**ALCOHOL DEPENDENCY**

**A molecular mechanism for choosing alcohol over an alternative reward**

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Alcohol addiction leads to increased choice of alcohol over healthy rewards. We established an exclusive choice procedure in which ~15% of outbred rats chose alcohol over a high-value reward. These animals displayed addiction-like traits, including high motivation to obtain alcohol and pursuit of this drug despite adverse consequences. Expression of the γ-aminobutyric acid (GABA) transporter GAT-3 was selectively decreased within the amygdala of alcohol-choosing rats, whereas a knockdown of this transcript reversed choice preference of rats that originally chose a sweet solution over alcohol. GAT-3 expression was selectively decreased in the central amygdala of alcohol-dependent people compared to those who died of unrelated causes. Impaired GABA clearance within the amygdala contributes to alcohol addiction, appears to translate between species, and may offer targets for new pharmacotherapies for treating this disorder.

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Alcohol use accounts for almost 5% of global disease burden (1), and the harm from alcohol use has been estimated to exceed that due to heroin or cocaine (2). Basic neuroscience has identified brain circuits and molecular mechanisms that contribute to drug and alcohol reward, craving, and relapse (3, 4). These advances have, however, had limited impact on the treatment of addictive disorders, suggesting that research in model organisms needs to incorporate additional processes (3, 5).

Once established, alcohol addiction—hereafter equated with alcoholism—is a chronic relapsing disorder in which alcohol use becomes compulsive, i.e., continues despite negative consequences (6). Understanding the transition from controlled to compulsive alcohol use is a critical challenge for addiction research. In humans, only a subset of users transition to compulsive drug use (7, 8).

In contrast, in commonly used animal models, nearly all rats learn to self-administer addictive drugs, including alcohol (9). This points to the possibility that focusing on self-administration may be insufficient to identify key mechanisms of addiction (5, 10).

This realization has prompted important conceptual advances. When rats are allowed to self-administer cocaine over a prolonged period of time, only a minority of them develop compulsive drug taking (11, 12). Furthermore, most rats will cease to self-administer cocaine when a high-value alternative becomes available, but a subset of animals continue self-administration despite the presence of an alternative (13, 14).

In epidemiological studies, only a minority of exposed people develop addiction (5, 10).

Here, we set out to identify molecular mechanisms underlying the choice of alcohol over a natural reward. Conceptually and methodologically, we built on the exclusive choice model pioneered with cocaine and heroin by Ahmed and colleagues (5, 13, 14). We first established an exclusive choice-based method to identify rats that continue to self-administer alcohol at the expense of a high-value alternative, a sweet solution, and assessed whether these animals show other characteristics of clinical alcoholism (15).

We then used gene expression profiling to identify a molecular mechanism that mediates compulsive alcohol drinking at the expense of other high-value options.

**Intense sweetness outcompetes alcohol reward in most but not all rats**

Sweetness is a fundamental reward that is highly conserved between humans and other animals (16). To tap into its value without the confound of caloric content, we used as alternative reward a solution of the noncaloric sweetener saccharin. In a first experiment (see fig. SI for experimental timelines), rats (n = 32) were trained to self-administer 20% alcohol for about 10 weeks until reaching stable response rates on a fixed-ratio 3 (FR-3) reinforcement schedule (fig. S2, A and B).

They were then offered daily sessions of mutually exclusive choice between alcohol and 0.04% saccharin (fig. 1A). Overall, despite not having previously been trained to respond to saccharin, rats quickly started to choose more saccharin than alcohol (fig. 1B). The percentage of choice for saccharin over alcohol became even higher when a more rewarding concentration of saccharin, 0.2%, was introduced (fig. IC, significant effect of the saccharin concentration: F1,131 = 21.56, p < 0.0001, η² = 0.41).

Once operant responding had stabilized, the rats only chose alcohol over the alternative reward 26.2% of the time (fig. IC). However, although the vast majority of rats strongly preferred saccharin, a minority, 4 rats out of 32 in the first experiment (12.5% of the population, fig. ID, left) continued to choose alcohol despite having access to a high-value alternative (alcohol-prefering, AP). Although this was a small number, it aligns well with human addiction rates (7, 8) and prompted us to expand the study of individual differences in choice behavior. Ultimately, this percentage was stable across a large number of rats from successive batches (see below; fig. ID, right).

Alcohol preference was not influenced by holding prior history of alcohol and saccharin self-administration identical (fig. S3).

