INDIVIDUAL SUSCEPTIBILITY

Genetic factors in addiction: QTL mapping and candidate gene studies implicate GABAergic genes in alcohol and barbiturate withdrawal in mice

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Abstract
Quantitative trait locus (QTL) mapping has allowed dramatic progress toward the detection and chromosome mapping of minor and major gene loci involved in murine responses to alcohol and other drugs of abuse. Here we focus on the identification of QTLs for one particular trait relevant to addiction, drug withdrawal following acute or chronic drug administration. To date, five significant QTLs (p < 5×10^{-5}) and six suggestive QTLs (p < 0.001) have been mapped to specific murine chromosomes for alcohol and pentobarbital withdrawal, indicating the presence of a relevant gene or genes at each location. Overlapping QTLs for alcohol withdrawal and pentobarbital withdrawal are identified on murine chromosomes 1, 4, and 11, and may detect the influence of common genes. For many QTLs, candidate genes with relevant neurobiological function lie within the mapped region. Notably, several QTLs for alcohol and pentobarbital withdrawal are in proximity to genes that directly or indirectly affect GABA_A receptor-mediated transmission, which has been implicated in some of the actions of alcohol and other drugs. These include a cluster of GABA_A receptor genes and genes encoding the enzymes steroid 5α-reductase-1 (involved in biosynthesis of the neuroactive steroid allopregnanolone) and glutamic acid decarboxylase-1 (involved in GABA biosynthesis).

This paper will discuss data that examines the involvement of GABAergic genes in withdrawal and other drug responses, including genetic variation in gene sequence, expression and function.

Introduction
Studies of strain and individual differences using animal models for addiction liability are useful as a means to identify potential genetic determinants of liability in humans. While addiction in humans is complex and encompasses symptoms such as compulsive drug use and the occurrence of a withdrawal syndrome, it is difficult to study addiction per se with a single animal model. However, animal models can be used to test various aspects of addiction, such as withdrawal, tolerance, preference and other behavioral re-
sponses to alcohol and other drugs of abuse that may be involved in addiction, as well as to dissect the contribution of genetic and environmental factors to the development of addiction.

A quantitative trait locus (QTL) is a site on a chromosome containing an allele (gene) that influences a continuously distributed or quantitative trait. Because of their typically polygenic and environmental determination, quantitative traits are also referred to as complex traits. The identification of those chromosomal regions where marker allelic and trait variation significantly covary, thus implicating the presence of a QTL, is now a standard method for detecting and mapping QTLs (see Belknap et al., 1997 for a recent review of QTL mapping methods). The majority of QTL analyses for behavioral responses to alcohol have used populations derived from the C57BL/6J (B6) and DBA/2J (D2) progenitor strains [e.g. BXD recombinant inbred (RI) strains and F2 mice]. The B6 and D2 strains differ markedly in their responses to many drug effects (Crabbe & Harris, 1991). QTL mapping using B6- and D2-derived animals has allowed dramatic progress toward the detection and chromosome mapping of minor and major gene loci involved in acute alcohol withdrawal (Buck et al., 1997), acute pentobarbital withdrawal (Buck et al., 1999), chronic alcohol withdrawal (Crabbe, 1998; K. J. Buck and J. C. Crabbe unpublished data) and other traits that may be involved in behavioral responses to alcohol and other drugs (see Crabbe et al., 1999).

Here we present recent progress made in identifying specific chromosome regions (i.e. QTLs) that are relevant to the development of physical dependence to alcohol and other drugs, and identify candidate genes of current and future interest in this research. Selective breeding studies suggest the influence of common (but anonymous) genes (Crabbe, Merrill & Belknap, 1991). We theorize that one or more common genes may be identified by overlapping QTLs for alcohol and barbiturate withdrawal. Because alcohol and pentobarbital have extensive pharmacological similarity, and are believed to produce many of their effects via modulation of GABAergic receptor-mediated transmission, genetic determinants of GABAergic transmission are plausible candidates for the genetic overlap between alcohol and barbiturate withdrawal. Moreover, a number of QTLs detected for acute ethanol and pentobarbital withdrawal, as well as for chronic ethanol withdrawal, map near GABAergic candidate genes. Here, we examine the possible contribution of GABAergic genes and their products as genetic factors for predisposition to the effects of ethanol, pentobarbital and other abused drugs.

