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Alcohol-related genes: contributions from studies with genetically engineered mice

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ABSTRACT

Since 1996, nearly 100 genes have been studied for their effects related to ethanol in mice using genetic modifications including gene deletion, gene overexpression, gene knock-in, and occasionally by studying existing mutants. Nearly all such studies have concentrated on genes expressed in brain, and the targeted genes range widely in their function, including most of the principal neurotransmitter systems, several neurohormones, and a number of signaling molecules. We review 141 published reports of effects (or lack thereof) of 93 genes on responses to ethanol. While most studies have focused on ethanol self-administration and reward, and/or sedative effects, other responses studied include locomotor stimulation, anxiolytic effects, and neuroadaptation (tolerance, sensitization, withdrawal). About 1/4 of the engineered mutations increase self-administration, 1/3 decrease it, and about 40% have no significant effect. In many cases, the effects on self-administration are rather modest and/or depend on the specific experimental procedures. In some cases, genes in the background strains on which the mutant is placed are important for results. Not surprisingly, review of the systems affected further supports roles for serotonin, γ-aminobutyric acid, opioids and dopamine, all of which have long been foci of alcohol research. Novel modulatory effects of protein kinase C and G protein-activated inwardly rectifying K^+ (GIRK) channels are also suggested. Some newer research with cannabinoid systems is promising, and has led to ongoing clinical trials.

Keywords Alcohol, genetics, knockout, mouse, sedative, self-administration.

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INTRODUCTION

Human risk of developing alcohol dependence is affected by both genetic and environmental factors. Some environmental risk factors can be specified with certainty (e.g. childhood abuse, extreme poverty, poorly functioning peers as role models), but it has proven more difficult to identify the specific genes affecting risk. While most studies suggest that genes contribute about 40–60% of total risk, their influence often depends upon the modulatory effects of environmental factors, a condition termed *gene × environment* interaction (Cloninger, Bohman & Sigvardsson 1981; Crabbe 2002). Despite the importance of inherited contributions, we know for certain of only two genes that affect alcoholism risk. A specific aldehyde dehydrogenase variant, common in South-east Asian populations, confers nearly 100% protection against an alcoholism diagnosis in homozygotes, and a variant of alcohol dehydrogenase exerts a more modest protective effect (Enoch & Goldman 2001). Both of these genes lead to an accumulation of the toxic alcohol metabolite acetaldehyde, and their relevance is attested to by the long usage of disulfiram (Antabuse®) to treat alcoholics, where it has found modest success in compliant individuals. If an alcoholic drinks alcohol while taking disulfiram, this acetaldehyde dehydrogenase inhibitor reproduces the flushing, nausea and other unpleasant side-effects experienced by those with the heritable protective alleles.

In the early 1980s, neurobiology was revolutionized by the demonstration that a gene could be inserted into the pronucleus of mouse eggs, and that adult mice grown from those embryonic cells expressed the gene's protein product (Palmiter *et al.* 1982). Since this dra-

matic demonstration of very large mice that clearly overexpressed the rat growth hormone gene, thousands of genes have been genetically engineered in mice in efforts to study their function. In most cases, a gene's sequence is altered so that it becomes a 'null mutant' or a 'knockout', because the gene's protein product is only marginally or not functional. As in the initial demonstration, many genes have been studied by the insertion of extra copies of a gene and the ensuing overexpression of the gene product. The purpose of this review is to summarize the existing reports of genetically engineered mice that have been used to study responses to ethanol. Earlier reviews have been limited to specific traits such as self-administration and withdrawal (Cunningham & Phillips 2003; Crabbe & Phillips 2004), or to specific collections of genes, such as those related to the function of γ-aminobutyric acid (GABA) neurotransmission (Boehm *et al*. 2004b) or opioid receptor-related genes (Oswald & Wand 2004).

The strengths and weaknesses of genetic engineering strategies have been discussed many times elsewhere and will not be reiterated in detail here. Nonetheless, certain caveats are of sufficient importance that we have tried to incorporate information about them in our tables. The first issues are strictly genetic, and involve the potential role of genes other than the mutated gene. In the usual strategies of gene deletion by homologous recombination, other genes are inevitably carried along with the engineered gene construct into the recipient genotype, and it is important not to misattribute the effects of such 'passenger genes' to the engineered gene. In addition, a null mutation may be placed by backcrossing onto any desired genotype for study, and the effects of a mutation may differ depending upon which strain the genotype is placed. Both of these may represent *gene–gene* interactions. In the first case, the effects of the mutated gene may interact with genes derived from the embryonic stem (ES) cell line (itself usually derived from one of the many substrains of 129 inbred mice). In the second, the background strain (usually the C57BL/6 inbred strain) contributes the interacting genes. The usual derivation of a null mutant by homologous recombination involves both 129 and C57BL/6, and if the mutant is backcrossed to a different strain, a third set of genes has the opportunity to interact. This is also true of many transgenic overexpression, inducible, or conditional expression lines. If a cre recombinase step has been used in preparing the mutant, it is often performed using the FVB/NJ inbred strain, and some background genes from this strain remain in the preparation. There are many effective strategies available for addressing these problems, but they are, unfortunately, rarely followed (Gerlai 1996; Wolfer, Crusio & Lipp 2002). Where possible, we have tracked the source of the genotypes used to prepare the mutants and report these in the tables, but not all papers, particularly those from the 1990s, report accurately on the strains used to generate the mutants (a frequent source of error is incorrectly naming the specific 129 substrain). Finally, genetic contamination of certain 129 substrains (129/Sv, 129/SvJ, 129X1/SvJ) from which certain ES cell lines have been developed (RW4, R1) raises the possibility of additional segregating alleles in mutants made from these ES cells (Simpson *et al*. 1997).

The second issue is the behavioral method employed. The characteristic response of a mouse genotype on a particular behavioral test may depend strongly on exactly how that test is conducted, while for some tests, such procedural variations may be relatively unimportant. For example, genotypic preference to drink ethanol solutions is highly reliable across many different variations of the basic test, as attested to by a pattern of inbred strain differences that has remained robust over more than 45 years. On the other hand, mouse assays of anxiety-like behavior are also influenced genetically, but the effective genes appear to vary according to the specific methods employed to perform the test (Wahlsten *et al*. submitted). Such sensitivity to the specifics of the tests employed to represent psychological or physiological constructs such as *reward*, *intoxication*, *ataxia* or *anxiety* is equally relevant for descriptions of a null mutant and for the background genotype on which it is studied. Of course, the gene–gene interactions and the procedural effects themselves can, and do, interact. Therefore, we have incorporated at least a modicum of information about test specifics and background genotypes of the 'wildtype' comparison genotypes into our tables.

A third major concern for null mutant data interpretation is the possibility of compensations that doubtless occur throughout post-embryonic development. Deletion of some proteins may trigger plastic changes that allow the mouse to be viable despite loss of a critical protein. For example, deletion of the α1 subunit of the $GABA_A$ receptor produces widespread compensatory changes in the brain (Ponomarev *et al*. 2006). One strategy that may avoid much of this compensation is construction of knock-in mice in which the protein is present, but has a mutation that removes only one aspect of the protein function. For example, mutation of the α1 subunit was successful in removing the potentiating action of ethanol on GABA function without otherwise impairing the function of these receptors (Borghese *et al*. in press; Werner *et al*. in press). On the other hand, these compensations provide fascinating study material in that they represent possible neuroadaptive mechanisms that could prove useful in treatment plans for neurological disorders.

In the summary tables to follow, we have broadly categorized the responses to ethanol studied in the various engineered mice, and include some other behavioral data

not involving alcohol when we believe it is relevant for interpreting the results. For example, interpretation of anxiety-like response to ethanol in the elevated plus maze test is equivocal without knowledge of the general exploratory activity of the animals. Completely inactive animals might never explore the two parts of an elevated plus maze, in which case their percent time spent in the open arms is not reliably indicating their anxiety-like behavior. Conversely, extremely active animals tend to show no choice between different parts of an apparatus, and excessive activity makes it likely that such an animal will not display a conditioned preference for an environment paired with drug injections. It should be noted also that we report in the tables our interpretation of the results from the publications. In some instances, our interpretations differ from those stated by the authors, and we leave it to the reader to decide between such alternative readings of the data. While we discuss data from most tables, we do not report specifics for all of the published data. Finally, we welcome corrections of any sort to the data summarized in these tables.

γ-AMINOBUTYRIC ACID (GABA) (TABLE 1)

The long-standing emphasis on GABAergic systems in alcohol research and the availability of mutants for many components of these systems, particularly subunits of $GABA_A$ receptors, provides many studies of GABA mutants and alcohol phenotypes (Boehm *et al.* 2004b). Specifically, we located 17 publications presenting 15 distinct mutant lines (note that the α1 null mutant was constructed independently in two laboratories). Both null mutants and overexpression transgenic lines for the GABA transporter have been studied. Many genes for subunits of the ionotropic $GABA_A$ receptor have been deleted and the mutants studied, although null mutants for the β1, γ1, α3 or α4 subunits have not been published. In addition, null mutants for metabotropic $GABA_B$ receptor subunits and the principal enzyme responsible for synthesis of GABA, glutamic acid decarboxylase, have not yet been explored for alcohol phenotypes.

It is useful to distinguish between the synaptic and extrasynaptic $GABA_A$ receptors, as the latter exert a tonic inhibition provided by low levels of GABA which diffuse from the synaptic region. Several recent papers propose that extrasynaptic $GABA_A$ receptors are particularly sensitive to alcohol and are critical for enhancement of GABAergic function by alcohol (Hanchar, Wallner & Olsen 2004, Hanchar *et al.* 2005), although not all studies support this conclusion (Borghese *et al.* 2006). Because the subunit composition of these receptors is distinct, null mutant mice should help to clarify their roles in alcohol action. Specifically, α4, α5, α6, β3 and δ subunits are most likely to form extrasynaptic receptors, whereas α1, α2, α3, β2 and γ2 are most prominent in synaptic receptors. In theory, we should be able to compare phenotypes of null mutants for these groups of subunits, but there are several complications. First, the α6 subunit is found only in cerebellar granule cells, so the lack of alcohol phenotypes in this mutant may merely reflect the limited presence of α6 in the brain. Second, the β3 null mutant is compromised by cleft palate and seizure disorders and is difficult to study behaviorally. The α4 mutant has only recently become available and has not been studied, so most of our interpretations of extrasynaptic receptors relies on the α5 and δ null mutant mice. Both of these mutations decreased alcohol consumption and decreased alcohol withdrawal signs (but not on all backgrounds), and the δ mutant decreased the anticonvulsant action of ethanol. It will be important to determine if these phenotypes are also present in α4 null mutants or in β3 conditional mutants where the deleterious effects might be avoided. It is of interest to note that gaboxadol has selectivity for extrasynaptic receptors and produces a loss of righting reflex (LORR) which is markedly reduced in δ null mutants (Boehm *et al.* 2006), but the ethanol LORR is not altered in δ mutants. This provides a conundrum in that it suggests that activation of δ receptors is sufficient to produce LORR, yet these receptors do not appear to be important for ethanol LORR, despite the assertion that a major effect of ethanol is to activate these receptors (Hanchar *et al.* 2004, 2005). It is also important to note that drugs that activate synaptic $GABA_A$ receptors, such as flunitrazepam and etomidate, also produce LORR, and this effect is reduced in mice lacking the α1 or β2 subunit (Blednov *et al.* 2003a,b). Ethanol LORR is also reduced in both of these null mutants, but this effect is seen only in males and is not as marked as for drugs with selectivity for synaptic $GABA_A$ receptors. Taken together, these data suggest that activation of either synaptic or extrasynaptic $GABA_A$ receptors can produce LORR, but these receptors have at most a minor role in the LORR produced by ethanol.

As noted above, most of the synaptic $GABA_A$ receptors contain α1 and β2 subunits. Deletion of either of these subunits reduces the number of brain $GABA_A$ receptors by about 50% (Sur *et al.* 2001). However, the null mutants provide another apparent contradiction: these two mutants do not show the same phenotypes for alcohol and other behaviors and in some instances show opposite phenotypes (Boehm *et al.* 2004b). Recent electrophysiological and molecular exploration of these mutants provides a likely explanation for these differences. In brief, absence of the α1 subunit allows α2 or α3 subunits to replace the α1 in some receptors. This switch prolongs the kinetics of the receptor by enhancing channel opening time and provides enhanced inhibitory function compared with the

Table 1 γ-Aminobutyric acid.