**Extensive pre-exposure to saccharin does not affect subsequent alcohol choice**

People who go on to develop addictive disorders have typically had extensive exposure to sweet reward prior to initiating alcohol use. We therefore tested whether this kind of preexposure would affect subsequent alcohol choice. A separate group of rats was offered the opportunity to drink a 0.2% saccharin solution or water (experiment 2, n = 32 per group) in their home cage for 4 weeks, before operant training was initiated. Rats that had access to the sweet solution consumed extensive amounts of saccharin during the first week of exposure (101.6 ± 6.8 ml/day) and maintained this consumption throughout their 4 weeks of exposure (week 4: 89.8 ± 5.4 ml/day; fig. S4A).

There was no significant difference in acquisition of alcohol self-administration between saccharin- and water-preexposed animals (fig. S4B), no main effect of group: saccharin versus water, F1,16 = 0.13, p = 0.72; main effect of sessions, F17,266 = 5.28, p < 0.001; η² = 0.08 but no interaction between group and sessions; F16,276 = 0.98, p = 0.48). Extensive preexposure to saccharin also left acquisition of the choice procedure unaffected (fig. S4C), no main effect of group: saccharin versus water, F1,17 = 0.40, p = 0.53; main effect of sessions, F17,266 = 7.82, p < 0.001; η² = 0.12 but no interaction between group and sessions: F16,276 = 1.00, p = 0.45).

Once responding had stabilized, both groups strongly favored the sweet solution (22.3 ± 3.8% alcohol choice for the saccharin-exposed group versus 20.6 ± 4.0% for the water-exposed group; fig. S4D), no main effect of group: saccharin versus water, F1,17 = 0.32, p = 0.577. Again, a subpopulation of animals chose alcohol over saccharin (fig. S4E, n = 3 in each group). Extensive pre-exposure to the sweet solution did not affect sampling (fig. S4F) or completed trials (F1,17 = 0.56, p = 0.46). Thus, the alcohol choice observed in experiment 1 was not sensitive to the individual’s prior history of sweet-reward exposure.

Finally, we investigated whether AP rats would persist in working for alcohol if the alternative reward was readily available. We used a modified choice paradigm in which rats had continuous access to both sources of reinforcement and found that AP rats maintained a strong preference for...
alcohol despite the concurrent availability of saccharin throughout five consecutive sessions (fig. S5).

**Alcohol-choosing rats show addiction-like behaviors**

Owing the low frequency of the AP phenotype, we repeated the first experiment on several batches of rats and screened them for preference during the choice procedure before carrying out further behavioral testing (Fig. 1D, right). We then characterized the subpopulation of animals that chose alcohol over saccharin with regard to addiction-like behaviors (experiments 3 and 4) and compared them to the much larger subpopulation of rats that stopped working for alcohol when an alternative high-value option was available (saccharin-preferring, SP). Only 95 rats out of 620 tested on the choice procedure continued to choose alcohol despite having access to a high-value alternative (15.3% of the population).

We first assessed the motivation of AP and SP rats to obtain and consume alcohol or saccharin using a progressive ratio reinforcement schedule (17). AP rats showed a higher motivation to obtain alcohol than SP rats (Fig. 1E, left bars), whereas the motivation to obtain saccharin did not statistically differ between the two groups (Fig. 1E, right bars; interaction between group (AP versus SP) × reinforcer (alcohol versus saccharin); $F_{1,90} = 31.13$, $p < 0.0001; \eta^2 = 0.26$; post hoc comparison AP versus SP rats for alcohol $p < 0.0001$, AP versus SP rats for saccharin $p = 0.33$).

We then investigated whether AP rats would maintain their alcohol drinking despite negative consequences: quinine adulteration or footshock punishment. AP rats showed a robust resistance to quinine adulteration (Fig. 1F, main effect of group, $F_{1,40} = 4.48$, $p < 0.05; \eta^2 = 0.10$). Their choice behavior remained unaffected even when alcohol was adulterated with a highly aversive concentration of quinine, 200 mg/liter. There was no statistical difference in the preference score for quinine alone when separately tested against water ($F_{1,40} = 0.09$, $p = 0.77$; fig. S6A), indicating that the resistance to quinine adulteration in AP rats was not due to an altered sensitivity to the quinine taste. Additionally, there was no statistical difference in preference score for 20% EtOH (left bars) or 0.2% saccharin (right bars; $n = 53$ for SP rats, 39 for AP rats).