### Quantitative trait locus (QTL) mapping in mice

#### Acute alcohol withdrawal QTLs

Physical dependence is operationally defined as the manifestation of physical disturbances (withdrawal syndrome) after alcohol administration is suspended. McQuarrie & Fingle (1958) first demonstrated a state of withdrawal central nervous system hyperexcitability following acute alcohol administration. The D2 strain is a well characterized animal model with severe acute (and chronic) alcohol withdrawal convulsions, whereas the B6 strain has mild withdrawal reactions (Crabbe, Young & Kosobud, 1983; Buck et al., 1997). In contrast, D2 mice show basal withdrawal convulsions that are either lower (Roberts, Crabbe & Kath, 1992) or do not differ (Crabbe, 1998) versus B6 mice. Using three populations derived from the B6 and D2 strains (BXD RI, F2 cross and selectively bred mice), Buck et al. (1997) mapped three significant QTLs associated with acute alcohol withdrawal severity (Alcw1–3) on chromosomes 1, 4, and 11 (Table 1). Together, these QTLs account for 68% of the genetic variability in acute alcohol withdrawal severity among mice derived from the B6 and D2 strains. Candidate genes in proximity to the chromosome 11 locus include genes encoding the GABA<sub>A</sub> receptor α<sub>1</sub>, β<sub>6</sub>, γ<sub>2</sub> and β<sub>2</sub> subunits (Fig. 1). In addition, suggestive linkage is indicated for two loci on mouse chromosome 2, one near Gad1 which encodes glutamic acid decarboxylase-1, and the other near the El2 locus which influences the seizure phenotype in the neurological mutant strain E1.

#### Acute pentobarbital withdrawal QTLs

Withdrawal following an acute injection of pentobarbital was assessed in order to focus on the central nervous system mechanisms of withdrawal, whereas chronic barbiturate treatment also induces metabolic tolerance (Flint & Ho, 1980). QTL analyses for acute pentobarbital
withdrawal convulsions used the same general methods to identify a QTL (*Pbw1*) in the same region of chromosome 1 where *Alcw1* was mapped (Buck et al., 1999). We also identified two suggestive pentobarbital withdrawal QTLs on chromosomes 4 and 11 ( provisionally referred to as *Pbw2* and *Pbw3*, see Table 1), which may overlap *Alcw2* and *Alcw3* (Fig. 1). Among BXD recombinant strains, acute alcohol withdrawal and pentobarbital withdrawal severity are highly correlated ($r = 0.79$, $p < 0.001$). Because this correlation is based on BXD strain means, it is predominantly genetic in origin (Hegmann & Possidente, 1981), and indicates $\sim 62\%$ genetic overlap between acute alcohol withdrawal and pentobarbital withdrawal severity. This suggests the presence of common genes, and provides supporting evidence that overlapping QTLs mapped for acute alcohol and pentobarbital withdrawal may identify some of the same genes.

### Chronic alcohol withdrawal QTLs

In the early 1970s, Dr Dora Goldstein developed a system to induce dependence on alcohol in mice by administering ethanol vapor continuously to animals confined in an inhalation chamber, and described and quantified the characteristic handling-induced convolution (HIC) displayed during withdrawal (see Goldstein & Pal, 1971). Genetic mapping analyses for chronic alcohol withdrawal using the BXD RI strains recently identified ten provisional QTLs (Crabbe, 1998), including a genetic correlation ($r = 0.66$, $p < 0.001$) between chronic alcohol withdrawal severity and a region of chromosome 13 to which the gene for the enzyme 5α-reductase-1 (*Srd5a1*) has been mapped (Jenkins et al., 1991). Subsequent analyses using an F$_2$ intercross definitively mapped a QTL (*Calw1*) to the distal region of chromosome 1 (Table 1), which may be identical to *Alcw1* and *Pbw1* (Fig. 1). Taken together, the BXD RI and F$_2$ experiments also identify suggestive QTLs (referred to as *Calw2* and *Calw3*) on chromosomes 13 and 4 (Table 1). *Calw3* maps to the same region of chromosome 4 as *Alcw2* and *Pbw2* (Fig. 1).