Gene	*Ref.*[a]	*Gender/ background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Anxiety*	*Tolerance*	*Withdrawal*	*Other*
GABA transporter overexpression	1	Male, female C57BL/6J × DBA/2J hybrid (data not analyzed by sex)	—	—	OX > WT (acute ethanol-induced activity, 1.75 g/kg)	OX ≤ WT (LORR latency, duration, 3.6 g/kg)	—	—	—	OX = WT (BEC, 60, 180 minutes after 3.6 g/kg) OX < WT (sensitivity to lethal effects of ethanol, 7.5 or 9 g/kg)
GABA transporter	2	Male F2 of CJ7 ES cells to C57BL/6J	(KO = WT) < HET (intake, PR ≥ 9%) 3%, 6%, 9%, 12%, 15% ethanol (4 days each) (KO = WT) < HET (saccharin preference) (KO = HET) > WT (quinine avoidance)	KO < (HET = WT) (CPP, 2 g/kg) KO ≤ WT (CTA, 2.5 g/kg)	(KO = WT) < HET (activation after 2 g/kg)	KO < (HET = WT) (LORR latency, duration, 3.6 g/kg)	—	—	—	KO = HET = WT (clearance, 3.6 g/kg, data not shown)
GABA-Aα1	3	Male, female F6-7 of 129/SvEv × C57BL/6J	KO < WT (intake, 2-hour limited access, 10%)	KO < WT (oral operant self-administration, 10% ethanol or 10% sucrose)	KO > WT (sensitivity to low-dose activation; genotypes not compared) KO < WT (sensitivity to high dose reduction in activity; genotypes not compared)	—	—	—	—	—

4	Female F6-7 of 129/SvEv × C57BL/6J	KO < WT (intake, PR) 3%, 6%, 9%, 12%, 15% ethanol (4 days each) KO < WT (saccharin PR) KO = WT (quinine avoidance)	KO = WT (CPP to 2 g/kg) KO > WT (CTA to saccharin after 2.5 g/kg)	KO > WT (ethanol-induced activation in CPP, 2 g/kg) KO > WT (activity after acute dose–response)	—	—	—	KO = WT (HIC after chronic ethanol liquid diet)	KO = WT (BEC, clearance, 4 g/kg)
5	Male, female F4-6 of C57BL/6J × FVB/NJ × 129Sv/SvJ mixed background	—	—	KO > WT (ethanol-induced activation, 1, 1.5 g/kg; no activation in WT, 0.75–1.5 g/kg)	KO = WT (accelerating rotarod, 1.5 g/kg; both showed ataxia) KO = WT (LORR duration, 3 and 3.5 g/kg)	KO ≤ WT (anxiolytic effect in elevated plus maze, 1.5 g/kg)	KO = WT (acute functional tolerance, stationary dowel test, 1.75 then 2.0 g/kg)	—	KO = WT (BEC, 60, 180 minutes after 3.5 g/kg) KO = WT (ethanol potentiation of muscimol-stimulated chloride uptake in cortical synapto-neurosomes) KO = HET = WT (sensitivity to anticonvulsant effect of ethanol on bicuculline-induced seizure threshold)
6	Male, female F6-7 of 129/SvEv × C57BL/6J	—	—	—	KO < WT (LORR duration, 3.4–3.8 g/kg, effect in males only)	—	—	—	KO = WT (ethanol potentiation of muscimol-stimulated chloride uptake in cortical microsacs)

Table 1 *Cont.*

Gene	*Ref.*[a]	*Gender/ background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Anxiety*	*Tolerance*	*Withdrawal*	*Other*
	7	Gender not specified 129Sv/SvJ × FVB/NJ × C57BL/6J, F8 mixed background	—	—	—	—	—	—	—	Inhibition of essential-like tremor in KO (0.25–2.5 g/kg; 0.25 g/kg *increased* tremor in WT)
	8	Male, female (two backgrounds) 129/SvEv × C57BL/6J; 129Sv/SvJ × FVB/NJ × C57BL/6J	—	—	—	—	—	—	—	KO and WT differ in cerebellar and cortical microarray expression of GABA and glutamate genes (among others)
GABA-Aα2	9	Male, female 129/SvEvJ × C57BL/6J heterozygous background	KO < WT (intake, PR) 3–15% ethanol, (4 days each) females only, no difference in males KO = WT (saccharin PR) KO ≥ WT (quinine avoidance)	—	KO = WT (activity increase after 1.5 g/kg in elevated plus maze)	KO < WT (LORR duration, 3.8 g/kg)	KO = WT (anxiolytic response to 1.5 g/kg in elevated plus maze)	—	KO ≤ WT (acute withdrawal HIC severity, 4 g/kg)	—
GABA-Aα5	9	Male, female 129SvEv × C57BL/6J	KO < WT (intake, PR) 3%, 6%, 9%, 12% ethanol (4 days each) males only, no difference in females KO = WT (saccharin PR, quinine avoidance)	—	KO = WT (activity increase after 1.5 g/kg in elevated plus maze)	KO = WT (LORR duration, 3.8 g/kg)	KO < WT (anxiolytic response to 1.5 g/kg in elevated plus maze)	—	KO < WT (acute withdrawal HIC severity, 4 g/kg)	—

	10	Female F3 of 129/SvEv × C57BL/6	KO < WT (intake, 10%, 16% trend) KO = WT (intake, 2–8%, PR, all concentrations) 2%, 4%, 6%, 8%, 10%, 16% ethanol (7 days each) KO < WT (intake, 8%, 2-hour test after 16-hour water deprivation)	KO = WT (operant self-administration, 10% sucrose + 10% ethanol after sucrose fading, FR4) KO = WT (no self-administration, 5% ethanol alone) KO < WT (self-administration, 10% sucrose alone)	—	KO = WT (sensitivity and duration of impairment on fixed speed rotarod, 2.5 g/kg) KO = WT (LORR latency, duration, and BEC at recovery, 3 g/kg)	—	—	—	—
GABA-Aα6	11	Male, female 129/SvJ × C57BL/6J	—	—	KO < WT (number of stairs climbed in staircase test 35 minutes after 2, 2.5 g/kg; but KO < WT at baseline)	KO = WT (accelerating rotarod, 2 and 2.5 g/kg; ataxia in KO, WT)	—	—	—	KO = WT (BEC, 15, 30, 90 minutes after 2 g/kg, male)
	12	Gender not specified F2-3 of 129 × C57BL/6J (129 substrain not specified)	—	—	—	KO = WT (LORR duration, 3.5 g/kg)	—	—	—	—
	13	Male, female F3-4 of 129/Sv × 129/SvJ × C57BL/6J	—	—	—	—	—	KO = WT (acute functional tolerance, stationary dowel test, 1.75, then 2.0 g/kg) KO = WT (protracted tolerance, LORR duration, 3.5 g/kg, during last withdrawal)	KO = WT (withdrawal-induced hyperexcitability, multiple withdrawals after vapor inhalation)	KO = WT (peak BEC, clearance, 3.5 g/kg)

Table 1 *Cont.*

Gene	*Ref.*[a]	*Gender/ background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Anxiety*	*Tolerance*	*Withdrawal*	*Other*
GABA-Aβ2	6	Male, female F6-7 of 129/SvEv × C57BL/6J	—	—	—	KO < WT (LORR duration, 3.4–3.8 g/kg, effect in males only)	—	—	—	KO < WT (ethanol potentiation of muscimol-stimulated Cl^- uptake in cortical microsacs)
	4	Female F6-7 of 129/SvEv × C57BL/6J	KO = WT (intake, PR) 3%, 6%, 9%, 12%, 15% ethanol (4 days each) KO < WT (saccharin PR) KO > WT (quinine avoidance)	KO < WT (CPP to 2.0 g/kg) KO = WT (CTA to saccharin after 2.5 g/kg)	KO = WT (ethanol-induced activity in CPP, 2 g/kg) KO = WT (activity after acute dose–response)	—	—	—	KO > WT (HIC after chronic ethanol liquid diet, but KO consumed more ethanol than WT)	KO = WT (BEC, clearance, 4 g/kg)
GABA-Aβ3	14	Male, female 129 × C57BL/6J (129 substrain not specified)	—	—	—	KO = WT (LORR duration, 3.5 g/kg)	—	—	—	—
GABA-Aγ2S overexpression	15	Gender not specified C57BL/6J × DBA/2J F1	—	—	—	OX = WT (dowel test sensitivity, 1.75 g/kg)	—	OX < control (acute functional tolerance, stationary dowel test, 1.75, then 2.0 g/kg)	—	—

GABA-Aγ2L	16	Gender not specified F2–4 of 129/SvJ × C57BL/6J hybrid	—	—	KO = WT (ethanol-stimulated activity in elevated plus maze, 1.5 g/kg)	KO = WT (LORR duration, 3.5 g/kg)	KO = WT (anxiolytic effect, elevated plus maze, 1.5 g/kg)	KO = WT (acute functional tolerance, stationary dowel test, 1.75, then 2.0 g/kg)	KO = WT (withdrawal-induced hyperexcitability, multiple withdrawals from vapor inhalation)	KO = WT (peak BEC, clearance, 3.5 g/kg) KO = WT (ethanol potentiation of GABA currents)
GABA-Aγ2L overexpression (two lines)	15	Gender not specified C57BL/6J × DBA/2J F1	—	—	—	OX = WT (LORR duration, BEC at righting, 4.2 g/kg) OX = WT (dowel test, 1.75 g/kg)	—	OX < WT (acute functional tolerance, stationary dowel test, 1.75, then 2.0 g/kg)	OX = WT (acute withdrawal severity, 4 g/kg)	OX = WT (ethanol potentiation of muscimol-stimulated chloride uptake in cortical and cerebellar microsacs)
GABA-Aδ	17	Male, female 129 × C57BL/6J hybrids (two different mixed backgrounds studied; most tests performed on one background only)	KO < WT (intake, PR; genetic difference in male > female) 3%, 5%, 7%, 9%, 11% ethanol (4 days each) KO = WT (saccharin PR) KO = WT (quinine avoidance)	—	KO = WT (activity in elevated plus maze; activity not altered by ethanol; 1.5 g/kg)	KO = WT (LORR duration, 3.5 g/kg) KO = WT (hypothermia time-course, 1–3 g/kg) KO < WT (dowel test sensitivity, 1.75 g/kg) KO < WT (latency to	KO = WT (anxiolytic effect in elevated plus maze, 1.5 g/kg)	KO = WT (acute functional tolerance, stationary dowel test, 1.75, then 2.0 g/kg) KO = WT (protracted tolerance, LORR duration, 3.5 g/kg, test during last withdrawal)	KO < WT (withdrawal-induced hyperexcitability; effect only seen on one background)	KO = WT (peak BEC, (clearance of 3.5 g/kg) KO < WT (sensitivity to anticonvulsant effect of ethanol on bicuculline-induced seizure threshold)

Table 1 *Cont.*

Gene	*Ref.*[a]	*Gender/ background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Anxiety*	*Tolerance*	*Withdrawal*	*Other*
						recover after 1.75 g/kg, but KO = WT in BEC at recovery of balance on dowel)		No tolerance on one background, tolerance on the other, KO = WT for both)		

[a]Reference nos. 1, Hu *et al*. (2004); 2, Cai *et al*. (2006); 3, June *et al.* (2006); 4, Blednov *et al*. (2003a); 5, Kralic *et al*. (2003); 6, Blednov *et al*. (2003c); 7, Kralic *et al*. (2005); 8, Ponomarev *et al*. (2006); 9, Boehm *et al*. (2004b); 10, Stephens *et al*. (2005); 11, Korpi *et al*. (1999); 12, Homanics *et al*. (1997); 13, Homanics *et al*. (1998); 14, Quinlan, Homanics & Firestone (1998); 15, Wick *et al*. (2000); 16, Homanics *et al*. (1998); 17, Mihalek *et al*. (2001).

The following footnotes also apply to Tables 2–16.

Behavioral test comparisons: Comparators (< ≤ = ≥ >) indicate relative sensitivity to ethanol. For example, KO > WT (loss of righting reflex [LORR] latency, duration, BEC at recovery) indicates knockout mice had shorter latencies to lose the righting reflex, a longer duration of LORR, and recovered righting reflex at lower BEC than wildtypes. Doses of ethanol are given as g/kg. WT, wildtype; KO, knockout; HET, +/– heterozygote; OX, overexpression transgenic. Gene manipulation is knockout unless specified.

Gender/Background: ES cells, embryonic stem cell line. Given where known. Often, only the 129 inbred mouse substrain is given. Offspring are then usually crossed to C57BL/6 inbreds to detect chimeras carrying the gene construct, and then backcrossed to another strain (usually C57BL/6). N#, number of backcross generations. F#, number of hybrid intercross generations. For behavioral traits, unless otherwise noted, mice of the indicated sex(es) were used and results were similar for both sexes.

Preference: Unless otherwise noted, two-bottle choice between 10% ethanol versus water with 24-hour continuous access in singly housed, adult mice. Intake, g/kg/day; PR, preference ratio (% of total fluid intake from ethanol bottle). Similar methods were generally used to test saccharin preference or quinine avoidance.

Other ethanol reward: This category includes oral operant self-administration schedules, CPP (conditioned place preference, conditioned by ethanol injections), CTA (conditioned taste aversions to a novel flavor such as saline conditioned by ethanol injections), and other tests related to reward.

Activity: Includes activity after acute ethanol, sensitization with repeated injections, and activity after ethanol assessed in other apparatus (e.g. elevated plus maze).

Sedation: Includes various tests of intoxication and motor incoordination.

Anxiety: Effect stated is for anxiolytic-like response to ethanol (e.g. percentage open arm entries or time in elevated plus maze).

Withdrawal: Withdrawal sign is generally handling-induced convulsion (HIC) severity, but sometimes includes anxiety-like behavior or self-administration.

Other: Most often includes blood ethanol concentration (BEC) after a fixed dose of ethanol. Sometimes includes clearance (rate of elimination), or other responses to ethanol that do not fit other categories.

wildtype receptor with α1 subunits. Thus, the result of deletion of a major $GABA_A$ receptor subunit is, paradoxically, increased inhibition (Vicini *et al.* 2001) as well as a number of other changes (Ponomarev *et al.* 2006). Increased ethanol-stimulated locomotor activity is one of the most striking phenotypes in the α1 mutants, but this is not seen in the β2 mutants and is likely due to plasticity secondary to loss of the α1 subunit rather than lack of GABAergic inhibition (Ponomarev *et al.* 2006).