**Fig. 1.** Addiction-like behaviors in rats that choose alcohol over a high-value alternative reward. (A) Schematic representation of the discrete choice procedure. (B) Percentage alcohol choice (±SEM) during acquisition ($n = 32$). (C) Stabilized percentage alcohol choice (±SEM; $n = 32$) (D) Individual distribution. (E) Selectively elevated motivation to obtain alcohol but not saccharin in alcohol-choosing rats [mean breakpoint ± SEM during separate sessions of progressive ratio responding for 20% EtOH (left bars) or 0.2% saccharin (right bars; $n = 53$ for SP rats, 39 for AP rats)]. (F) Compulsive alcohol drinking in alcohol-choosing rats, shown by resistance to quinine adulteration of the alcohol solution (percentage change ± SEM in quinine-adulterated alcohol drinking; $n = 28$ for SP rats, 14 for AP rats). (G) Compulsive alcohol drinking, shown by resistance to footshock (percentage change ± SEM in footshock-punished alcohol self-administration; $n = 66$ for SP rats, 41 for AP rats).
a 0.2% solution of saccharin ($F_{1,40} = 0.51, p = 0.48$) or 20% ethanol ($F_{1,40} = 0.48, p = 0.49$), indicating that alcohol preference during choice could not be explained by preexisting individual differences in preference for the taste of saccharin or alcohol.

Consistent with the quinine adulteration experiment, AP, but not SP, rats also maintained their responding for alcohol when drug delivery was paired with a contingent footshock punishment (16) (Fig. 1G; Kruskal–Wallis nonparametric analysis of variance, significant group effect: $H(3,218) = 73.7, p < 0.001$; significant difference between AP and SP rats at 0.2 mA ($p < 0.001$), but not at 0.1 mA ($p = 1$). This was not caused by any difference in pain sensitivity between AP and SP rats (fig. S6B). Shock-resistant alcohol consumption in this procedure per se could potentially be explained by complex processes such as counterconditioning or latent inhibition (19). However, because of the strong correlation of shock resistance with alcohol choice and also with resistance to quinine adulteration, we believe that such a mechanism is less likely. Instead, the most parsimonious explanation appears to be that increased appetitive motivation of AP rats for alcohol allows them to overcome the aversion to footshock punishment.

Together, these results show that AP rats display a constellation of behavioral traits that resemble those considered diagnostic for addiction (15): (i) The user gives up valuable social and recreational activities because of substance use, here modeled by an increase in choice of alcohol over the high-value alternative reward, saccharin; (ii) the user shows an increased motivation to obtain and take the drug, spending excessive time and effort on its procurement and consumption, here modeled using an elevated breakpoint on a progressive-ratio schedule (II, 27); and (iii) the user continues drug use despite its harmful and negative consequences, here modeled using continued alcohol self-administration despite quinine adulteration or delivery of a shock punishment contingent with drug delivery (II, 12).

Notably, the difference in total alcohol exposure during self-administration training and choice was minimal (fig. S7), making it unlikely that addiction-like behavior in AP rats resulted from alcohol exposure per se.

**Locomotor reactivity to novelty does not predict alcohol choice**

Because locomotor reactivity to a novel environment predicts aspects of cocaine seeking and taking (20, 21), we investigated whether this behavioral marker would also be associated with alcohol choice and other alcohol-related behaviors (experiment 5). We found that neither alcohol self-administration nor alcohol choice was associated with reactivity to novelty (figs. S8 and S9); nor was reactivity to novelty associated with anxiety-like behaviors (fig. S10).

**The GABA transporter GAT-3 is down-regulated in the amygdala of AP rats**

To identify molecular substrates of alcohol choice, we carried out a gene expression screen in several brain regions thought to be involved in alcohol-taking behaviors in rodent models (22) (experiment 6). We used a custom Nanostring nCounter array that contains probes targeting 310 transcripts previously hypothesized to be involved in drug addiction (23, 24). Using this highly sensitive and quantitative tool, we compared gene expression of AP and SP rats ($n = 7$ to 8 per group) in tissue punches from the nucleus accumbens (NAcc), caudate-putamen (CPU), prelimbic prefrontal cortex (PRL), infralimbic prefrontal cortex (IL), hippocampus (HIPP), and amygdala (AMG). This analysis found the highest number of significant changes in gene expression within the amygdala (Fig. 2A), with relatively few genes differentially expressed in the other five regions studied.