### QTLs and candidate gene testing

While QTL mapping cannot provide direct evidence for which specific genes are involved in a particular trait, mapping experiments can identify the probability that a gene exists at the chromosomal location (typically expressed as logarithm of the odds of linkage, or LOD score) as well as the confidence interval that surrounds

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### Table 1. Identification of five definitively mapped quantitative trait loci (QTLs) and six suggestive QTLs for alcohol and pentobarbital withdrawal in mice

<table>
<thead>
<tr>
<th>Drug response</th>
<th>QTL$^b$</th>
<th>LOD</th>
<th>Chr</th>
<th>1-LOD support interval (cM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute alcohol withdrawal$^c$</td>
<td><em>Alcw1</em></td>
<td>5.6</td>
<td>1</td>
<td>66-ter</td>
</tr>
<tr>
<td></td>
<td><em>Alcw2</em></td>
<td>5.6</td>
<td>4</td>
<td>$\sim 20$–58</td>
</tr>
<tr>
<td></td>
<td><em>Alcw3</em></td>
<td>4.1</td>
<td>11</td>
<td>16–28</td>
</tr>
<tr>
<td></td>
<td><em>(Alcw4)</em></td>
<td>2.3</td>
<td>2</td>
<td>27–49</td>
</tr>
<tr>
<td></td>
<td><em>(Alcw5)</em></td>
<td>2.1</td>
<td>2</td>
<td>$\sim 50$–80</td>
</tr>
<tr>
<td>Acute pentobarbital withdrawal$^d$</td>
<td><em>Pbw1</em></td>
<td>6.2</td>
<td>1</td>
<td>88-105</td>
</tr>
<tr>
<td></td>
<td><em>(Pbw2)</em></td>
<td>3.3</td>
<td>4</td>
<td>38-ter</td>
</tr>
<tr>
<td></td>
<td><em>(Pbw3)</em></td>
<td>2.3</td>
<td>11</td>
<td>cen-26</td>
</tr>
<tr>
<td>Chronic alcohol withdrawal$^e$</td>
<td><em>Calw1</em></td>
<td>$\sim$6.0</td>
<td>1</td>
<td>$\sim 80$–ter</td>
</tr>
<tr>
<td></td>
<td><em>(Calw2)</em></td>
<td>$\sim$3.3</td>
<td>13</td>
<td>$\sim 26$–47</td>
</tr>
<tr>
<td></td>
<td><em>(Calw3)</em></td>
<td>$\sim$2.5</td>
<td>4</td>
<td>$\sim 21$–62</td>
</tr>
</tbody>
</table>

$^a$Chr, chromosome; LOD, logarithm of the odds of linkage; cM, centiMorgan; ter, terminus; cen, centromere. $^b$Significant or suggestive linkage using Lander & Kruglyak criterion (1995). Most likely QTL positions are shown in Fig. 1. Locus names are assigned to QTLs that have been definitively mapped ($p < 5 \times 10^{-5}$), whereas loci shown in parentheses indicate suggestive linkages ($p < 1 \times 10^{-5}$). $^c$Data from Buck et al., 1997. $^d$Data from Buck et al., 1999. $^e$Data from Crabbe, 1998 and J. C. Crabbe and K. J. Buck (unpublished observations). LOD scores are approximate pending the testing of additional animals.
the gene’s location. Once a plausible candidate gene(s) has been identified in the QTL region, the next crucial step is to show that the gene differs in protein coding sequence and/or expression among the appropriate genetic animal models (e.g., those used to map the QTL). Allelic differences in the gene’s coding sequence may result in different gene products which may differ in biological function. Ideally, a coding sequence difference is identified that could clearly have an impact on function (e.g., stop codons, non-conservative amino acid changes in active sites). Allelic differences that produce a large functional impact can provide tremendous insight into how a candidate gene could affect drug action. Alternatively, differences in gene expression can affect protein expression and function, and are more frequently detected than allelic differences in coding sequences.

