important aspect of ethanol (EtOH) is its effect on the reward system in the mammalian brain, because it may help us to understand and help people afflicted with alcohol abuse and addiction better. In PNAS, Patricia Janak, Dorit Ron, and their colleagues (1) at the Ernest Gallo Clinic and Research Center associated with the University of California, San Francisco, are making progress on both aspects by using the technique of viral-mediated RNAi to knock down the expression of a specific inhibitory neurotransmitter receptor protein implicated in low- to moderate-dose EtOH effects on brain. This manipulation was carried out in vivo on rats in a specific mesolimbic brain region implicated in the reinforcing effects of EtOH, producing a significant reduction in oral uptake of EtOH.

The gene product implicated by Nie et al. (1) using the knockdown technique with viral-mediated RNAi (2, 3) is the extrasynaptic $GABA_A$ receptor ($GABA_AR$) δ-subunit, which has been shown to confer unique sensitivity to enhancement by concentrations of EtOH found in the blood of humans drinking one or a few drinks, in recombinant heterologous cell expression, in brain slices, and in vivo (4–9). The brain region implicated by Nie et al. (1) where extrasynaptic $GABA_A$Rs are critical for EtOH oral intake is the dorsomedial shell of the nucleus accumbens (NAc), an area noted for dopamine-mediated reward mechanisms involved in virtually all drugs of abuse (e.g., cocaine, nicotine, opiates; Fig. 1).

This demonstration takes advantage of a unique technique for temporary reduction of a specific gene product by in vivo microinjection of siRNA into a specific anatomical brain region to determine the role of that gene product in a specific behavior; here, oral EtOH intake regulated by $GABA_A$Rs in the NAc.

Further support for an involvement of the δ-subunit–containing $GABA_AR$ subtypes in EtOH effects has come from three lines of investigation. First, rats exhibiting a naturally occurring allelic variation in the $GABA_AR$ α6-cerebellar subunit

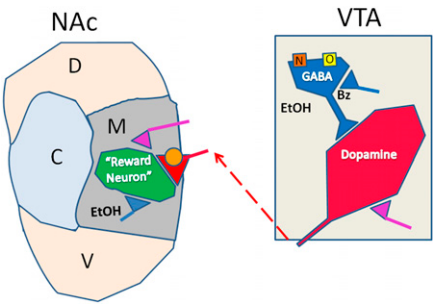

Fig. 1. Reward circuit (schematic), including dopamine neurons in the VTA projecting to "reward neurons" in the NAc. The NAc is divided into the core (C) and the shell, which is subdivided into ventral (V), medial (M), and dorsal (D) parts. The reward system is affected by most categories of drugs of abuse and involves numerous transmitters in the two main regions. The extended amygdala, not shown for simplicity, is sometimes considered part of the circuit. Color code: red, dopamine; violet, glutamate; blue, GABA. Acetylcholine, 5-hydroxytryptamine (5HT), and cannabinoid cells are not shown. Receptors are indicated for N (brown, nicotine and nicotinic acetylcholine receptor), O (yellow, opioid peptides and opiate drug receptor), and the orange ball (cocaine sites on dopamine transporter on dopamine nerve endings); BZ (sites implicated for the action of benzodiazepines on GABA interneurons in VTA) (16); and EtOH (sites implicated for action of EtOH). The author acknowledges input to this figure (17–19).

R100Q exhibit greater than normal sensitivity to the motor-incoordinating actions of moderate doses of EtOH; this hypersensitivity to EtOH is also seen in tonic inhibitory currents mediated by the α6βδ-type $GABA_A$Rs measured by patch-clamp recordings from granule cells in cerebellar slices (7, 9). Recombinant $GABA_A$Rs expressing the α6R100Qβ3δ in oocytes also showed higher sensitivity to EtOH modulation (1– 10 mM) than the already sensitive WT α6R100β3δ (10–30 mM) (7). Second, the residue R100 in the $GABA_AR$ α-subunit that affects EtOH sensitivity is part of the benzodiazepine ligand-binding pocket on the α6-subunit, consistent not only with the discovery of a previously unappreciated benzodiazepine (BZ) site on the δ-subunit–containing $GABA_A$Rs but with the demonstration that these unique BZ sites mediate antagonism by the BZ ligand Ro15-4513 of in vivo EtOH behaviors (10) and in vitro antagonism of low- to moderate-dose EtOH enhancement of δ-subunit–containing $GABA_A$Rs (7). Third, the observation that the α4βδ $GABA_AR$ subtypes are the most rapidly regulated in plastic mechanisms triggered by high-dose EtOH or chronic exposure to EtOH in rats (8) is consistent with these extrasynaptic $GABA_A$Rs being among the first responders to EtOH in the brain.