A strategy that can reduce or avoid the problems of subunit substitution and compensation when a $GABA_A$ subunit is deleted is substitution of a mutated subunit for the wildtype (knock-in mice). This was used elegantly to define $GABA_A$ subunits responsible for different actions of benzodiazepines (Rudolph & Mohler 2004). Recently, mice were constructed with α1 subunits that contain two mutated amino acids; these allow the receptor to retain normal GABA sensitivity, but eliminate the response to alcohol (Borghese *et al.* 2006). Studies of these mice indicate that most behavioral effects of alcohol are not changed, but recovery of motor coordination following injection of ethanol is enhanced (Werner *et al.* in press). Thus, synaptic $GABA_A$ receptors may be particularly important for some aspects of ethanol ataxia, and this domain should be studied further in other $GABA_A$ mice, such as in β2 null mutants.

DOPAMINE (TABLE 2)

Five dopamine receptors (one with short and long isoforms) have been identified through molecular cloning techniques (Monsma *et al.* 1990; Sokoloff *et al.* 1990; Sunahara *et al.* 1991; Van Tol *et al.* 1991). Three of these receptors—D_1, D_2, D_3—have been implicated in drug reward in general through extensive pharmacological studies; a smaller literature addresses the involvement of D_4 and D_5 receptors. For example, studies with psychostimulants have shown that antagonists of D_1, D_2 and D_3 receptors block the reinforcing actions of cocaine (Koob & Le Moal 2006), and knockouts of D_1 and D_2 receptors have shown that D_1 knockout mice do not show a deficit in acquisition of a conditioned place preference for cocaine (Miner *et al.* 1995) but are impaired in acquisition of intravenous cocaine self-administration (Caine *et al.* 2002). D_2 knockout mice show a blockade of morphine-induced place preference (Maldonado *et al.* 1997). D_2 knockout mice actually self-administer more cocaine than wildtype controls (Caine *et al.* 2002). D_4 knockout mice showed an accentuation of psychostimulant activity (Rubinstein *et al.* 1997). Concerns about motor effects in D_2 knockouts cloud interpretation of some effects in these mice (Baik *et al.* 1995; Fowler *et al.* 2002).

D_1 knockout mice on a mixed 129S4/SvJae × C57BL/6 background show decreased ethanol intake in 1-hour limited access preference tests and in 24-hour access (El-Ghundi *et al.* 1998). Most studies of D_2 knockout mice have been performed with 129S2/SvPas backcrossed (N5–10) onto C57BL/6J. D_2 knockouts showed a decrease in ethanol intake (Phillips *et al.* 1998; Palmer *et al.* 2003; Thanos *et al.* 2005), decreased operant self-administration of ethanol (Risinger *et al.* 2000), and absence of place preference to ethanol (Cunningham *et al.* 2000). The decreases in ethanol intake with D_2 knockout mice were correlated with an increased sensitivity to ethanol-induced activity and its sensitization, and prior sensitization obviated the differences in drinking (Palmer *et al.* 2003). Effects of the mutation on ethanol's activating and sensitizing effects were not seen on a 129S2/SvPas × 129S6 background. The absent place preference was observed in D_2 knockouts from 129S2/SvPas first backcrossed (N5) to C57BL/6J and then intercrossed (F2) with DBA/2 mice (Cunningham *et al.* 2000). This step was taken because the C57BL/6J strain shows very weak ethanol-conditioned place preference, while the DBA/2J strain has a robust conditioned response. D_2 knockout mice also showed decreased food intake and decreased saccharin intake (Risinger *et al.* 2000). Administration of an adenoviral vector containing cDNA coding for the D_2 receptor into the nucleus accumbens of D_2 knockout mice backcrossed to C57BL/6J transiently increased ethanol consumption and preference in a two-bottle choice test but *decreased* ethanol consumption and/or preference in wildtype and heterozygous mice, suggesting to the authors an inverted U-shaped function relating D_2 receptor activity to ethanol consumption (Thanos *et al.* 2005). D_2 knockout mice were also less ataxic after ethanol in the grid test than heterozygous or wildtype mice on the mixed B6 background only (Palmer *et al.* 2003), and showed markedly reduced striatal overflow of dopamine after 2 g/kg than wildtypes on the B6 background (Job *et al.* 2006).

Two experiments have been reported with D_3 knockout mice on an N5 backcross to C57BL/6J from 129S4/SvJae. These knockouts showed no difference from wildtypes in ethanol consumption in two-bottle preference; nor did they differ in operant self-administration of ethanol or in ethanol-conditioned place preference (Boyce-Rustay & Risinger 2003). However, the same D_3 knockout mice did show an increased ethanol LORR and increased physical signs of withdrawal from chronic ethanol after a liquid diet dependence induction procedure (Narita *et al.* 2002), despite ingesting less of the liquid diet than wildtypes. D_4 knockout mice on a 129/Ola × C57BL/6J background showed normal ethanol intake in a two-bottle preference test (Falzone *et al.* 2002), but D_4 knockouts showed reduced basal locomotor activity and increased locomotor stimulation to ethanol (Rubinstein *et al.* 1997).

Overall, these results show that knockout of D_1 or D_2 dopamine receptors, but not D_3 or D_4 receptors, can have

Table 2 Dopamine.

Gene	*Ref.*[a]	*Gender/background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Anxiety*	*Withdrawal*	*Other*
D_1	1	Male J1 ES cells (129S4/SvJae) to C57BL/6 background, heterozygote matings	KO < (HET = WT) (intake) 3%, 6%, 12% ethanol (≥6 days each) (1-hour limited access without fluid deprivation) Same pattern with periodic 12% access for 24 hours, and forced 12% for 24-hour test	—	—	—	—	—	KO = HET = WT (peak BEC, clearance of 2.5 g/kg)
D_2	2	Male, female 129S2/SvPas × C57BL/6J F2, then N5 backcross to C57BL/6J; Gender effects not analyzed	KO < (HET = WT) (intake, PR) 3%, 6%, 10% ethanol (4 days each) KO = HET = WT (saccharin PR, quinine avoidance, total fluid intake)	—	KO < HET < WT (decreased activity, 2 g/kg; no ethanol effect in KO; KO also shows less basal activity than WT)	KO < HET < WT (grid test ataxia, 2 g/kg)	—	—	KO = HET = WT (BEC, 15 and 30 min after 2 g/kg)
	3	Male, female 129S2/SvPas on two different backgrounds N5 backcross to C57BL/6J, and a complex mixed 129 background	KO < (HET = WT) (intake, PR) KO = HET = WT (after sensitization, 2.5 g/kg/day for 10 days; tested on C57BL/6J only; 10% ethanol, 8 days)	—	KO > (HET = WT) (activation with 2 g/kg, on C57BL/6J) KO = HET = WT (activation with 2 g/kg, on 129) KO ≥ (HET = WT) (sensitization, on C57BL/6J) (KO = HET) ≥ WT (sensitization, on 129) KO < WT < HET (reduced activity after 2 g/kg in the grid test; tested on 129 only)	KO < (HET = WT) (2 g/kg-induced grid test ataxia, on 129)	—	—	KO = WT (BEC, 2 g/kg)

	4	Male 129S2/SvPas, N10 backcross to C57BL/6J	KO < HET < WT (intake, PR) D_2 receptor viral vector infusion (nucleus accumbens) transiently decreased intake in WT & HET (intake and PR *increased* in KO) 10% ethanol, 14 days	—	—	—	—	—	—
	5	Male 129S2/SvPas, N10 backcross to C57BL/6J	—	KO < WT (operant self-administration; 5%, 10%, 20%, 30%, ethanol, FR4; 5%, 10%, 20% in saccharin, FR4) KO < WT (food responding, saccharin intake)	—	—	—	—	—
	6	Male, female 129S2/SvPas, N13 backcross to C57BL/6J	—	—	—	—	—	—	KO < WT (striatal overflow of dopamine after 2 g/kg)
	7	Male, female 129S2/SvPas, N5 backcross to C57BL/6J, then F2 intercross with DBA/2J	—	KO < (HET = WT) (CPP, 2 g/kg; CPP absent in KO)	(KO = HET) > WT (increased activity during CPP, 2 g/kg)	—	—	—	—
D_3	8	Male 129S4/SvJae, N5 backcross to C57BL/6J	KO = WT (intake, PR) 3%, 10% ethanol (8 days each)	KO = WT (operant self-administration, 10% ethanol, FR4) KO = WT (CPP, 3 g/kg)	—	—	—	—	—

Table 2 *Cont.*

Gene	*Ref.*[a]	*Gender/background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Anxiety*	*Withdrawal*	*Other*
	9	Gender not specified 129S4/SvJae, N5 backcross to C57BL/6J	—	—	—	KO > WT (LORR sensitivity; 3.5 g/kg)	—	KO > WT (composite withdrawal index after 7% chronic liquid diet for 4 days; no BECs provided, but KO ≤ WT in diet ingested)	—
D_4	10	Male, female 129/Ola × C57BL/6J, N10 intercross	KO = WT (intake, PR) 10% ethanol, 9 days; tested in males only)	—		—	KO ≥ WT (anxiolytic effect in elevated plus maze, 1 g/kg but not 2 g/kg)	—	—
	11	Female 129/OlaHsd × C57BL/6J, F2 intercross	—	—	KO > WT (increased activity, 2 g/kg)	—	—	—	—
Dopamine transporter (DAT)	12	Male, female 129/SvJ × C57BL/6J mixed background	KO < (HET = WT) (intake, PR, females) 3%, 6%, 10%, 20% ethanol (8–10 days each; no difference in males) KO < (HET = WT) (saccharin PR, female; quinine avoidance, male and female)	—	—	(KO = HET) > WT (LORR onset sensitivity, but not duration; 3.8 g/kg; BECs at recovery equal among genotypes)	—	—	—
	13	Male, female J1 ES cells (129S4/SvJae) × C57BL/6J mixed background, >F10	(KO = HET) >WT (intake of 24%, not PR, male) 1–32% ethanol (2–3 days each) HET > (KO = WT) (PR, female)	—	—	—	—	—	—

	14	Male 129/SvJ × C57BL/6J mixed background	—	—	—	—	—	—	KO = WT (increase in dopamine dialysate in caudate-putamen, 2 g/kg) KO = WT (decreased dopamine signal by voltammetry in caudate-putamen slices)
Brain vesicular monoamine transporter (VMAT2)	12	Male, female AB1 ES cells (129S5/SvEvBrd) × C57BL/6J mixed background KO was HET (deletion is lethal in homozygotes)	HET > WT (intake, not PR, ≥16%) 1–32% ethanol (2–3 days each; males only; no difference in females) HET ≥ WT (food consumption, males)	—	—	—	—	—	—
DARPP-32	15	Male E14 ES cells (129/Ola) to C57BL/6J, N10 backcross to C57BL/6J	—	KO < WT (oral operant self-administration), 0–30% ethanol KO = WT (operant self-administration of food, water) KO < WT (CPP, 2 g/kg; no CPP in KO) KO = WT (CTA to NaCl, 2 and 4 g/kg)	KO > WT (activation, 2 g/kg, in CPP test)	—	—	—	—
	16	Gender not specified E14 ES cells (129/Ola) to C57BL/6J, N10 backcross to C57BL/6J	—	—	—	—	—	—	KO > WT [inhibition by D_1 receptor agonist of ethanol-induced *N*-methyl-D-aspartate (NMDA) currents in nucleus accumbens medium spiny neurons]

[a]Reference nos. 1, El-Ghundi *et al.* (1998); 2, Phillips *et al.* (1998); 3, Palmer *et al.* (2003); 4, Thanos *et al.* (2005); 5, Risinger *et al.* (2000); 6, Job *et al.* (2006); 7, Cunningham *et al.* (2000); 8, Boyce-Rustay & Risinger (2003); 9, Narita *et al.* (2002); 10, Falzone *et al.* (2002); 11, Rubinstein *et al.* (1997); 12, Savelieva *et al.* (2002); 13, Hall *et al.* (2003); 14, Mathews *et al.* (2006); 15, Risinger *et al.* (2001); 16, Maldve *et al.* (2002).

significant effects on ethanol intake that parallel to some extent pharmacological studies with selective dopamine D_1 and D_2 antagonists. However, these phenotypes are accompanied by changes in intake of food and saccharin, and in responding in general, raising the perennial issue of general motor or motivational effects from disruption of the dopamine systems (Salamone & Correa 2002). The degree of motor effect in dopamine knockouts appears to be determined by several factors, including gene dosage, genetic background and developmental adaptations. One preparation was able to acquire a rotarod task to the same degree as wildtype (Kelly *et al.* 1998). To this point, in none of the studies cited above was ethanol intake abolished, the largest effects being approximately a 60–65% decrease in intake in D_1 and D_2 knockout mice (El-Ghundi *et al.* 1998; Phillips *et al.* 1998), but up to 90% decrease in operant responding for ethanol on a higher response schedule (fixed-ratio 4) and elimination of conditioned place preference characterized D_2 knockouts (Cunningham *et al.* 2000; Risinger *et al.* 2000).

Two studies with dopamine transporter (DAT) knockout mice showed conflicting responses on preference drinking. DAT knockout female, but not male, mice on a mixed 129/SvJ × C57BL/6J background showed decreased ethanol intake in a two-bottle choice test, decreased preference for saccharin, and decreased quinine avoidance, but also increased water intake. Latency to LORR after ethanol was also shorter in these DAT knockouts (Savelieva *et al.* 2002). In a separate study, DAT knockout male, but not female, mice on a 129S4/SvJae × C57BL/6J mixed background showed increased ethanol intake, and female heterozygous knockout mice showed increased preference in a two-bottle choice test (Hall, Sora & Uhl 2003). It is difficult to reconcile these opposite findings, but the 129/SvJ source is known to be segregating at many loci (Simpson *et al.* 1997), so background genes other than the DAT knockout could be affecting results.