Genetic polymorphism in GABAergic genes
Biochemical and genetic studies suggest GABA$_A$ receptors as a site for some actions of alcohol, barbiturates, benzodiazepines and other drugs, but the molecular mechanisms responsible for genetic differences in initial drug sensitivity, and adaptation following acute or chronic drug treatment remain to be elucidated. GABA$_A$ receptor-operated chloride ion channels are thought to be an oligomeric complex composed of multiple subunits, and different subunit combinations affect GABA$_A$ receptor pharmacology (reviewed by Whiting, McKernan & Wafford, 1995). Molecular cloning has identified a number of distinct but related genes coding for a number of subunit isoforms (e.g. $\alpha_1$–$\alpha_6$, $\beta_1$–$\beta_4$, $\gamma_1$–$\gamma_3$, $\delta$, $\varepsilon$, $\pi$ and $\rho_1$–$\rho_2$). Allelic variation in GABA$_A$ receptor subunit gene sequences have been identified, and in some cases shown to have a significant impact on GABA$_A$ receptor function (Table 2). Genetic variation in genes that indirectly affect GABA$_A$ receptor-mediated activity (e.g. steroid 5$\alpha$-reductase-1) may also contribute to genetic diversity in alcohol and drug responses.

GABA$_A$ receptor $\gamma_2$ subunit polymorphism among B6, D2 and BXD RI mice. The $\gamma_2$ subunit is expressed widely throughout the brain (see McKernan & Whiting, 1996) and is required for normal channel conductance (Gunther et al., 1995). Buck & Hood (1998) showed recently that the GABA$_A$ receptor $\gamma_2$ subunit gene

Figure 1. Alcohol and pentobarbital withdrawal quantitative trait loci (QTLs) and candidate genes. QTLs identified on mouse chromosomes 1, 2, 4, 11, and 13 and X are indicated, in their most likely location as determined by mapping studies. QTL locations are given in centimorgans (cM) relative to marker loci, and also indicated to the left of each chromosome (positions reproduced from Silver & Nadeau, 1997). QTL abbreviations: Alcw, acute alcohol withdrawal; Pbwt, acute pentobarbital withdrawal; Calw, chronic alcohol withdrawal. Candidate genes: Atp2b4, ATPase, Ca$^{2+}$ transporting; Atp1b1, Na$^+$/K$^+$ ATPase, $\beta_1$ polypeptide; Atpla2, Na$^+$/K$^+$ ATPase, $\alpha_2$ polypeptide; Gad2, glutamic acid decarboxylase-2; Scn1a, sodium channel, voltage-gated, type 1, $\alpha$ polypeptide; Gad1, glutamic acid decarboxylase-1; Grik3, glutamate receptor, ionotropic, kainate 3; Gabrg2, GABA receptor, $\gamma_2$ subunit; Gabra1, GABA$_A$ receptor, $\alpha_1$ subunit; Gabra6, GABA$_A$ receptor, $\alpha_6$ subunit; Drd1a, dopamine receptor D$_1$; Srd5a1, steroid $5\alpha$-reductase; Dat1, dopamine transporter.
Table 2. Sequence comparison of GABAergic candidate genes between different animal models

<table>
<thead>
<tr>
<th>Gene</th>
<th>Product</th>
<th>Mice compared</th>
<th>Sequence variation</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA_A receptor genes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gabra1</td>
<td>a1 subunit</td>
<td>LS/SS</td>
<td>none</td>
<td>Keir, Deitrich &amp; Sikela, 1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B6/D2</td>
<td>SNP(5); no Δ aa sequence</td>
<td>Wang et al., 1992a</td>
</tr>
<tr>
<td>Gabra2</td>
<td>a2 subunit</td>
<td>B6/D2</td>
<td>none</td>
<td>Wang et al., 1992a</td>
</tr>
<tr>
<td>Gabra3</td>
<td>a3 subunit</td>
<td>B6/D2</td>
<td>none</td>
<td>Wang et al., 1992a</td>
</tr>
<tr>
<td>Gabra6</td>
<td>a6 subunit</td>
<td>AT/ANT (rat)</td>
<td>SNP(1); Δ aa sequence</td>
<td>Korpi et al., 1993</td>
</tr>
<tr>
<td>Gabrh1</td>
<td>β1 subunit</td>
<td>B6/D2</td>
<td>none</td>
<td>Kamatchi et al., 1995</td>
</tr>
<tr>
<td>Gabrh2</td>
<td>β2 subunit</td>
<td>B6/D2</td>
<td>none</td>
<td>Kamatchi et al., 1995</td>
</tr>
<tr>
<td>Gabrh3</td>
<td>β3 subunit</td>
<td>B6/D2</td>
<td>SNP(3); no Δ aa sequence</td>
<td>Kamatchi et al., 1995</td>
</tr>
<tr>
<td>Gabrg1</td>
<td>γ1 subunit</td>
<td>B6/D2</td>
<td>none</td>
<td>Wang et al., 1998</td>
</tr>
<tr>
<td>Gabrg2</td>
<td>γ2 subunit</td>
<td>B6/D2, BXD</td>
<td>SNP(3); Δ aa sequence(1)</td>
<td>Buck &amp; Hood, 1998; Hood &amp; Buck, 2000</td>
</tr>
<tr>
<td>Gabrd</td>
<td>δ subunit</td>
<td>B6/D2</td>
<td>SNP(1)^{2}</td>
<td>Wang, Kofufi &amp; Burt, 1992b</td>
</tr>
<tr>
<td>Neuroactive steroid biosynthesis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Srd5a1</td>
<td>sterol 5α-reductase-1</td>
<td>B6/D2</td>
<td>RFLP(1)</td>
<td>Jenkins et al., 1991</td>
</tr>
</tbody>
</table>