What is not so consistent with this picture is the phenotypes of mice lacking the $GABA_AR$ α4, α6, and δ subunits. None of these three KO mice show seriously altered effects of EtOH in vivo: The α6-, α4-, and δ-KOs show no changes in sensitivity to EtOH effects on anxiety and sedation (11–14), despite decreased sensitivity to EtOH of $GABA_AR$-mediated tonic inhibitory currents in brain neurons (7). The α4-KO shows reduced sensitivity to the motor incoordinating effects and reduced enhancement of $GABA_AR$-mediated inhibitory tonic currents by the GABA agonist THIP (4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol) (13). The δ-KO does show reduced sensitivity to behavioral effects of GABAergic neurosteroids and also exhibits reduced EtOH self-administration compared with WT (12).

The complexity of phenotypes produced by global KOs of genes in which an individual lacks the gene product throughout life in all anatomical regions can make it difficult to demonstrate a suspected gene function in a given behavior under study, even if the deletion is not fatal. Effects of global KOs are often masked by compensatory changes in genes serving similar functions. For example, in the $GABA_AR$ α6-KO mouse, which shows unimpaired sensitivity to behavioral effects of EtOH (11), there is a total loss of the δ-subunit, whose obligatory subunit partner is α6 and changes in the levels of other $GABA_AR$ subunits; these animals show compensatory up-regulation of a voltage-independent K^+ channel (15), generating a compensatory tonic inhibitory current in cerebellar granule cells. In this abnormal environment, it is not surprising that it is difficult to establish a clear role for the

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$GABA_AR$ δ-subunit in EtOH actions. Also worth noting might be the rather large differences in EtOH sensitivity among mouse strains and that the vast majority of detailed positive results implicating extrasynaptic $GABA_ARs$ in behavioral alcohol action were obtained using rats rather than mice (including the current study).

Some success in overcoming this lack of specificity in genetic engineering has come from conditional KOs, gene deletions induced only at a specific anatomical location and specific age. Similarly, knock-ins introduce a specific point mutation in a single gene that is demonstrated in vitro to have a functional consequence for a specific behavior, for example, rendering $GABA_ARs$ insensitive to a drug action, such as benzodiazepine or anesthetic modulation (16). Alternatively, behaviors with a well-defined anatomical localization and implicated gene product function can be studied by knocking down the gene expression by introducing appropriate siRNA constructs into specific identified cells using suitable viral vectors.

The dopamine reward circuit, including the ventral tegmental area (VTA) and NAc (Fig. 1), is a specific anatomical area involved in the reinforcing effects of drugs of abuse suitable for manipulation of genetic expression regulation with siRNA using viral vectors.

Such an opportunity has been identified by Nie et al. (1). Previous workers had shown that various drugs of abuse stimulate the well-known dopamine reward circuit, in which the drugs stimulate dopamine neurons situated in the VTA and projecting to the NAc and amygdala (Fig. 1). The activity of this circuit is modulated by EtOH but also by nicotine, cannabinoids, opiates, cocaine, and methamphetamine as well as by the club drug γ-hydroxybutyrate and benzodiazepines, with drug targets located at various places for different drugs (17). The circuit is modulated by excitatory and inhibitory inputs to presynaptic and postsynaptic receptors using glutamate, GABA, acetylcholine (nicotinic receptors), 5-hydroxytryptophan, opioid peptides, and cannabinoids (18). Addiction is thought to require plastic changes in the synaptic glutamate receptor activity of this reward circuit following chronic activation by the drug of abuse (19, 20). Alternatively, plasticity in the circuits mediating negative reinforcement resulting from the absence of the previously present drug of abuse (withdrawal) could contribute to addiction.

Several drugs of abuse stimulate dopamine neurons in the VTA. Tan et al. (16) demonstrated that benzodiazepines, abused GABAergic drugs, potentiate $GABA_AR$-mediated inhibition in the VTA associated with induction of synaptic plasticity, and thus consistent with addiction potential. They showed that a gene knock-in mouse for the $GABA_AR$ α1-subunit was critical for this addictive action of the BZs in the VTA. Could this same target in the VTA mediate reinforcement to EtOH? Do we need another target for EtOH? Reinforcing effects of many drugs of abuse have been shown to involve the NAc (17, 18). Rewal et al. (2) previously demonstrated a linkage of EtOH effects with GABA, also in the NAc, showing that selective reduction of the GABAR α4-subunit in the NAc shell, but not in the core, reduces EtOH self-administration. Jeanblanc et al. (3) further used the gene knock-down approach utilizing siRNA to implicate BDNF in the NAc shell for EtOH reinforcement.