Pathway analysis showed that several genes implicated in γ-aminobutyric acid (GABA)-mediated transmission were expressed at significantly lower levels in the amygdala of AP rats (Fig. 2B and table S1). In particular, the expression of the GABA transporter GAT-3 ($Slc6a3$) was decreased. The mammalian genome contains four genes that encode high-affinity GABA transporters (GAT-3, $Slc6a3$; GAT-2, $Slc6a2$; GAT-3, $Slc6a3$; and BGT-1, $Slc6a12$) (25, 26). GABA transporters are sodium- and chloride-dependent members of the solute carrier family 6 (Slc6) and mediate rapid clearance of GABA to maintain low extracellular GABA concentrations (27). These transporters are therefore likely to play an important role in controlling the actions of GABA, the principal inhibitory neurotransmitter of the brain (28).

Using quantitative polymerase chain reaction (qPCR), we confirmed the differential expression of GAT-3 detected by the Nanostring analysis. Because the three other genes of the GABA transporter family were not represented on our Nanostring panel, we assessed their expression using qPCR and found that GAT-1 and BGT-1 were also significantly down-regulated in the amygdala of AP rats; there was also a trend for lower GAT-2 expression (Fig. 2C). We used qPCR to

**Fig. 2. The GABA transporter GAT-3 is down-regulated in the amygdala of AP rats.** (A) A gene expression screen points to the amygdala (AMG) as a region of most prominent differential gene expression between AP and SP rats (other regions analyzed: nucleus accumbens (NAcc), caudate putamen (CPU), prelimbic prefrontal cortex (PRL), infralimbic prefrontal cortex (IL), and hippocampus (HIPP)). (B) Pathway analysis shows that a gene network comprising several genes involved in GABAergic transmission is down-regulated in the amygdala of AP rats (green color indicates down-regulation in AP rats, whereas red color indicates up-regulation). (C) mRNA expression of GAT-3 and other GABA transporters is decreased in the amygdala of AP rats ($n = 7$ to 8 per group). (D) mRNA expression of several other genes involved in GABAergic transmission is decreased in the amygdala of AP rats ($n = 7$ to 8 per group).
confirm the down-regulation of additional genes involved in GABA-mediated transmission identified by the Nanostring screen. The expression of genes encoding several GABA<sub>A</sub> receptors subunits, Gabrbq, Gabrg3, Gabbr1, was also lower in AP rats, presumably reflecting adaptations to an increased GABA tone due to down-regulated expression of transporters that clear extracellular GABA (Fig. 2D; for detailed statistics, see table S2). Results of the Nanostring and qPCR analyses were highly correlated (fig. S11). Thus, up-regulated GABA-mediated transmission in the amygdala represents a candidate mechanism for mediating alcohol choice, which is in agreement with and expands on prior work (29, 30).

In further support of a role for GAT-3 in preclinical models of dependence, this transcript was also selectively down-regulated in the amygdala of the Indiana alcohol preferring rats P-rats (compared to their nonpreferring counterpart; p < 0.01, fig. S12). This strain has been selectively bred from Wistar rats for high alcohol drinking and has been proposed to model aspects of human alcoholism (31, 32).

### AP rats show increased tonic inhibition in central amygdala (CeA)

To establish whether decreased expression of GAT-3 results in an increased GABA tone in the amygdala of AP rats, we performed slice electrophysiology experiments (experiment 7). Field potential recordings revealed a significantly greater synaptic output from the CeA in AP rats compared to brain slices from SP rats (main effect of group: AP versus SP: F<sub>1,34</sub> = 17, p < 0.01) (Fig. 3A). There was no group effect on neurotransmitter release probability, as assessed by paired pulse stimulation (Fig. 3B; SP versus AP: F<sub>5,9</sub> = 0.83, p > 0.05). Disinhibition induced by the GABA<sub>A</sub> receptor antagonist bicuculline (20 μM) was significantly greater in slices from AP rats, indicating an enhanced GABA tone (AP versus SP: F<sub>1,23</sub> = 6.03, p < 0.05) (Fig. 3C). These data provide consistent support for an increased tonic inhibition caused by elevated levels of extracellular GABA in AP rats, similar to that seen after induction of alcohol dependence (30). Because GABA-mediated transmission in the amygdala is believed to play an important role in the modulation of anxiety responses (33–35), we also assessed anxiety-like behaviors of AP and SP rats using the elevated plus-maze (36, 37). In agreement with our electrophysiology findings, we observed higher anxiety-like behavior in AP rats (fig. S13).