*LS/SS, long-sleep vs. short-sleep selectively-bred mice; B6/D2, C57BL/6J vs. DBA/2J inbred mice; BXD, B6 × D2 recombinant inbred strains; AT/ANT, alcohol tolerant vs. alcohol non-tolerant selectively-bred rats; WSP/WSR, withdrawal seizure-prone vs. -resistant selectively-bred mice; SNP, single nucleotide polymorphism; aa, amino acid; RFLP, restriction fragment length polymorphism. ^{a}None of the five SNPs affect amino acid sequence, but one does create a Nsi I restriction site. ^{b}AT express α6 (R100); ANT express both α6 (Q100); devoid of diazepam-insensitive high-affinity [^3]H]Ro15-4513 binding) and α6 (R100). ^{c}Long and short variants are detected in both the B6 and D2 strains, and appear to be homologous to alternatively spliced variants detected in AT and ANT rats that are generated by the alternative use of 3′ acceptor sites at the end of intron 3 (Korpi et al., 1994). ^{d}None of the three SNPs affect amino acid sequence, but two create RFLPs (PrI and RsaI). ^{e}One of the three SNPs affects amino acid sequence; the B6 derived form is γ3 (A11) whereas the D2 derived variant is γ3 (T11). ^{f}Substitution of His (H229) for a conserved Tyr (Y229) was observed among clones from both the D2 and B6 inbred strains.
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(Gabrg2) is polymorphic between the B6 and D2 strains (Ala/Thr-11), and that the resulting difference in amino acid sequence significantly alters the predicted secondary structure of the mature γ2 peptide. Among BXD RI strains, polymorphism in Gabrg2 is genetically correlated with acute alcohol withdrawal and other alcohol response traits that may generally be characterized as debilitating or motivationally negative (e.g. ethanol-conditioned taste aversion, ethanol-induced motor incoordination, ethanol-induced hypothermia) (Hood & Buck, 2000). Transgenic heterozygous mice (Gabrg2<sup>2+/−</sup>) show enhanced behavioral inhibition toward aversive stimuli and heightened responsiveness in trace fear conditioning and ambiguous cue discrimination learning (Crestani et al., 1999), but it remains to be determined whether reduced expression/function of the γ2 subunit affects alcohol response traits of interest in Gabrg2<sup>2+/−</sup> mice.

To date, alcohol response traits have been examined in mice with targeted disruption of the long splice variant of the γ2 subunit (Homanics et al., 1999). These γ2<sub>L</sub> knockout mice have a normal complement of functional receptors, and show normal behavioral effects of ethanol (Homanics et al., 1999). Future studies will test whether polymorphism in the Gabrg2 coding sequence (resulting in allelic variation in the γ2 subunit, Ala/Thr-11) affects the actions of ethanol on GABA<sub>A</sub> receptors, or whether this polymorphism represents a marker in linkage disequilibrium with functionally relevant polymorphism elsewhere in the Gabrg2 gene (e.g. the promoter) or a nearby gene.