Finally, brain vesicular monoamine transporter heterozygote male, but not heterozygote female, knockout mice on a 129/SvEvBrd × C57BL/6J background showed increased ethanol intake in a two-bottle choice test but also increased food intake (Hall *et al.* 2003). The phosphoprotein DARPP-32 knockout shows reduced operant self-administration of ethanol and conditioned place preference, and enhanced locomotor activation, but no difference from wildtype in conditioned taste aversion or operant self-administration of food and water (Risinger *et al.*, 2001). This suggests that this protein, which is phosphorylated by D_1 receptor activation, may partially mediate dopamine's role in ethanol seeking behavior. In all, interpretation of the ethanol-related results for the knockout of dopamine and vesicular transporters is complicated by the involvement of pathways modulated by these proteins that influence critical behaviors such as locomotion.

CORTICOTROPIN-RELEASING FACTOR (CRF) (TABLE 3)

Alcohol has been established as a powerful activator of the hypothalamic-pituitary-adrenal axis, and this activation is largely due to stimulation of corticotropin-releasing factor (CRF) release from the paraventricular nucleus through the portal system (Rivier, Bruhn & Vale 1984; Ogilvie, Lee & Rivier 1997; Lee *et al.* 2004). However, CRF is heavily localized extrahypothalamically in the basal forebrain (central nucleus of the amygdala, bed nucleus of the stria terminalis) and brainstem where it has been hypothesized to have a role in mediating behavioral and sympathetic responses to stressors (Heinrichs & Koob 2004). Two CRF receptors have been characterized—CRF_1 and CRF_2—and they have been hypothesized to mediate the effects of CRF and other stress-related peptides, urocortin 2 and 3, respectively. Urocortin 1 activates both CRF_1 and CRF_2 receptors, while urocortin 2 and 3 are selective for CRF_2 receptors. CRF_1 and CRF_2 receptors have different distributions in the rodent brain, with overlap in some basal forebrain regions such as the bed nucleus of the stria terminalis and amygdala (Chalmers, Lovenberg & De Souza 1995). Pharmacological studies using selective agonists and antagonists of the CRF_1 and CRF_2 receptor have shown a predominantly antistress-like effect of blockade of CRF_1 receptors and activation of the CRF_2 receptor (Zorrilla & Koob 2004). However, blockade of CRF_2 receptors has produced mixed results, with low doses of an antagonist producing stress-like effects but higher doses and selective blockade in certain basal forebrain regions producing antistress like effects (Kishimoto *et al.* 2000; Takahashi *et al.* 2001; Bakshi *et al.* 2002). Knockout of the CRF_1 receptor results in phenotypes of decreased stress-like responses in most measures (Timpl *et al.* 1998; Contarino *et al.* 1999), and knockout of the CRF_2 receptor results in largely the opposite phenotypes of increased stress-like responses in some but not all hormonal and behavioral tests (Bale *et al.* 2000; Coste *et al.* 2000; Kishimoto *et al.* 2000).

Transgenic mice overexpressing CRF consumed significantly less ethanol in a two-bottle preference test but had slightly less ethanol-conditioned taste aversion (Palmer *et al.* 2004). The transgenics also showed an increased sensitivity to ethanol as measured by the LORR, and a slightly faster rate of ethanol clearance. CRF null mutants showed enhanced preference and less conditioned place preference for ethanol, but resembled an approximately matched control genotype in other responses to ethanol (Olive *et al.* 2003). CRF_1 knockout mice show a mixed pattern of responses to ethanol,

Table 3 Corticotropin-releasing factor (CRF).

Gene	*Ref.*[a]	*Gender/background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Anxiety*	*Withdrawal*	*Other*
CRF	1	Gender not specified 129S2/SvPas × C57BL/6J, N2 backcross to C57BL/6J, then F5-8 intercross as background ('WT' = F2 hybrid from 129S1/SvImJ × C57BL/6J)	KO > WT (intake, PR, >4%) 2%, 4%, 8%, 10% ethanol (4 days each) KO = WT (saccharin PR, quinine avoidance, total fluid intake) KO > WT (intake, 10%, 2-hour limited access)	KO < WT (CPP to 2 g/kg; no CPP in KO) KO = WT (CPP to 3 g/kg)	KO < WT (increase in activity, 2 g/kg; no response in KO)	KO = WT (LORR latency, duration, 4 g/kg)	—	—	KO = WT (clearance, 4 g/kg)
CRF overexpression	2	Male, female Rat CRF gene into SJL × C57BL/6J; N9 backcross to C57BL/6J	OX < WT (intake, 10%, 20% ethanol) OX < WT (PR, ≥6% ethanol) 3%, 6%, 10%, 20% ethanol (4 days each) OX ≤ WT (saccharin PR, quinine avoidance)	OX < WT (CTA to 3 g/kg but not 1.5 g/kg)	—	OX > WT (LORR duration, BEC at recovery, 3.6 g/kg; tested in males only)	—	—	OX > WT (clearance, 3 g/kg; tested in males only)
CRF_1	3	Male F2 hybrid background of 129/Ola × CD-1	KO = WT (intake) 2%, 4%, 8% ethanol (6 days each) 3 weeks after 8 weeks of 8% ethanol or water habituation, three repeated social defeat/ forced swim stresses increased intake in KO (not WT) (remained elevated for 6 months)	—	—	—	—	—	KO = WT (clearance, 3.5 g/kg)

Table 3 *Cont.*

Gene	*Ref.*[a]	*Gender/background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Anxiety*	*Withdrawal*	*Other*
	4	Gender not specified F2 hybrid of KO chimeras × 129/Ola or CD-1	—	—	Cf. withdrawal	—	Cf. withdrawal	KO = HET = WT (reduced open field activity post 20% ethanol forced consumption, 18 days) KO > (HET = WT) (entries and time spent in light in light-dark box during withdrawal; no change from baseline in any genotype during withdrawal) (KO = HET) >WT (increased latency to enter light compartment in light-dark box during withdrawal) KO < (WT = HET) Increased percentage of WT and HET (not KO) avoided light during withdrawal	KO = WT (clearance, 3.5 g/kg, tested in males only)
CRF_2	5	Male, female 129/SvJ to C57BL/6J N8 backcross to C57BL/6J	KO = WT (intake, PR) 3%, 6%, 10%, 20% ethanol (4 days each; tested only in females) KO > WT (intake, limited access, 7.5%, 10% only; 0–10%, 30 minutes to 2 hours, in dark (tested in males and females)	KO = WT (CTA to 2, 4 g/kg)	—	KO = WT (hypothermia, 3 g/kg) KO = WT (LORR duration, BEC at recovery, 4 g/kg)	—	—	KO = WT (clearance, 3 g/kg)

[a]Reference nos. 1, Olive *et al.* (2003); 2, Palmer *et al.* (2004); 3, Sillaber *et al.* (2002); 4, Timpl *et al.* (1998); 5, Sharpe *et al.* (2005).

possibly consistent with reduced anxiety-like behavior during alcohol withdrawal (Timpl *et al.* 1998). Activity was reduced in the open field equally in knockout and wildtype mice, and knockouts were equal to wildtypes in the increased latency to enter the light compartment of the light-dark box. However, fewer knockout mice avoided the compartment than wildtype or heterozygote mice. CRF_1 knockout mice also show a lesser increase in ethanol consumption during withdrawal from ethanol dependence (Chu *et al.* submitted). The phenotype of other CRF knockout mice on ethanol drinking without withdrawal is less robust and somewhat counterintuitive. Mice lacking the CRF_1 receptor showed no changes in drinking of ethanol in a two-bottle preference test for 8% ethanol during baseline or immediately after exposure to a social stressor, but a significant increase in drinking 3 weeks after repeated social stress. The authors linked this change to an up-regulation of the glutamate NR2B subunit in the basal forebrain (Sillaber *et al.* 2002). However, levels of ethanol intake were extremely low in this study. CRF_2 knockout mice showed no phenotypic differences from wildtypes in continuous-access ethanol preference, and only a modest reduction with limited access. Neither did wildtypes and knockouts differ in ethanol-conditioned taste aversion, or in sensitivity to hypothermia or LORR (Sharpe *et al.* 2005).

These results, combined with the CRF pharmacological studies, suggest that the role of CRF in ethanol drinking may be complex, with CRF mediating drinking in different ways depending on the stress/anxiety interaction. Acute withdrawal-induced anxiety-like responses are blocked by CRF_1 antagonists and may be attenuated in CRF_1 knockouts. When these anxiety-like responses lead to excessive drinking during acute withdrawal, ethanol self-administration is also blocked. However, under non-withdrawal conditions, knockout of CRF_1 receptors can also lead to increased ethanol self-administration. No effect of CRF_2 knockout is observed on baseline drinking, and no data are yet available on CRF_2 receptors and the increased drinking associated with ethanol withdrawal. The role of the CRF system may differ in animals with a history of drinking or during withdrawal, an area that should be further explored.

OPIOIDS (TABLE 4)

Opioid peptides have long been hypothesized to have a role in ethanol reward based on animal and human studies showing efficacy of the opioid antagonist naltrexone in blocking ethanol self-administration (Altshuler, Phillips & Feinhandler 1980; O'Malley *et al.* 1992; Volpicelli *et al.* 1992). Knockout of the μ opioid receptor on a 129/Sv × C57BL/6J background resulted in decreased ethanol self-administration in a number of paradigms. μ opioid receptor knockout mice show decreased drinking in preference tests (Roberts *et al.* 2000; Hall, Sora & Uhl 2001; Becker *et al.* 2002). The effects on two-bottle preference depend on the background and may be gender-specific (Hall *et al.* 2001). μ opioid knockouts also show decreased operant self-administration (Roberts *et al.* 2000) and decreased place preference, but in females only using a biased procedure (Hall *et al.* 2001). Hall *et al.* (2001) saw decreases in intake only in female μ knockout and heterozygous mice on a 129/SvEv × C57BL/6J background. Roberts *et al.* (2000) saw decreases in intake or preference, but only after operant training for ethanol or forced ethanol intake in male homozygous μ knockout mice on a different 129/Sv × C57BL/6J background. Others observed an approximate 50% decrease in intake of ethanol 3–4 weeks into preference testing in male mutants on a 129/Ola × C57BL/6J background (Becker *et al.* 2002). However, the effects on ethanol reward were more robust under operant limited access tests (Roberts *et al.* 2000; Hall *et al.* 2001) and Hall *et al.* (2001), but not Becker *et al.* (2002), saw an effect on conditioned place preference. μ opioid receptor knockout mice did not show differences in anxiety-like responses to acute ethanol in the elevated plus maze (LaBuda & Fuchs 2001), though they were compared with C57BL/6J or C57BL/6J × 129/SvEvTac F1 hybrids, rather than matched wildtype controls. μ opioid mutants had a blunted stimulant and anxiolytic response to ethanol, and developed handling-induced convulsions during withdrawal earlier than wildtypes during repeated periods of exposure and withdrawal. μ knockouts also had greater anxiety-like responses in the light-dark test during withdrawal (Ghozland *et al.* 2005).

In contrast, mice with knockout of the δ opioid receptor on a 129/Sv × C57BL/6Orl background showed increases in operant self-administration of ethanol and in preference during a retest after the operant studies (Roberts *et al.* 2001). κ receptor knockouts on a 129/SvJ × C57BL/6Orl background, but backcrossed to C57BL/6Orl mice, showed decreased ethanol intake in preference testing (Kovacs *et al.* 2005). κ receptor knockout mice also showed a decrease in saccharin preference and lesser quinine avoidance, suggesting effects on orosensory systems (Kovacs *et al.* 2005). κ receptor knockout mice on a different mixed background showed enhanced dopamine overflow in the nucleus accumbens after 2 g/kg ethanol (Zapata & Shippenberg 2006). δ receptor knockout mice showed an anxiogenic-like phenotype in the light-dark test that was reversed by ethanol (Roberts *et al.* 2001). Two reports showed a tendency toward a reduced effect of ethanol to stimulate activity in μ knockout mice (Hall *et al.* 2001; Ghozland *et al.* 2005). Thus, μ opioid receptor knockout mice showed decreased ethanol reward and an anxiogenic-like response, and δ

Table 4 Opioids.