The ability to influence a specific gene with siRNA in a specific anatomical region and at a specific age (not to mention a specific animal species) using viral vectors, and a specific behavior already linked to the brain region, allows a very specific conclusion about gene function, certainly more specific than the global KO mouse. In particular, in the current work, we have a totally identified location that allows a more unambiguous demonstration of a role for this gene product in this behavior. Not only is the NAc implicated, but, specifically, the dorsomedial shell, rather than the ventral or lateral shell or the core, is shown to be involved. Furthermore, the ingestion of sucrose was not affected. In this case, the specificity may be added to by the unusual extrasynaptic localization of the δ-subunit–containing $GABA_ARs$, and their unique physiology and pharmacology. Thus, we can tentatively conclude, for example, because of the detailed rationale for the gene, tissue, and function analyzed, that other $GABA_AR$ subunits, although they were not similarly studied with gene knockdown, are not really likely to be involved in this function just because the δ-subunit is. This makes results obtained in this manner quite compelling. Of course, the study also unambiguously shows that the gene in question is knocked down and specifically in the medial shell of the NAc, using siRNA techniques already familiar to the authors.

To quote Nie et al. (1): "In conclusion, the current findings indicate that δ-containing $GABA_ARs$ in medial NAc shell play an important role in alcohol drinking behavior, strengthening the hypothesis that the $\alpha4\beta\delta$ $GABA_AR$ (mediating tonic inhibition) in a restricted region of the NAc shell is a key brain substrate for the reinforcing properties of oral alcohol."

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1. Nie H, Rewal M, Gill TM, Ron D, Janak PH (2011) Extrasynaptic δ-containing $GABA_A$ receptors in the nucleus accumbens dorsomedial shell contribute to alcohol intake. *Proc Natl Acad Sci USA* 108:4459–4464.
2. Rewal M, et al. (2009) Alpha4-containing GABA(A) receptors in the nucleus accumbens mediate moderate intake of alcohol. *J Neurosci* 29:543–549.
3. Jeanblanc J, et al. (2009) Endogenous BDNF in the dorsolateral striatum gates alcohol drinking. *J Neurosci* 29: 13494–13502.
4. Sundstrom-Poromaa I, et al. (2002) Hormonally regulated alpha(4)beta(2)delta GABA(A) receptors are a target for alcohol. *Nat Neurosci* 5:721–722.
5. Wallner M, Hanchar HJ, Olsen RW (2003) Ethanol enhances alpha 4 beta 3 delta and alpha 6 beta 3 delta gamma-aminobutyric acid type A receptors at low concentrations known to affect humans. *Proc Natl Acad Sci USA* 100:15218–15223.
6. Wei W, Faria LC, Mody I (2004) Low ethanol concentrations selectively augment the tonic inhibition mediated by delta subunit-containing GABA(A) receptors in hippocampal neurons. *J Neurosci* 24:8379–8382.
7. Wallner M, Hanchar HJ, Olsen RW (2006) Low dose acute alcohol effects on GABA. A receptor subtypes. *Pharmacol Ther* 112:513–528.
8. Liang J, et al. (2007) Mechanisms of reversible GABA(A) receptor plasticity after ethanol intoxication. *J Neurosci* 27:12367–12377.
9. Santhakumar V, Wallner M, Otis TS (2007) Ethanol acts directly on extrasynaptic subtypes of GABA(A) receptors to increase tonic inhibition. *Alcohol* 41:211–221.
10. Paul SM (2006) Alcohol-sensitive GABA receptors and alcohol antagonists. *Proc Natl Acad Sci USA* 103: 8307–8308.
11. Homanics GE, et al. (1997) Gene knockout of the alpha6 subunit of the gamma-aminobutyric acid type A receptor: Lack of effect on responses to ethanol, pentobarbital, and general anesthetics. *Mol Pharmacol* 51: 588–596.
12. Mihalek RM, et al. (2001) GABA(A)-receptor δ subunit knockout mice have multiple defects in behavioral responses to ethanol. *Alcohol Clin Exp Res* 25:1708–1718.
13. Chandra D, et al. (2006) GABA(A) receptor alpha 4 subunits mediate extrasynaptic inhibition in thalamus and dentate gyrus and the action of gaboxadol. *Proc Natl Acad Sci USA* 103:15230–15235.
14. Chandra D, et al. (2008) Normal acute behavioral responses to moderate/high dose ethanol in GABA(A) receptor alpha 4 subunit knockout mice. *Alcohol Clin Exp Res* 32:10–18.
15. Brickley SG, Revilla V, Cull-Candy SG, Wisden W, Farrant M (2001) Adaptive regulation of neuronal excitability by a voltage-independent potassium conductance. *Nature* 409:88–92.
16. Tan KR, et al. (2010) Neural bases for addictive properties of benzodiazepines. *Nature* 463:769–774.
17. Lüscher C, Ungless MA (2006) The mechanistic classification of addictive drugs. *PLoS Med* 3:e437.
18. Koob GF, et al. (1998) Neurocircuitry targets in ethanol reward and dependence. *Alcohol Clin Exp Res* 22:3–9.
19. Saal D, Dong Y, Bonci A, Malenka RC (2003) Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. *Neuron* 37:577–582.
20. Heikkinen AE, Moykkynen TP, Korpi ER (2009) Long-lasting modulation of glutamatergic transmission in VTA dopamine neurons after a single does of benzodiazepine agonists. *Neuropsychopharmacology* 34: 290–298.