GABA<sub>A</sub> receptor α<sub>6</sub> subunit variant in AT and ANT rats. ANT (Alcohol-Non-Tolerant) rat cerebella are generally devoid of diazepam-insensitive high-affinity [<sup>3</sup>H]Ro15-4513 binding (Siegart et al., 1987), but in non-selected strains such binding is associated with a granule-cell-specific GABA<sub>A</sub> receptor complex containing the α<sub>6</sub> subunit (Luddens et al., 1990). This profound effect on GABA<sub>A</sub> receptor function is due to a point mutation in the α<sub>6</sub> gene in ANT rats that results in a single amino acid substitution (Korpi et al., 1993). In vitro studies show that the mutant recombinant α<sub>6</sub>(Q100)β<sub>2</sub>γ<sub>2</sub> receptors show diazepam-sensitive [<sup>3</sup>H]Ro15-4513 binding and diazepam potentiation of GABA<sub>A</sub> receptor-mediated currents, whereas wild-type cerebellar GABA<sub>A</sub> receptor and α<sub>6</sub>β<sub>2</sub>γ<sub>2</sub> recombinant receptors are diazepam-insensitive (Korpi et al., 1993). Notably, this mutation was only detected in about half of the ANT clones, suggesting that pharmacologically atypical ANT rats may be heterozygous for the mutation. Targeted disruption of the α<sub>6</sub> subunit gene (Gabra6) does not seem to affect ethanol or pentobarbital-induced loss of righting reflex (Homanics et al., 1997) or ethanol tolerance and withdrawal responses (Homanics et al., 1998) in knockout mice on a mixed genetic background of the 129/SvJ and C57BL/6J strains.

Steroid 5α-reductase-1 polymorphism in B6- and D2-derived mice. The neuroactive steroid allopregnanolone (3α-hydroxy-5α-pregnan-20-one) is a potent positive modulator of GABA<sub>A</sub> receptors (see Paul & Purdy, 1992; Lambert et al., 1995). Endogenous allopregnanolone concentrations can reach levels in both males and females that are in the range of concentrations shown to potentiate the in vitro action of GABA at GABA<sub>A</sub> receptors (Morrow, Suzdak & Paul, 1987; Gee et al., 1988; Paul & Purdy, 1992; Barbaccia et al., 1996). These findings suggest that fluctuations in endogenous allopregnanolone levels may modulate GABA<sub>A</sub> receptors. Restriction fragment length polymorphism analysis has demonstrated that the steroid 5α-reductase-1 gene (Srd5a1) is polymorphic between B6 and D2 mice and the BXD RI strains (Jenkins et al., 1991). While it is not known whether this polymorphism alters the coding sequence, 5α-reductase-1 enzyme activity in hippocampus and liver is significantly different between alcohol naïve B6 and D2 mice (D. Finn and C. Roselli, unpublished data), suggesting that these two genotypes may have functionally different gene products or differ in enzyme expression (see below).

GABAergic candidate gene expression

Chronic alcohol exposure produces well documented changes in GABA<sub>A</sub> receptor subunit gene expression (Buck et al., 1991a; Montpied et al., 1991; Mhatre et al., 1993; Mhatre & Ticku, 1994; Devaud et al., 1995, 1996; Mahmoudi et al., 1997; for reviews, see Grobin et al. 1998; Reilly et al., 2001). In general, chronic ethanol exposure results in decreased α<sub>1</sub> and γ<sub>2</sub> subunit mRNA and peptide content, and increased α<sub>6</sub> and γ<sub>1</sub> subunit mRNA and peptide content.
More variable results are obtained for the $\alpha_4$, $\beta_2$ (see below) and $\beta_3$ subunits for which mRNA and peptide content is unchanged or increased. These differences in ethanol regulation may be due in part to differences in the degree of physical dependence induced (e.g., in different genetic models). Overall, comparison between the ethanol-induced changes in GABA$_A$ receptor subunit mRNA levels vs. peptide levels suggests that they are highly correlated during alcohol dependence (Devaud et al., 1997). Since studies utilizing recombinant receptors demonstrate that GABA$_A$ receptor subunit composition influences their pharmacological properties (see Sieghart, 1995), it is possible that chronic ethanol-induced changes in GABA$_A$ receptor subunit mRNA and peptide levels would lead to alterations in assembly of receptors and in sensitivity of these receptors to positive and/or negative modulators.