**GAT-3 knockdown in the CeA mimics the increased tonic inhibition of AP rats**

We next examined whether the functional consequences of GAT-3 down-regulation in AP rats at a synaptic level could be mimicked using a viral knock-down (KD) approach. We injected rats with a short hairpin-mediated RNA (shRNA) adeno-associated virus (AAV) vector targeting Slc6a11 in the amygdala (see fig. S14 for in vitro validation of the construct), and performed ex vivo electrophysiology in amygdala slices to assess the resulting changes in GABA-mediated neurotransmission. In agreement with our findings in AP rats, we observed a significantly greater synaptic output from the CeA in GAT-3 KD rats compared to brain slices from rats injected with the scrambled control vector (Fig. 3D; GAT-3 KD versus Scrambled: F<sub>1,40</sub> = 19, p < 0.001). Again, there was no group effect on neurotransmitter release probability, as assessed by paired pulse stimulation (Fig. 3E; GAT-3 KD versus Scrambled: F<sub>1,57</sub> = 0.44, p > 0.05); whereas disinhibition induced by the GABA<sub>A</sub> receptor antagonist bicuculline (20 μM) was significantly greater in slices from GAT-3 KD rats, indicative of an enhanced GABA tone (Fig. 3F; GAT-3 KD versus scrambled versus: F<sub>1,256</sub> = 7.70, p < 0.05). Whole-cell recordings revealed no effect of GAT-3 KD on recorded spontaneous (sIPSCs) or miniature (mIPSCs) inhibitory postsynaptic currents, indicating that baseline activity of GABA-releasing neurons is unaltered (fig. S15, A to D). In contrast, there was no significant effect of bicuculline administration in the basolateral amygdala (main effect of group: F<sub>1,20</sub> = 1.43, p = 0.25; main effect of time: F<sub>10,200</sub> = 4.82, p < 0.0001; group × time interaction: F<sub>10,200</sub> = 0.35, p = 0.09; fig. S15). These results show that a KD of GAT-3 mimics the increased GABA tone in the CeA of AP rats.

**A causal role of GAT-3 in amygdala for alcohol choice behavior**

To establish a causal role of GAT-3 for alcohol choice, we injected a separate group of SP animals (experiment 8, n = 31) with the shRNA AAV vector targeting Slc6a11 in the amygdala (fig. S16) and examined the consequences of down-regulated GAT-3 expression on choice between alcohol and the alternative high-value saccharin reward. Fluorescent in situ hybridization using RNAscope confirmed that Slc6a11 was down-regulated in the CeA of GAT-3 KD animals compared to scrambled controls (Fig. 4, A and B; Mann-Whitney U = 1, two-sided exact p = 0.009). Using mass spectrometry (fig. S17), we also found that the knockdown of the Slc6a11 transcript resulted in a significant decrease of the transporter protein (Fig. 4C; t test followed by a Benjamini-Hochberg multiple testing correction, p < 0.001).

We tested the effects of GAT-3 KD in the amygdala of rats preselected for high (~75% of baseline) levels of saccharin choice. In the first posturgical week, we observed an initial increase of alcohol choice that was nonspecific, but behavior of control vector-injected animals returned to baseline within a week and remained at this level throughout the experiment. In contrast, beginning during week 2, we observed a shift of choice behavior toward alcohol in GAT-3 KD animals. This shift increased during week 3, coinciding with full expression of the shRNA vector, and was maintained for the remainder of the sessions (Fig. 4D, main effect of group, F<sub>1,27</sub> = 11.02, p < 0.01; η<sup>2</sup> = 0.29; interaction between group and sessions, F<sub>14,378</sub> = 2.01, p < 0.05; η<sup>2</sup> = 0.07). Once behavior was stable, the preference of GAT-3 KD animals was reversed, and they chose significantly more alcohol compared to control vector-treated animals (Fig. 4E, 47.1% alcohol choice versus 18.9%, F<sub>1,27</sub> = 18.71, p < 0.001).
out alcohol dependence (from deceased human subjects with and without alcohol dependence measured by RNAseq. Red dots show the expression of Slc6a11 (GAT-3). Blue labeling represents DAPI (4′,6-diamidino-2-phenylindole) staining, and green labeling confirms virus injection. (B) Slc6a11 (GAT-3) mRNA levels after a viral-mediated knockdown (n = 5 to 6 per group). (C) Fold change in GAT-3 protein levels measured by mass spectrometry (n = 7 to 8 per group). (D) Percentage alcohol choice during acquisition. (E) Stabilized percentage alcohol choice (mean ± SEM) (n = 14 to 15 per group).