Knockout	*Ref.*[a]	*Gender/background*	*Preference*	*Other ethanol reward*	*Activity*	*Anxiety*	*Withdrawal*
μ	1	Male 129/Sv × C57BL/6J F1	KO < WT (intake, PR) 10% ethanol, one bottle (3 days) then two bottle preference (3 days)	KO < WT (operant self- administration, 10% ethanol across 8 days, after saccharin fading)	—	—	—
	2	Male, female 129/SvEv × C57BL/6J F1	(KO = HET) < WT (intake; effect in female only) 2%, 4%, 8%, 12%, 16%, 24%, 32% ethanol (2–3 days each) KO = WT (food intake)	(KO = HET) < WT (CPP, 2 g/kg; effect in WT females only; no CPP in males; note biased apparatus)	(KO = HET) < WT (ethanol-increased activity after 2 g/kg in WT only; differences non-significant overall)	—	—
	3	Male 129/Ola × C57BL/6J F1	KO < WT (intake) 10% ethanol; WT increases from 15 to 30 g/kg in weeks 3–4; KO remains at 15 g/kg	KO = WT (CPP, 4 g/kg; note biased apparatus)	—	—	—
	4	Male 129/Sv to C57BL/6J	—	—	KO < WT (ethanol-induced activity after 0.75, 1.25, 1.75 g/kg; no increase in activity in KO)	KO < WT (anxiolytic effect in light/dark test after 1.25, 1.75 g/kg; no effect in KO)	KO > WT (withdrawal signs with multiple withdrawals; ethanol liquid diet; three withdrawals; KO exhibited withdrawal with all three tests; WT exhibited withdrawal with 2nd, 3rd tests only) KO > WT (anxiogenic effect of withdrawal in light/dark test; ethanol liquid diet; withdrawal effect in 2nd and 3rd tests only; genetic difference in 2nd test only)

	5	Male, female 129/Sv × C57BL/6J F1	—	—	—	KO = C57BL/6 = B6129F1 (Anxiolytic response in elevated plus maze 1.0, 1.6 g/kg; no littermate controls tested)	—
δ	6	Male 129/Sv × C57BL/6Orl F1	KO = WT (intake, PR) 10% ethanol, (3 days) KO > WT (intake, PR after operant self-administration in 3-day retest)	KO > WT (operant self-administration of 10% ethanol across 20 days, after saccharin fading)	KO = WT (activation in light-dark test after ethanol self-administration; cf. anxiety)	KO > WT (baseline anxiety-like behavior in light/dark test; WT unresponsive to ethanol self-administration; KO exhibited anxiolytic effect; KO reached > BEC than WT (trend); $n = 4$/genotype/group)	—
κ	7	Male, female 129/SvJ, N10 backcross to C57BL/6Orl Separate WT and KO lines tested	KO < (HET = WT) (intake, PR, 12% ethanol, males only) KO < HET < WT (intake, PR, females only) 3% (3 days), 6% (3 days), 12% ethanol (12 days); only 12% data reported KO < WT (saccharin PR, quinine avoidance)	—	KO = WT (ethanol-reduced activity after 1.5 g/kg in photocell chamber; decreased activity KO, WT during 5–15 minutes post ethanol period)	—	—
	8	Male F7 of 129S6/SvEvTac × C57BL/6	—	—	—	KO = WT (dopamine overflow in nucleus accumbens, 24 hours after 4-day inhalation)	KO > WT (dopamine overflow in nucleus accumbens after acute 2 g/kg)

Table 4 *Cont.*

Knockout	*Ref.*[a]	*Gender/background*	*Preference*	*Other ethanol reward*	*Activity*	*Anxiety*	*Withdrawal*
β-Endorphin	9	Male, female 129S2/SvPas Backcross to C57BL/6N, then N7-10 to C57BL/6J	(KO = HET) > WT (intake, PR; 7% ethanol) (KO = WT) < HET (intake, PR; 10% ethanol) 7%, 10% ethanol (8 days each)	—	—	—	—
	10	Male 129S2/SvPas Backcross to C57BL/6N, then N9 to C57BL/6J	KO = WT (intake) 10% ethanol (28 days) KO > WT (2-hour/day limited access, 10 days) KO > WT (2-hour/day limited access after 2-day deprivation) KO = WT (2-hour/day limited access after 5-week deprivation)	—	—	—	—
Preproenkephalin	11	Male 129X1/SvJ × 129/Sv N10 backcross to C57BL/6J	KO = WT (intake, PR) 2%, 4%, 8%, 10% ethanol (4 days each) KO = WT (total fluid intake)	KO = WT (CPP, 2 g/kg; both exhibited CPP)	—	—	—

[a]Reference nos. 1, Roberts *et al.* (2000); 2, Hall, Sora & Uhl (2001); 3, Becker *et al.* (2002); 4, Ghozland *et al.* (2005); 5, LaBuda & Fuchs (2001); 6, Roberts *et al.* (2001); 7, Kovacs *et al.* (2005); 8, Zapata & Shippenberg (2006); 9, Grisel *et al.* (1999); 10, Grahame *et al.* (2000); 11, Koenig & Olive (2002).

knockouts showed increased ethanol consumption and an anxiolytic-like response.

The ethanol drinking phenotype of β-endorphin knockout mice on a background of 129S2/SvPas × C57BL/6N (then backcrossed to C57BL/6J) is less robust and somewhat counterintuitive. β-Endorphin synthesis was eliminated via site-directed mutagenesis in ES cells. Heterozygous β-endorphin knockout mice showed increased drinking in preference tests compared with wildtype mice, but homozygous mice showed increases only at a lower concentration of ethanol (7%) (Grisel *et al.* 1999). The homozygotes also showed greater ethanol intake initially during daily 2-h tests and after 2 days of alcohol deprivation compared with wildtype mice, but no difference from wildtypes during 28 days of continuous access (Grahame *et al.* 2000). No differences in ethanol drinking or conditioned place preference were observed in mice lacking the preproenkephalin gene on an R1 embryonic cell line background then backcrossed to a C57BL/6J (Koenig & Olive 2002). However, recent results suggest a decrease in ethanol intake in these knockouts, accompanied by a greater conditioned taste aversion to ethanol, but no difference in ethanol-conditioned place preference (Blednov *et al.* submitted). These results, combined with the opioid receptor knockout studies, suggest redundancy within the opioid system regarding ethanol reward. In addition, the results suggest a role for the μ opioid receptor system with possible modulatory roles for δ and κ receptors through indirect pathways such as those involved in anxiety and orosensory processing. Similar to the results for D_1 and D_2 receptor null mutants, ethanol intake in two-bottle choice studies was not abolished—it was reduced by about 50% in κ knockouts (Kovacs *et al.* 2005) and by up to 65% in μ receptor knockout mice, depending upon the amount of past ethanol experience (Roberts *et al.* 2000; Becker *et al.* 2002). However, operant self-administration of ethanol was virtually negligible in μ receptor knockout mice (Roberts *et al.* 2000).

CANNABINOIDS (TABLE 5)

The cannabinoid-1 (CB_1) receptor appears to mediate the psychoactive effects of cannabinoids and is widely distributed throughout the brain, with very high concentrations in the extrapyramidal motor system (Freund, Katona & Piomelli 2003). Alcohol intake of young mice but not older mice was blocked by administration of a CB_1 antagonist (Wang *et al.* 2003). A CB_1 antagonist blocked ethanol self-administration in alcohol-preferring rats (Colombo *et al.* 2004; Gessa *et al.* 2005) and blocked cue-induced reinstatement of ethanol-seeking behavior in rats (Cippitelli *et al.* 2005). Cannabinoid antagonists also have been shown to block responding for opioids, nicotine, cannabinoids and food, suggesting a more general effect on reward or reinforcement mechanisms (Martellotta *et al.* 1998; Cohen *et al.* 2002; Solinas & Goldberg 2005). Place preference for nicotine is also blocked by CB_1 antagonists (Le Foll & Goldberg 2004). Finally, cue-induced reinstatement of nicotine-seeking behavior was blocked by a CB_1 antagonist (Cohen, Kodas & Griebel 2005). Overall, these results suggest a wider role for cannabinoid receptors in responses to several addictive substances.

CB_1 knockout mice on three different genetic backgrounds have been tested for ethanol intake in six preference experiments. In five of these studies, knockouts showed less ethanol intake or preference than wildtypes, while in the other, knockouts showed no difference or perhaps enhanced intakes. On a 129/Ola × C57BL/6 F2 background, CB_1 knockouts showed decreased ethanol intake in a two-bottle preference test with 10% ethanol (Poncelet *et al.* 2003). This test was limited to offering ethanol during four daily, 6-hour periods of limited access, and sucrose intake (5%) was also lower. An extended (14-day) two-bottle unrestricted access comparison reported no difference in intake between knockouts and wildtypes on this background but a decrease in ethanol preference (Lallemand & De Witte 2005). In knockout mice on a 129/Sv × C57BL/6J background, there was decreased ethanol intake in a two-bottle preference test for 12% ethanol and during withdrawal from ethanol vapor inhalation (Hungund *et al.* 2003). No differences in food intake or total fluid intake were observed. However, in another study, CB_1 knockout mice on this background actually had greater ethanol consumption (8% versus water) than the C57BL/6J wildtype in a two-bottle choice paradigm during initial exposure. However, these mice showed decreased stress-induced increases in ethanol consumption and showed no ethanol withdrawal responses after forced drinking of 4–16% solutions for 4 weeks (Racz *et al.* 2003). Finally, two studies showed similar decreases in ethanol intake versus wildtypes in two-bottle preference in CB_1 knockouts developed on a 129/Sv background, then maintained as a heterozygous cross with the outbred CD-1 strain (Naassila *et al.* 2004; Thanos *et al.* 2005). These CB_1 knockout mice showed increased sensitivity to the sedative effects of ethanol as measured by LORR and ethanol-induced hypothermia, and lacked the locomotor stimulant response to ethanol seen in wildtypes. They also had increased severity of ethanol withdrawal-induced convulsions after removal of a chronic liquid diet containing ethanol (Naassila *et al.* 2004). A third withdrawal experiment showed greater intake of knockouts during withdrawal after extended vapor inhalation exposure, but also an increased blood ethanol concentration after vapor exposure in knockouts (Lallemand & De Witte 2005). CB_1 knockout mice

Table 5 Cannabinoids.

Gene	*Ref.*[a]	*Gender/ background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Anxiety*	*Tolerance*	*Withdrawal*	*Other*
CB_1	1	Male 129/Ola × C57BL/6 F2	KO < WT (intake) 6-hour limited access; 10% ethanol, dark cycle for 4 days KO < WT (5% sucrose intake) KO = WT (food intake)	—	—	—	—	—	—	—
	2	Male, female 129/Sv × C57BL/6J HET matings, N5 'WT' = C57BL/6J	KO < (HET = WT) (intake; no PR data, bigger difference in females) 3% ethanol (3 days); 6% (3 days), 12% (12 days; only 12% data reported) KO = WT (food intake, total fluid intake)	—	—	—	—	—	—	KO < WT (dopamine levels in nucleus accumbens after 1.5 g/kg; only males tested)

3	Male 129/Sv × C57BL/6J HET matings, N5 'WT' = C57BL/6J	KO > WT (intake, PR) 8% ethanol Dark cycle × 5 weeks Difference in week 1 only, KO = WT by week 3 Footshock stress increased intake for 1-day test in WT only	—	—	KO = WT (accelerating rotarod, 2 and 4 g/kg) KO = WT (hypothermia, 1–4 g/kg)	KO = WT (elevated zero maze, anxiolytic effect of 2 g/kg)	KO = WT (hypothermic tolerance to 2 and 4 g/kg after 3-week chronic forced intake)	KO < WT (HIC after chronic 4–16% ethanol, forced for 4 weeks) Little withdrawal effect 3 hours later (intake and BEC not provided) KO < WT (withdrawal-induced activity in open field)	—
4	Male, female R1 ES cells to 129/Sv, crossed to CD-1 outbred, F15 heterozygous matings as background	KO < WT (intake, PR for ≥10%) 3%, 6%, 10%, 20% ethanol (6 days each) KO = WT (sucrose PR, quinine avoidance, total fluid intake)	—	KO < WT (activity after ethanol dose–response; WT had increased activity, KO *decreased* or no change)	KO > WT (hypothermia 1.5 g/kg, not 3 g/kg) KO > WT (LORR duration, 4 g/kg)	—	—	KO > WT (HIC after chronic liquid diet; only tested in males; effect opposite to ref. 3)	KO = WT (clearance, 4 g/kg)
5	Male 129/Sv × C57BL/6J HET matings, 'WT' = C57BL/6J	—	—	—	—	—	—	—	KO > WT (reduction in FR10 responding for milk after 0.5–2.0 g/kg ethanol)
6	Male R1 ES cells to CD-1, F5 heterozygous matings as background	KO < (HET = WT) (PR) 10% ethanol (2 weeks)	KO < WT (CPP to 2 g/kg; KO showed no place preference)	—	—	—	—	—	—

Table 5 *Cont.*

Gene	*Ref.*[a]	*Gender/ background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Anxiety*	*Tolerance*	*Withdrawal*	*Other*
	7	Male 129/Ola × C57BL/6J, F2	KO = WT (intake) 10% ethanol (14 days) KO < WT (PR)	—	KO = WT (activity during withdrawal Day 1)	—	—	—	KO < WT (PR) 10% ethanol (during 40 days of withdrawal after 13 days of vapor inhalation) KO ≥ WT in g/kg intake during withdrawal KO > WT in total BEC during inhalation phase	KO > WT (peak BEC after 5 g/kg, not 1 or 3 g/kg) KO = WT (clearance of all three doses)
	8	Male R1 ES cells to CD-1, F15 heterozygote matings as background	—	KO < WT (CPP to 0.5, 1, 1.5, but not 2 g/kg)	KO = WT (activity increase after 1.5 g/kg in elevated plus maze)	—	KO = WT (anxiolytic response to 1.5 g/kg in elevated plus maze)	—	—	—
Fatty acid amidohydrolase (FAAH)	9	Male, female 129/SvJ × C57BL/6, N5 backcross to C57BL/6J	KO > WT (intake, PR; effect in females only) 3%, 6% ethanol (3 days each) then 12%, 20% ethanol (12 days each) KO = WT (saccharin PR, quinine avoidance, food intake)	—	—	KO < WT (hypothermia to 1.5 but not 3 g/kg; effect in females only) KO < WT (LORR duration, 4 g/kg; effect in females only)	—	—	—	KO = WT (clearance, 2 g/kg) KO < WT (down-regulation of limbic forebrain CB_1 receptor binding after ethanol intake; effect in females only)

[a]Reference nos. 1, Poncelet *et al.* (2003); 2, Hungund *et al.* (2003); 3, Racz *et al.* (2003); 4, Naassila *et al.* (2004); 5, Baskfield, Martin & Wiley (2004); 6, Thanos *et al.* (2005); 7, Lallemand & De Witte (2005); 8, Houchi *et al.* (2005); 9, Basavarajappa *et al.* (2006).

showed reduced ethanol-induced conditioned place preference in two studies (Houchi *et al.* 2005; Thanos *et al.* 2005), which was correlated with an overexpression of striatal dopamine D_2 receptors in one study (Houchi *et al.* 2005). The relative sedative sensitivity also differed in different backgrounds. Thus, the effect of CB_1 knockout is a decreased intake of ethanol and decreased ethanol reward as measured by conditioned place preference, similar to that observed with CB_1 receptor antagonists (Economidou *et al.* 2006). The place preference results must be tempered by the fact that conditioned place preference was only tested on a CD-1 background. Some differences in sensitivity to ethanol withdrawal and ethanol pharmacokinetics have been reported but cannot yet be considered well established. A recent study compared mice lacking fatty acid amidohydrolase (FAAH), which increases endogenous cannabinoids, and reported significant effects in females, but not males. FAAH knockouts were less sensitive to ethanol's sedative effects, and ingested more ethanol. After self-administration, they did not show the down-regulation of limbic CB_1 receptors seen in wildtypes (Basavarajappa *et al.* 2006).