GABA$_A$ receptor subunit expression in WSP and WSR mice. With regard to genetic differences in the expression of specific GABA$_A$ receptor subunits following exposure to chronic alcohol, alcohol dependent as well as alcohol-naïve WSP and WSR mice also differ in the expression of specific GABA$_A$ receptor subunit mRNAs (Buck et al., 1991a; Keir & Morrow, 1994). Previous studies suggest that differences in alcohol withdrawal between selectively bred withdrawal seizure-prone (WSP) and -resistant (WSR) lines of mice may be correlated with differences in $\alpha_1$ or $\alpha_6$ subunit mRNA content following chronic alcohol treatment (Buck et al., 1991a). Some of the functional properties of GABA$_A$ receptor in these lines are also differentially affected by alcohol treatment (i.e., alcohol-induced sensitization to benzodiazepine receptor inverse agonists), providing additional evidence that differences in GABA$_A$ receptor expression or function may contribute to genetic variation in withdrawal (Buck, McQuilken & Harris, 1991b). In general these studies suggest that genetic differences in alcohol withdrawal severity may be associated with allelic differences in GABA$_A$ receptor genes, thereby affecting receptor function and/or expression. It is noteworthy that a decrease in expression of the $\alpha_1$ subunit mRNA, which was reported in WSP but not WSR mice, also has been consistently demonstrated in mice, rats and neuronal cell cultures that were exposed to chronic ethanol (reviewed in Morrow, 1995; Crews et al., 1996). This finding suggests the possibility of a genetic link between subsequent alcohol withdrawal severity and decreased level of the $\alpha_1$ subunit mRNA, but has not yet been examined using B6D2-derived mice.

Ethanol regulation of GABA$_A$ receptor $\beta_2$ subunit expression in mouse models of alcohol withdrawal severity. GABA$_A$ receptor $\beta$ subunits are critical to the GABA binding site (Amin & Weiss, 1993). Recent findings indicate that chronic alcohol administration alters $\beta_2$ subunit expression in rats and mice, but suggest that alcohol may differentially increase $\beta_2$ subunit expression in different animals (genotypes) and brain regions. Chronic ethanol treatment results in either no change or increased $\beta_2$ subunit mRNA and peptide in rat cerebral cortex and cerebellum (Mhatre & Ticku, 1994; Devaud et al., 1995, 1997). Alcohol-naïve WSR mice show greater mRNA content in the cerebellum compared to naïve WSP mice (Keir & Morrow, 1994). In addition, following chronic ethanol treatment, D2 mice with moderate blood ethanol concentrations (~1.0–1.4 mg/ml) show a larger increase in $\beta_2$ subunit mRNA content than B6 mice with comparable blood ethanol concentrations (Reilly & Buck, 2000). Notably, $\beta_2$ subunit mRNA content was highly correlated with blood ethanol concentration in B6 and D2 mice ($r = 0.79, p < 0.002$). Collectively, these findings suggest that genetic models of severe alcohol withdrawal (e.g., WSP and D2) differ in $\beta_2$ expression compared to animals with mild withdrawal (e.g., WSR and B6).

Ethanol regulation of steroid 5α-reductase-1 expression and function. Recent findings indicate that chronic alcohol administration alters endogenous allopregnanolone in rats and mice. Cerebral cortical allopregnanolone levels were significantly reduced in alcohol-dependent, but not alcohol-withdrawing male rats (Janis et al., 1998). In two different mouse models of alcohol withdrawal severity, exposure to chronic alcohol differentially altered plasma allopregnanolone concentration in the selectively bred WSP and WSR mice, as well in the B6 and D2 inbred strains (Finn, Gallaher & Crabbe, 2000). Specifically, plasma allopregnanolone levels decreased in all alcohol-dependent mice, but persisted during alcohol withdrawal only in the seizure prone genotypes (i.e., WSP and D2).
Therefore, the sustained reduction in endogenous allopregnanolone to levels below the physiologically relevant range during alcohol withdrawal in WSP and D2 mice may contribute to their more severe withdrawal convulsions. Consistent with this assumption, recent findings in alcoholic patients suggest that plasma allopregnanolone was significantly decreased between the first and 15th day of alcoholic withdrawal (Romeo et al., 1996). This decrease in endogenous allopregnanolone was correlated with an increase in subjective ratings of anxiety and depression during withdrawal. Therefore, results in two different animal models of alcohol withdrawal severity, and in humans, are suggestive of a relationship between endogenous allopregnanolone levels and behavioral changes in excitability during alcohol withdrawal.