Fig. 5. GAT-3 is selectively down-regulated in the CeA of postmortem brains from individuals with confirmed alcohol dependence. Normalized read counts were extracted from an RNA-sequencing experiment of postmortem human brain samples. P values indicate a generalized linear model, with alcohol dependence as main factor, while simultaneously controlling for sex, ethnicity, smoking, and age. CeA, central nucleus of amygdala; BLA, basolateral amygdala; CTX, cortex; NAcc, nucleus accumbens. n = 29 for controls, 9 for alcohol-dependent individuals.

p < 0.001; η² = 0.41). GAT-3 down-regulation did not affect control behaviors (fig. S18). Thus, decreased expression of GAT-3 in the amygdala is sufficient to promote choice of alcohol at the expense of a high-value alternative. This effect of GAT-3 KD was specific for choice behavior and was achieved in the absence of effects on alcohol- or saccharin self-administration (experiment 9, fig. S20).

GAT-3 is specifically down-regulated in the CeA of alcohol-dependent people

Finally, we examined the potential translational validity of our findings. To do so, we carried out RNA sequencing of postmortem tissue samples from deceased human subjects with and without alcohol dependence (38). After correcting for common confounders such as age and smoking (table S3), patients with confirmed alcohol dependence showed down-regulation of GAT-3 in the CeA compared to controls (generalized linear model, p < 0.01), but not in other brain regions (Fig. 5).

Concluding remarks

We developed a procedure in which rats are allowed a mutually exclusive choice between alcohol and a high-value alternative, a highly rewarding concentration of the noncaloric sweetener saccharin. Using this procedure, we found that the vast majority of outbred rats stopped responding to alcohol when offered the opportunity to access the high-value alternative reward. However, a subpopulation continued to choose alcohol despite the presence of the alternative. The frequency of this population was stable in multiple batches of animals, as confirmed by screening a total of 620 rats on the choice procedure.

Rats that chose alcohol over the high-value alternative displayed addiction-like behaviors, increased motivation to obtain alcohol, and continued use despite adverse consequences. These traits mimic key clinical diagnostic criteria for alcoholism. Furthermore, the percentage of AP rats (15.3%) is similar to human alcoholism rates (7, 8).

The addiction-like phenotype in rats was associated with decreased expression of the GABA transporter GAT-3 in the amygdala, accompanied by down-regulation of several GABA_A receptor subunit transcripts. The latter observation presumably reflects increased GABA tone due to lower clearance of extracellular GABA. Accordingly, AP rats showed increased GABA-mediated inhibition in the CeA in slice electrophysiology experiments. A viral-vector-mediated knockdown of Slc6a11 mimicked this effect at the synaptic level and converted SP rats into AP rats in vivo. The latter finding demonstrates a causal role of Slc6a11 for alcohol choice. Thus, increased GABAergic tone in the CeA due to reduced GABA uptake by GAT-3 transporters contributes to behaviors that are key for alcohol addiction.

Our findings are convergent with prior reports of increased GABA tone in CeA as a result of alcohol dependence and with results in which GABA_A receptor agonists administered into CeA promote, whereas antagonists inhibit, alcohol self-administration (29, 30). Our data provide strong support for a causal contribution of neuroadaptations affecting GABA signaling within the amygdala to the development of alcohol addiction. Furthermore, our findings suggest that preexisting differences in GABAergic gene expression in the CeA may also influence susceptibility to developing alcohol addiction. Collectively, these experiments identify impaired GABA clearance within CeA as a molecular mechanism that contributes to behavioral traits central to alcohol addiction.
REFERENCES AND NOTES


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SUPPLEMENTARY MATERIALS

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Materials and Methods

Figs. S1 to S20
Tables S1 to S3
References (39–54)

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Finding the vulnerable minority

"Only" about 10 to 15% of people exposed to alcohol develop alcohol-related problems. The behavioral repertoire of people confronted with opportunities to consume alcohol involves numerous choices between this drug reward and healthy alternatives. Augier et al. established a choice procedure that begins to address alcohol addiction in rats (see the Perspective by Spanagel). They found that a minority of outbred rats continued to self-administer alcohol even when a high-value alternative (such as sugar) was available. That minority displayed a remarkable constellation of behavioral traits resembling the human clinical condition, including a high motivation to obtain alcohol and continued use despite adverse consequences. The cause was impaired GABA (γ-aminobutyric acid) clearance in the central amygdala. Postmortem tissue analysis supported the possibility of a similar pathology in human alcoholism.

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