SEROTONIN (TABLE 6)

Multiple serotonin receptors have been identified through molecular cloning techniques (Humphrey, Hartig & Hoyer 1993). Five of these receptor subtypes—5-HT_{1B}, 5-HT_2, 5-HT_{1A}, 5-HT_3, 5-HT_4—have been implicated in drug reward in general through pharmacological studies. For example, studies with ethanol have shown that antagonists of 5-HT_{1B}, 5-HT_2, 5-HT_3 and 5-HT_4 receptors decrease ethanol intake (Fadda *et al.* 1991; Panocka *et al.* 1995; Roberts *et al.* 1998; Tomkins & O'Neill 2000). 5-HT_3 receptor antagonists decrease ethanol intake in 24-hour two-bottle choice preference tests and in limited access operant tests (Fadda *et al.* 1991; Hodge *et al.* 1993). However, a 5-HT_2 receptor antagonist can selectively decrease operant responding for ethanol and not responding for saccharin (Roberts *et al.* 1998). 5-HT_3 receptor antagonists have been effective in decreasing relapse in clinical trials (Johnson *et al.* 2000), while the 5-HT_2 antagonist ritanserin has not been effective (Johnson 2004).

5-HT_{1B} knockout mice on a mixed 129 substrain background were reported to show increased ethanol intake in two-bottle choice tests with no difference in food, water or saccharin intake, or quinine avoidance (Crabbe *et al.* 1996). The mice were also less sensitive than wildtypes on a test of ethanol-induced ataxia, and developed less tolerance, but showed equivalent withdrawal severity and ethanol clearance. However, this initial finding of a drinking difference was not replicated (Crabbe, Wahlsten & Dudek 1999; Bouwknecht *et al.* 2000). In fact, another study with 5-HT_{1B} knockout mice derived from the same mixed 129 background showed a blunted place preference to ethanol and taste aversion equivalent to wildtypes (Risinger, Bormann & Oakes 1996). Risinger, Doan & Vickrey (1999) also showed greater levels of operant responding for 10% ethanol, but not for other concentrations, or when the ethanol was sweetened. This study also reported generally quite low absolute levels of self-administration. One study noted differences in fluid intake, with 5-HT_{1B} knockout mice consuming 50% higher total fluid intake (Bouwknecht *et al.* 2000) and is consistent with evidence suggesting an inhibitory role of 5-HT_{1B} receptors in the regulation of body weight and fluid intake (Kennett, Dourish & Curzon 1987). The differences in outcomes among the self-administration studies have been attributed to the changing mixture of 129 substrains used to maintain the populations of knockouts (Phillips, Hen & Crabbe 1999). In a subsequent study (Phillips & Belknap 2002), mice from the F2 cross of the knockout and mixed 129 wildtype genotypes were selectively bred for four generations for high or low ethanol preference. In this population, the frequency of the 5-HT_{1B} mutant allele was 0.5 at the start. The lines diverged substantially in intake, and by the fourth generation the frequency of the mutation had increased to 0.75 in the high drinkers, and decreased to about 0.25 in the low drinkers. The mutant allele accounted for only 18% of the individual differences in this population, strongly suggesting that the remainder of the wide divergence in drinking between the high and low selected lines was due to the epistatic (interactive) influences of other genes segregating in the mixed 129 background population (Phillips & Belknap 2002). Thus, the effect of deletion of the 5-HT_{1B} gene on ethanol drinking depends on the genotype at other genes. 5-HT_3 receptor overexpression in mice on a B6SJL F1 background did not alter the discriminative stimulus effects of ethanol or the effects of GABA-positive modulators and *N*-methyl-D-aspartate (NMDA) antagonists in producing ethanol-like stimulus effects (Shelton, Dukat & Allan 2004). Null mutants for the 5-HT_6 receptor did not differ from wildtypes in intake, but were more stimulated; they were also less sedated in two of three tests than wildtypes (Bonasera *et al.* 2006).

Serotonin transporter knockout mice on a CD-1 background (Bengel *et al.* 1998) backcrossed to C57BL/6J showed decreased ethanol intake in a two-bottle choice preference test (Kelai *et al.* 2003). However, in a subsequent study, this effect was much less pronounced and there was no difference in sensitivity to ethanol-conditioned place preference (Boyce-Rustay *et al.* in press). Serotonin transporter knockouts did have greater sensitivity to ethanol-induced LORR, but did not differ in hypothermic sensitivity. It may be that the greater sensitivity of knockouts to rotarod ataxia may have been

Table 6 Serotonin.

Gene	*Ref.*[a]	*Gender/ background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Tolerance*	*Withdrawal*	*Other*
5-HT_{1B}	1	Male, female 129 substrain mixture as background	KO > WT (intake, PR) 3%, 6%, 10%, 20% ethanol (8–12 days each) KO = WT (saccharin, sucrose PR, quinine avoidance, food intake)	—	—	KO < WT (ataxia in grid test, 1.5, 2, 2.5 g/kg)	KO < WT (rate and magnitude of ataxia tolerance in grid test, 2 g/kg)	KO = WT (HIC after acute withdrawal from 4 g/kg, and chronic withdrawal after vapor inhalation)	KO = WT (clearance of 3.5 g/kg)
	2	Male, female 129 substrain mixture as background	KO = WT (intake, PR) 6% ethanol (4 days)	—	—	—	—	—	—
	3	Male 129 substrain mixture as background	KO = WT (intake, PR) 3%, 6%, 10%, 20% ethanol (6–8 days each)	—	—	—	—	—	—
	4	Male, female 129 substrain mixture as background	—	KO = WT (CTA, dose–response, 1–4 g/kg) KO < WT (CPP, 2 g/kg; no CPP in KO)	KO < WT (ethanol-induced activity, late trials during CPP)	—	—	—	—

	5	Male, female 129 substrain mixture as background	—	KO > WT (operant self-administration, 10% ethanol, FR4, 30 days) KO = WT (serial 0, 5%, 10%, 20% ethanol, alone and in saccharin)	—	—	—	—	—
5-HT_3 overexpression	6	Male C57BL/6J × SJL/J F2 background	—	OX = WT (operant self-administration, 0.56, 1.0, 1.8 g/kg, 5.5% ethanol, FR5)	—	—	—	—	OX = WT (aggression in resident-intruder; no effect of ethanol self-administration)
	7	Gender not specified C57BL/6J × SJL/J F1 background	—	—	—	—	—	—	OX = WT (drug discrimination, 1.5 g/kg ethanol versus water)
5-HT_3	8	Gender not specified C57BL/6J × SJL/J; F2 backcrossed to C57BL/6J and DBA/2J; N1, N3 and N5 generations were studied	KO < WT (intake) 10% ethanol (10 days, on C57BL/6J background) KO = WT (intake) 10% ethanol (10 days, on DBA/2J background)	—	KO = WT (activity increase, 1.5 g/kg, N5 vs both backgrounds, and N5 vs B6SJL F2 transgenic)	—	—	—	—
5-HT_6	9	Male JM1 ES cell to C57BL/6J, F1 hybrid with C57BL/6J	KO = WT (intake, PR) 3%, 6%, 10%, 20% ethanol (4 days each)	—	KO > HET > WT (activation, 2 g/kg, 5 minutes test)	KO < (HET = WT) (rotarod, 2 g/kg; LORR duration, 3.5 g/kg) KO = HET = WT (hypothermia, 2 g/kg)	—	—	KO = HET = WT (clearance, 3.5 g/kg)

Table 6 *Cont.*

Gene	*Ref.*[a]	*Gender/ background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Tolerance*	*Withdrawal*	*Other*
5-HT transporter	10	Male R1 ES cells to CD-1 background, N7 backcross to C57BL/6J	KO < WT (intake; >6% only) 3%, 6%, 10%, 15%, 20% ethanol, 4–24 days each	—	—	—	—	—	—
	11	Male, female R1 ES cells to CD-1 background, >N15 backcross to C57BL/6J	KO < WT (intake, not PR, 7% only) 3%, 5%, 7%, 9%, 11% (4–12 days each; only tested in females) KO = WT (saccharin intake, quinine avoidance)	KO = HET = WT (CPP, 2 g/kg; tested only in females)	—	KO > (HET = WT) (LORR duration, 3 g/kg) KO = HET = WT (hypothermia, 2 and 4 g/kg) KO ≥ WT (rotarod ataxia, 1.5–2.5 g/kg, but KO > WT in basal ataxia) KO = HET = WT (hypothermia, 2 g/kg) Tested only in males in all four tests	—	—	KO = HET = WT (tail suspension test immobility, 1 and 1.5 g/kg; tested only in males) BEC not reported
	12	Male R1 ES cells to CD-1 background, >N15 backcross to C57BL/6J	—	—	—	—	—	—	KO > WT (sensitivity to ethanol-induced inhibition of 5-HT clearance from hippocampus)

[a]Reference nos. 1, Crabbe *et al.* (1996); 2, Crabbe *et al.* (1999); 3, Bouwknecht *et al.* (2000); 4, Risinger *et al.* (1996); 5, Risinger *et al.* (1999); 6, McKenzie-Quirk *et al.* (2005); 7, Shelton *et al.* (2004); 8, Metz *et al.* (2006); 9, Bonasera *et al.* (2006); 10, Kelai *et al.* (2003); 11, Boyce-Rustay *et al.* (in press); 12, Daws *et al.* (2006).

due to basal performance differences and floor effects (Boyce-Rustay *et al.* in press). In summary, there is mixed evidence for ethanol intake phenotypes in alteration of individual serotonin receptor subtypes, and the effect of 5-HT_{1B} deletion depends heavily on the presence of other unknown genes. However, global increases in serotonin produced by knockout of the serotonin transporter have effects in decreasing ethanol intake similar to those observed with selective serotonin reuptake inhibitors (Sellers, Higgins & Sobell 1992).

ION CHANNELS (TABLE 7)

Most of the studies of ion channel mutants have used mice lacking ligand-gated ion channels, such as $GABA_A$ receptors (Table 1 above). Despite the importance of voltage-gated ion channels in the brain, we find only three studies in the literature manipulating potassium and one targeting calcium channels. Null mutants for one member of the G protein-activated inwardly rectifying potassium channel family (GIRK2) were examined by several groups after recombinant GIRK channels were found to be activated by ethanol. These studies addressed the question of which behavioral actions of ethanol might be mediated by activation of GIRK channels: one answer is analgesia (Blednov *et al.* 2003b). This is a behavioral effect of ethanol not often studied, but GIRK channels appear critical for analgesic effects of many drugs (Blednov *et al.* 2003b), and it is reasonable that reduction of pain is a consequence of ethanol activation of GIRK2. In a more extensive characterization of the GIRK2 null mutants (Blednov *et al.* 2001), the mutation clearly did not affect preference for ethanol or sensitivity to high dose effects such as LORR. Multiple tests of ethanol withdrawal gave different answers, as did tests of ethanol's effects on activity, which differed according to the specific procedures used. GIRK2 mutants may have showed a lesser anxiolytic response and did not differ in acute functional tolerance. However, Hill *et al.* (2003) were unable to condition a place preference to 2 or 3 g/kg ethanol in GIRK2 KO mice, while conditioning was robust in wildtypes. KO mice were also less sensitive than wildtypes to ethanol conditioned taste aversion.

The *N*-type calcium channels are also G-protein modulated channels as they are inhibited by activation of G-protein coupled receptors. They are also inhibited by acute exposure to ethanol and up-regulated by chronic exposure (Newton & Messing 2006). Deletion of *N*-type calcium channels (by targeting Cav2.2) substantially reduced the ethanol LORR but slightly increased the ataxic effects of ethanol. These mutant mice consumed much less ethanol but were less sensitive to the aversive effects of ethanol and developed conditioned place preference with low doses of ethanol (which did not produce a place preference in wildtypes) (Newton *et al.* 2004). This is consistent with ethanol inhibition of calcium channel function contributing to LORR. The inhibition of voluntary drinking combined with decreased aversive effects and enhanced place preference suggests that gene deletion enhanced the rewarding effects of alcohol, and the mutant mice did not need to consume as much alcohol to achieve the same level of reward.