Chronic alcohol exposure may alter endogenous allopregnanolone levels by either altering biosynthesis or elimination of this neuroactive steroid. With regard to steroid biosynthesis, steroid 5α-reductase is the rate-limiting enzyme in the biosynthesis of allopregnanolone from progesterone (see Celotti, Melcangi & Martini, 1992). While there are two isoforms of steroid 5α-reductase, the type-1 isoform is widely expressed in the rodent central nervous system; the expression is similar in males and females and does not appear to be controlled by androgens (Melcangi et al., 1998).

Glutamic acid decarboxylase (GAD) mRNA content and function. Two suggestive QTLs for acute alcohol withdrawal are detected on chromosome 2 (Table 2). The 2proximal locus is detected by markers in the ~27–45 cM region near Gad1, whereas the 2distal locus is associated with markers located ~69 cM distal to the centromere (Buck et al., 1997). These two QTLs have opposing effects on alcohol withdrawal, with D2 alleles at the 2proximal locus associated with higher withdrawal scores ($r = 0.61, p = 0.005$), and D2 alleles at the 2distal locus instead associated with lower risk for withdrawal ($r = −0.65, p = 0.0046$). Glutamic acid decarboxylase (GAD) is the rate-limiting enzyme in GABA biosynthesis (Roberts & Eidelberg, 1960). There are two GAD isoforms (i.e. 67 kD and 65 kD) encoded by distinct genes, Gad1 and Gad2, respectively (for reviews, see Erlander & Tobin, 1991; Martin & Rimvall, 1993). Gad1 is expressed in neuronal terminals, soma and dendrites. Gad2 is expressed only in neuronal terminals, and also maps to the proximal region of mouse chromosome 2. The D2 and B6 strains differ in total brain GAD activity (which is 31% greater in B6 than in D2 mice, $p < 0.05$), suggesting that differences in alcohol withdrawal severity among mice derived from these strains could be associated with differences in GAD enzyme activity and/or gene expression (Buck et al., 1997). Reduced plasma GABA levels have been reported in chronic alcoholics (Coffman & Petty, 1985), suggesting that decreased GABA synthesis could contribute to physical dependence.

Interestingly, a QTL for alcohol preference drinking has also been definitively mapped to this region of chromosome 2 (Phillips et al., 1998), and it is possible that a common gene in this chromosomal region may contribute to the genetic correlation observed between alcohol preference drinking and withdrawal (Metten et al., 1998). Markers in this chromosomal region are also identified by QTL analysis using BXD RI mice tested for withdrawal from nitrous oxide (Belknap et al., 1993), suggesting that a gene in this region of chromosome 2 might influence withdrawal from a variety of CNS depressants including alcohol and gaseous anesthetics.

Conclusion
The search for molecular targets that underlie the pharmacological effects of alcohol has identified multiple ion channels as sites for alcohol’s action. After termination of chronic alcohol exposure, the resultant increase in neuronal excitability that is characteristic of the withdrawal syndrome represents a summation of ion channel adaptations (see Crews et al., 1996). For example, GABAergic inhibitory neurotransmission is reduced, and glutamatergic excitatory transmission is increased following chronic alcohol administration (see Morrow, 1995; Tabakoff & Hoffman, 1996).

QTL mapping studies have made dramatic progress toward the detection and localization of minor and major gene loci involved in murine responses to alcohol and other drugs of abuse, and can implicate specific genes in alcohol and drug responses such as withdrawal. Candidate gene studies provide additional evidence for the involvement of GABAergic mechanisms in acute and chronic alcohol withdrawal and barbiturate.
withdrawal, via differences in sequence or expression of specific GABAA receptor subunit genes or biosynthetic enzymes for GABA or the neurosteroid allospregnanolone. Once a gene is identified and shown to be causally linked to a QTL, its relevance in human populations can be explored.

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References
Buck, K. J. & Hood, H. M. (1998) Genetic association of a GABAA receptor γ2 subunit variant with severity of acute physiological dependence on alcohol, Mammalian Genome, 9, 975–978.


HOOD, H. M. & BUCK, K. J. (2001) Allelic variation in the GABA<sub>A</sub> receptor γ<sub>2</sub> subunit is associated with ethanol-induced motor incoordination and hypothermia, -conditioned taste aversion, and withdrawal in BXD/Ty recombinant inbred mice, *submitted*.


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