The other ion channels included in Table 7 are two classes of ligand-gated channels, neuronal nicotinic and glycine-activated chloride channels. There is considerable evidence of overlapping actions of alcohol and nicotine, ranging from common actions on nicotinic receptor function to the high rate of nicotine use by alcoholics (Dani & Harris 2005). In view of the proposed role of nicotinic receptors in alcohol action, and the complexity of this receptor system, it is surprising that only one subunit has been studied in mutant mice. Deletion of the $\alpha 7$ subunit, which is primarily presynaptic and regulates neurotransmitter release, increased three behavioral effects of ethanol: hypothermia, duration of LORR, and stimulated motor activity (Bowers *et al.* 2005). Ethanol enhances function of the glycine-gated chloride channel, and the role of this channel in alcohol action was addressed by construction of transgenic mice expressing an $\alpha 1$ subunit rendered alcohol-resistant by a single amino acid mutation. This subunit is a component of almost all glycine-activated chloride channels in adult animals. This transgenic overexpression reduced the effect of alcohol on strychnine seizures, LORR, and rotarod performance. This suggests these receptors as important targets of alcohol action, but it should be noted that the transgenic approach has the shortcoming of adding the mutant receptor in addition to the existing wildtype subunits. A better approach is to replace the wildtype receptor with the mutant construct (knock-in). This was attempted, but the mutation was lethal, and the $\alpha 1$ null mutant mouse also dies within the first few weeks after birth, limiting genetic tools for behavioral study of glycine-activated chloride channels (Findlay *et al.* 2003).

ADENOSINE (TABLE 8)

Adenosine A2A receptors are well positioned in the basal ganglia to influence reward-related behaviors (Ferre *et al.* 1997). A2A receptor activation may oppose some effects mediated by dopaminergic systems (Ferre *et al.* 1992, 1994). However, some have reported that A2A receptors cooperate in the enhancing effects of ethanol on cyclic adenosine monophosphate/protein kinase (cAMP/PKA) signaling (Yao *et al.* 2002; Mailliard & Diamond 2004). An A2A receptor null mutant has been characterized as hypodopaminergic (Dassesse *et al.* 2001). Table 8 summarizes studies of ethanol-related effects in this A2A

Table 7 Ion channels.

Gene	*Ref.*[a]	*Gender/ background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Anxiety*	*Tolerance*	*Withdrawal*	*Other*
G-protein-activated inwardly rectifying K^+ channel-2 (GIRK2)	1	Male, female 129/SvJ × C57BL/6J hybrid	KO = WT (intake, PR) 3%, 6%, 10% ethanol (8 days each) KO > WT (intake, 8%, when presented on favored side; tested only in females) KO > WT (total fluid intake) KO ≥ WT (saccharin PR, quinine avoidance; tested only in females)	—	KO > WT (ethanol-induced activity in open field after 0.5–2.25 g/kg) KO < WT (increased activity after 1.25 g/kg (but not 1 or 2 g/kg) in home cage after habituation) KO > WT (decreased activity after 2 g/kg in home cage; WT not affected) KO > WT (baseline activity in home cage)	KO = WT (LORR duration, BEC at righting, 3.8 g/kg)	KO ≤ WT (anxiety-like behavior in elevated plus maze after 1 g/kg in WT, not KO) Lack of KO response may be limited by ceiling effect	KO = WT (acute functional tolerance, fixed speed rotarod, 1.75 then 2 g/kg)	KO < WT (acute withdrawal severity after 4 g/kg) KO > WT (baseline HIC) KO > WT (withdrawal severity after chronic ethanol liquid diet; KO > WT in intake) KO = WT (withdrawal severity after chronic ethanol liquid diet when pair-fed)	—
	2	Male, female 129/SvJ × C57BL/6J hybrid, F2–13	—	—	—	—	—	—	—	KO < WT (analgesia, hot plate test, 1.75, 2.0 g/kg)

	3	Male, female 129/SvJ × C57BL/6J hybrid	—	KO < WT (CTA to 2, 2.5 g/kg) KO = WT (CTA to 3.5 g/kg) KO < WT (CPP to 2 and 3 g/kg; no CPP in KO)	KO > WT (ethanol-induced activation during CPP trials, 2 and 3 g/kg)	—	—	—	—	—
Shaw-like potassium K^+ channels (Kv3.1/Kv3.3 double KO)	4	Male 129/SvJ × C57BL/6J hybrid	—	—	—	KO > (WT = HET) (sideways falls in an open field, 0.5, 1, 2 g/kg)	—	—	—	—
Nicotinic α7	5	Gender not specified B6.129S7-Acra7-tm1Bay; C57BL/6J background	—	—	—	—	—	—	—	(KO = HET) > WT cells (sensitivity to ethanol neurotoxicity; MTT assay; 50–150 mM) KO > (HET = WT) cells (sensitivity to ethanol neurotoxicity; calcein-AM assay; 50–200 mM)
	6	Male, female 129/SvEv ES cells to C57BL/6J, then > N10 backcross to C57BL/6J	—	—	KO > WT (open field activation, 1 g/kg, but not 1.5 or 2 g/kg) KO = WT (ethanol-induced reductions in Y-maze crosses and rearing, 1–2 g/kg)	KO > WT (hypothermia; 3 g/kg) KO > WT (LORR duration; 3.8 g/kg)	—	—	—	KO = WT (clearance, 3.8 g/kg) KO = WT (ethanol reduction of acoustic startle and prepulse inhibition; 0.75–2.5 g/kg)

Table 7 *Cont.*

Gene	*Ref.*[a]	*Gender/ background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Anxiety*	*Tolerance*	*Withdrawal*	*Other*
Glycine receptor α1 subunit S267Q mutant	7	Male, female Point mutation was placed on FVB/NJ background. Subjects were from F2 intercross of TG × GlyR α1 KO mice on C57BL/6J	—	—	—	TG = WT (hypothermia, 3.0, 3.4, 3.8 g/kg) TG < WT (LORR duration, male, 3.8, 4.2, not 3.4 g/kg; also less sensitive by ED_{50} to lose righting reflex; effect only in males) TG < WT (sensitivity to fall from fixed speed rotarod, 2.5 r.p.m., assessed by ED_{50})	—	TG = WT (acute functional tolerance, 5 r.p.m. fixed speed rotarod, 1.75 then 2 g/kg)	—	TG < WT (sensitivity to anticonvulsant effect of ethanol against strychnine seizures, 0.5–1 g/kg) TG = WT (sensitivity to anticonvulsant effect of ethanol against pentylenetetrazol seizures, 1–1.25 g/kg) TG = WT (clearance, 4 g/kg)
N-type Ca^{2+} channels	8	Male 129/SvJae × C57BL/6J hybrids	KO < WT (intake, PR) 3%, 6%, 10%, 14%, 20% ethanol (4 days each) KO = WT (saccharin intake, quinine intake, water intake)	KO > WT (CPP, 1.2 g/kg) KO < WT (CPP, 2 g/kg) KO = WT (CPP, 2.8 g/kg) KO < WT (ethanol-induced CTA to saline, 2 g/kg)	—	KO = WT (fixed speed rotarod ataxia, 20 r.p.m., 1.5 or 2 g/kg) KO > WT (accelerating rotarod ataxia, 4–40 r.p.m. over 5 minutes, 2 g/kg) KO < WT (LORR duration, 3.6 g/kg; and ED_{50} to LORR; LORR duration difference was confirmed on a 129/SvJae background)	—	—	—	KO = WT (clearance, 2 g/kg)

[a]Reference nos. 1, Blednov *et al.* (2001); 2, Blednov *et al.* (2003b); 3, Hill *et al.* (2003); 4, Espinosa *et al.* (2001); 5, de Fiebre & de Fiebre (2005); 6, Bowers *et al.* (2005); 7, Findlay *et al.* (2002); 8, Newton *et al.* (2004).

Table 8 Adenosine-related genes.

Knockout	*Ref.*[a]	*Gender/background*	*Preference*	*Sedation*	*Tolerance*	*Withdrawal*	*Other*
A2A receptor	1	Male 129/SvJ × CD-1 N5 backcross to CD-1	KO = WT (ethanol liquid diet intake, 11 days)	—	—	KO < WT (HIC during 9-hour withdrawal from 11 days of ethanol liquid diet)	KO = WT (clearance, 3.5 g/kg)
	2	Male, female 129/SvJ × CD-1 N15 backcross to CD-1	KO > WT (intake, PR) 3%, 6%, 10%, 20% ethanol (6 days each) KO = WT (sucrose PR, quinine avoidance, total fluid intake)	KO < WT (LORR duration, 3 g/kg, but not 4 g/kg) KO < WT (hypothermia, 1.5, 3, 4 g/kg)	KO = WT Male KO < WT Female (hypothermia, 3 g/kg for 4 days)	—	KO = WT (clearance, 4 g/kg)
	3	Male 129/SvJ × CD-1 > N15 backcross to CD-1	—	KO < WT (LORR duration, 3.5 and 4 g/kg) KO > WT (pentobarbital LORR duration, 30 and 40 mg/kg) KO = WT (hypothermia, 3.5 g/kg)	—	—	—
Equilibrative nucleoside transporter type 1 (ENT1) (*Slc29a1*)	4	Male 129X1/SvJ × C57BL/6J hybrid	KO > WT (intake, PR) 3%, 6%, 10% ethanol (2-week period) KO = WT (saccharin PR, quinine avoidance)	KO < WT (20 r.p.m. fixed speed rotarod, 1 and 1.5 g/kg) KO < WT (LORR duration, 3.6 and 4 g/kg)	—	—	KO = WT (clearance, 3.6 g/kg)

[a]Reference nos. 1, El Yacoubi *et al.* (2001); 2, Naassila, Ledent & Daoust (2002); 3, El Yacoubi *et al.* (2003); 4, Choi *et al.* (2004).

receptor mutant and a null mutant for the nucleoside transporter that regulates extracellular adenosine concentrations. When examined for ethanol preference drinking, both A2A and nucleoside transporter knockout mice consumed more ethanol in a standard two-bottle choice procedure. Both mutants also displayed reduced sensitivity to several sedative-ataxic effects of ethanol across most tests, in the absence of differences in blood ethanol clearance. In addition, A2A knockout mice exhibited reduced ethanol withdrawal severity (El Yacoubi *et al.* 2001, 2003; Naassila *et al.* 2002). These results are consistent with the negative genetic correlation that has been found between ethanol consumption and withdrawal severity (Metten *et al.* 1998) and with the proposal that higher ethanol consumption may be associated with reduced ethanol sensitivity. Although heightened ethanol consumption often is interpreted as an indication of increased sensitivity to the rewarding effects of ethanol, Choi *et al.* (2004) showed that phosphorylated cAMP response element binding protein (pCREB) is increased in the striatum of nucleoside transporter knockout mice. Previous research has shown that increased pCREB is associated with diminished cocaine reward, when measured using the conditioned place preference procedure (Carlezon *et al.* 1998), and decreased pCREB is associated with increased ethanol intake and increased anxiety (see *Neuropeptide Y* section). Data for other reward-related traits are needed to clarify whether ethanol reward is enhanced or reduced in these mice.

G PROTEINS AND CYCLASE-RELATED GENES (TABLE 9)

Many of ethanol's responses appear to be modulated through effects on intracellular signaling cascades (Newton & Messing 2006). We discussed in the previous section effects initiated by interactions of ethanol with adenosine A2A receptors and the type I equilibrative nucleoside transporter. Downstream effects on protein kinases A and C are discussed below. Here, we discuss the effects of targeting adenylate cyclase-1 (AC1), AC8 and Gsα. Of the two AC isoforms stimulated by calcium, only deletion of AC8 reduced ethanol intake, while either deletion led to increased sensitivity to ethanol's sedative effects. Heterozygous knockdown of the Gsα gene enhanced sensitivity to ethanol sedation and reduced intake. Importantly, these effects were seen on three genetic backgrounds, albeit to different degrees. On C57BL/6J background, knockdown heterozygotes developed less tolerance than WT to LORR, but not to hypothermia. A similar pattern of results in mice transgenically overexpressing a dominant negative for the regulatory subunit of protein kinase A (PKA) suggested that the Gsα effects were modulated through PKA. However, mice transgenic for a point mutation in Gsα leading to overexpression and increased adenyl cyclase activity showed only an attenuated sedative response, but no difference in self-administration of ethanol from wildtypes. These results are consistent with data from *Drosophila* showing that reduction of PKA activity by genetic or pharmacological approaches causes increased sedation (Moore *et al.* 1998).

PROTEIN KINASES (TABLE 10)

There is considerable evidence that intracellular signaling systems in general, and protein kinases in particular, are critical for alcohol actions (Newton & Messing 2006). Four classes of protein kinases were studied with mutant mice: PKA, PKC, PKG and Fyn kinase. PKA consists of catalytic and regulatory subunits (which bind cAMP), and genetic manipulation of the regulatory subunits was used to alter PKA activity. Transgenic expression of a dominant negative regulatory subunit [R(AB)], which reduced PKA activity in the forebrain (CaMKIIα promoter), reduced alcohol consumption and increased alcohol sedation (Wand *et al.* 2001; see Table 9). Conversely, activation of PKA by deletion of the regulatory subunit RIIβ increased alcohol consumption and decreased alcohol sedation. Deletion of other regulatory proteins did not affect these behaviors, suggesting that PKA in forebrain regions, which is regulated by the RIIβ subunit, is particularly important for alcohol consumption and sedation. As noted above, these results are consistent with manipulations of PKA activity in *Drosophila* which showed that reduction of PKA activity caused increased sedation (Moore *et al.* 1998).

The PKC family is activated by phospholipids and consists of nine gene products which are grouped into three classes: conventional, novel and atypical. Only two PKCs, γ and ∈, which are conventional PKCs, have thus far been implicated in alcohol action (Newton & Messing 2006). The PKCγ mouse was one of the first null mutants examined by alcohol researchers, and seven studies have reported ethanol responses in mice with this mutation. Deletion of PKCγ reduced ethanol LORR and hypothermia in multiple studies, and increased ethanol preference in one experiment. It also reduced development of tolerance to the sedative and hypothermic effects of alcohol, but the tolerance phenotypes were dependent upon genetic background (Bowers *et al.* 1999). Deletion of PKC∈ increased ethanol LORR and reduced alcohol consumption. Thus, the two isoforms of PKC had opposite effects on LORR and alcohol consumption. Although there are many targets for PKC-mediated phosphorylation, there is evidence that both PKCγ and PKC∈ can regulate the function of $GABA_A$ receptors. PKCγ null mutant

Table 9 G proteins/cyclase-related genes.

Gene	*Ref.*[a]	*Gender/background*	*Preference*	*Sedation*	*Tolerance*	*Other*
Adenylyl cyclase-1	1	Male 129/Sv × C57BL/6J ≥N9 backcross to C57BL/6J	KO = WT (intake, PR) 3%, 6%, 10%, 20% ethanol (6 days each) KO = WT saccharin PR, quinine avoidance, food intake, total fluid intake)	KO > WT (LORR duration; 3.2, 3.6 and 4.0 g/kg)	—	KO = WT (clearance, 3.6 g/kg)
Adenylyl cyclase-8	1	Male 129/Sv × C57BL/6J ≥N9 backcross to C57BL/6J	KO < WT (intake, PR) 3%, 6%, 10%, 20% ethanol (6 days each) KO = WT (saccharin PR, quinine avoidance, total fluid intake) KO > WT (food intake)	KO > WT (LORR duration; 4 g/kg, but not 3.2, or 3.6 g/kg)	—	KO = WT (clearance, 3.6 g/kg)
Adenylyl cyclase-1/ Adenylyl cyclase-8 double KO	1	Male 129/Sv × C57BL/6J ≥N9 backcross to C57BL/6J	KO < WT (intake, PR) 3%, 6%, 10%, 20% ethanol (6 days each) KO = WT (saccharin PR, quinine avoidance, food intake, total fluid intake)	KO > WT (LORR duration; 3.2, 3.6 and 4.0 g/kg) KO = WT (grid test ataxia, 2 g/kg) KO = WT (balance beam missteps, 1.25 g/kg) KO = WT (vertical screen test, 1.75 g/kg) KO = WT (stationary dowel test, 1.75 g/kg) KO = WT (observer-rated wobble and splay, 3 g/kg)	—	KO = WT (clearance, 3.6 g/kg)
Adenylyl cyclase-7 transgenic human AC7 overexpression	2	Male unspecified SJL × C57BL/6 background, N8-9 backcross to C57BL/6	—	—	—	OX > WT (induction of AC7 and phosphorylation of DARPP-32 in amygdala and nucleus accumbens, not striatum, after 2 g/kg ethanol)
Gsα subunit (*Gnas*)	3	Male, female J1 ES cell × C57BL/6J, then backcrossed to C57BL/6J,	HET < WT (intake, PR, C57BL/6J background) 3%, 6%, 10%, 20% ethanol (3 days each)	HET > WT (LORR duration, 3.5 g/kg, all three backgrounds)	—	HET = WT (BEC at 15, 30, and 60 minutes, 3.5 g/kg, C57BL/6J background)

Table 9 *Cont.*

Gene	*Ref.*[a]	*Gender/background*	*Preference*	*Sedation*	*Tolerance*	*Other*
		129/SvEv, or 129/SvEv × CD-1 (all backcrosses N3-7)	HET < WT (PR, 129/SvEv and 129/SvEv × CD-1 backgrounds) 6% ethanol HET = WT (sucrose PR, quinine avoidance, food intake, fluid intake on C57BL/6J background) HET = WT (food intake, fluid intake, 129/SvEv and 129/SvEv × CD-1 backgrounds)	—	—	—
	4	Male, female J1 ES cell × C57BL/6J, then backcrossed to C57BL/6J	—	HET > WT (LORR duration, 3 g/kg) HET > WT (hypothermia, 3 g/kg)	HET < WT (LORR tolerance, 3rd daily 3 g/kg injection) HET = WT (hypothermia tolerance, 3rd daily 3 g/kg injection)	—
Gsα Q222L transgenic	3	Male, female Backcrossed (N7–N9) to C57BL/6J	TG = WT (intake, PR) 3%, 6%, 10%, 20% ethanol (3 days each)	TG < WT (LORR duration, 3.5 g/kg)	—	TG = WT (BEC at 10, 20, and 30 minutes, 3.5 g/kg)
R(AB) transgenics	3	Male, female Backcrossed (N7–N9) to C57BL/6J	TG < WT (intake, PR) 3%, 6%, 10%, 20% ethanol (3 days each) TG = WT (sucrose PR, quinine avoidance, food intake, fluid intake)	TG > WT (LORR duration, 3.5 g/kg)		TG = WT (BEC at 10, 30, and 60 minutes, 3.5 g/kg)
Pituitary adenylate cyclase-activating polypeptide	5	Male 129/SvJ × ICR hybrid	—	KO < WT (hypothermia, 2.5 g/kg) KO < WT (LORR duration, 4 g/kg)	—	KO = WT (clearance, 2.5 g/kg)
Pituitary adenylate cyclase-activating polypeptide type-1 receptor	6	Male C57BL/6J (75%) × 129/Ola (25%) hybrid	—	KO = WT (LORR duration, 3.5 g/kg)	—	—

[a]Reference nos. 1, Maas *et al.* (2005); 2, Donohue, Hoffman & Tabakoff (2005); 3, Wand *et al.* (2001); 4, Yang *et al.* (2003); 5, Tanaka *et al.* (2004); 6, Otto *et al.* (2001).

Table 10 Protein kinases.

Knockout	*Ref.*[a]	*Gender/background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Anxiety*	*Tolerance*	*Other*
Protein kinase A (PKA) PKA RIβ	1	Male, female 129/SvJ × C57BL/6J hybrid	KO = WT (intake) 3%, 6%, 10%, 20% ethanol (8 days each)	—	—	—	—	—	—
PKA RIIβ	1	Male, female 129/SvJ × C57BL/6J hybrid	KO > WT (intake, PR, ≥6%) 3%, 6%, 10%, 20% ethanol (8 days each) KO = WT (sucrose PR, quinine avoidance, food intake, water intake)	—	—	KO < WT (LORR duration, 4.0 g/kg)	—	—	KO = WT (clearance, 4 g/kg)
	2	Male, female 129/SvJ × C57BL/6J, N8 backcross to C57BL/6J	KO > WT (intake, PR, ≥15%) 3%, 5%, 8%, 10%, 13%, 15%, 18%, 20% ethanol (4 days each)	KO < WT (operant self-administration, 10%, 14%, 18%, after sucrose fading; effect in females only) KO < WT (operant self-administration of food; effect in females only) Both results attributed by authors to excessive bar pressing by WT females without consumption of all delivered ethanol or food	—	—	—	—	—

Table 10 *Cont.*

Knockout	*Ref.*[a]	*Gender/background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Anxiety*	*Tolerance*	*Other*
	3	Male, female 129/SvJ × C57BL/6J, N8 backcross to C57BL/6J (also versus 129/SvEv × C57BL/6J F1)	—	—	KO ≥ WT (activation, 1.4 g/kg, F1 background; no activation in WT) KO = WT (sensitization after chronic 1.4 g/kg in test chamber, F1 background) KO > WT (sensitization after repeated 2 g/kg in test chamber, C57BL/6J background) KO < WT (reduced activity after 2 g/kg in home cage, C57BL/6J background) KO = WT (sensitization after repeated 2 g/kg in home cage, C57BL/6J background)	—	—	—	KO = WT (BEC, 20 minutes after 1.4 g/kg, F1 background)
	4	Male, female 129Sv/J × C57BL/6J, N8 backcross to C57BL/6J (also versus 129/SvEv × C57BL/6J F1)	KO > WT (intake, PR) 3%, 5%, 8%, 10%, 15%, 18%, 20% ethanol (4 days each) Effect seen on both backgrounds	—	—	KO < WT (LORR duration, 3.8 g/kg, both backgrounds)	—	—	KO = WT (clearance, 3.8 g/kg, only C57BL/6J background tested)

PKA Cβ1	1	Male, female 129/SvJ × C57BL/6J hybrid	KO = WT (intake) 3%, 6%, 10%, 20% ethanol (8 days each)	—	—	—	—	—	—
Protein kinase C (PKC) PKC γ	5	Male 129/SvJae × C57BL/6J hybrid, F4-5	—	KO = WT (operant oral sucrose/ethanol self-administration, 10%) KO < WT (operant oral ethanol self-administration, 10%)	—	—	—	—	KO < WT (ethanol-induced increase in nucleus accumbens dopamine, 1 and 2 g/kg) KO < WT (ethanol-induced increase in nucleus accumbens taurine, 1 and 2 g/kg; no increase in KO)
	6	Gender not specified 129/SvJae (25%) × C57BL/6J (73.4%) × FVB/N (1.6%) hybrid (conditional, tissue-specific KO also tested, with and without rescue)	KO < WT (intake, PR, ≥6%) 3%, 6%, 10% ethanol (4 days each) KO = WT (saccharin PR, quinine avoidance) Doxycycline-treated conditional KO = KO (all drinking traits) Rescued KO = WT (all drinking traits)	—	—	KO > WT (LORR duration, 3.6 g/kg) Doxycycline-treated conditional KO = KO Rescued KO = WT	—	—	(KO = Conditional KO = Rescued KO = WT) (BEC, clearance, 3.6 g/kg)
	7	Male 129/SvJae × C57BL/6J hybrid, F2–4	KO < WT (intake, PR) 2%, 4%, 6%, 10%, 14% ethanol (4 days each) KO = WT (saccharin PR, quinine avoidance, food intake, water intake)	—	KO > WT (ethanol activation, 2 g/kg)	KO > WT (LORR duration, 3.2, 3.6, 4 g/kg)	—	—	KO = WT (clearance, 4 g/kg) KO > WT (ethanol-induced increase of muscimol-stimulated chloride uptake, 20 mM ethanol)

Table 10 *Cont.*

Knockout	*Ref.*[a]	*Gender/background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Anxiety*	*Tolerance*	*Other*
	8	Male 129/SvJae × C57BL/6J hybrid, N10 backcross to C57BL/6J, then HET matings with 129/SvJae	KO < WT (intake, PR) 10% ethanol (16–24-hour sessions) KO < WT (sensitivity to mGluR5 antagonist to reduce ethanol intake, PR) KO = WT (water intake)	—	—	—	—	—	—
	9	Male, female 129SvJae × C57BL/6J hybrid	—	—	—	KO > WT (LORR duration, BEC at recovery, 3.5 g/kg)	—	—	KO > WT (ethanol-enhanced GABA-A IPSCs) KO = WT (ethanol-induced increase in holding current)
	10	Male 129S4/SvJae × C57BL/6J, N10 backcross to C57BL/6J, used HET × C57BL/6J F2	KO < WT (intake, PR; data for 10% shown) 3%, 6%, 10% ethanol (3–6 days each) KO < WT (intake, PR; 10%, tested after chronic 2.5 g/kg injections bid, 6 days; no change in either genotype after chronic injection)	—	—	KO > WT (rotarod ataxia, 2 g/kg) KO = WT (dowel test, 1.5 g/kg) KO > WT (LORR duration, 3.2,4 g/kg; also by ED_{50}, BEC at recovery)	—	KO < WT (acute functional tolerance, dowel test, 1.5, then 1.5 g/kg) KO > WT (chronic tolerance, LORR duration, after 2.5 g/kg bid, 6 days) KO > WT (increase in acute functional tolerance after chronic tolerance-inducing injections)	KO < WT (acute functional tolerance after i.p. injection of 2 or 4 g/kg to *in vitro* ethanol potentiation of muscimol-stimulated chloride ion flux in cerebellar microsacs; no acute functional tolerance in KO) KO = WT (clearance, 3.6 g/kg, before or after chronic tolerance)

PKCγ	11	Male, female 129/Ola × C57BL/6, N6 backcross to C57BL/6J, then crossed to 129/SvEvTac Maintained thereafter as HET × C57BL/6J hybrid	—	—	KO = WT (ethanol effects on closed arm entries, total entries in elevated plus maze, 1–1.5 g/kg)	—	KO < WT (sensitivity to anxiolytic effects of ethanol in elevated plus maze, 1, 1.25 and 1.5 g/kg) KO = WT (lack of anxiolytic responses to ethanol in light-dark box or mirrored chamber)	—	—
	12	Male, female 129/Ola × C57BL/6, N6 backcross to C57BL/6J, then crossed to 129/SvEvTac Maintained thereafter as HET × C57BL/6J	—	—	—	KO < WT (LORR duration, 3.5 g/kg) KO < WT (hypothermia, 1, 2, 3 g/kg)	—	—	KO < WT (ethanol-induced increase of muscimol-stimulated chloride uptake, 15 mM ethanol, cerebellum and cortex) KO = WT (BEC, clearance, 3.5 g/kg)
	13	Male, female 129/Ola × C57BL/6, N6 backcross to C57BL/6J, then crossed to 129/SvEvTac Maintained thereafter as HET × C57BL/6J	KO > WT (intake, PR) 3%, 5%, 7%, 9%, 11% ethanol (4 days each) KO = WT (saccharin PR, total fluid intake)	—	—	—	—	—	—

Table 10 *Cont.*

Knockout	*Ref.*[a]	*Gender/background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Anxiety*	*Tolerance*	*Other*
	14	Male, female 129/Ola × C57BL/6, N6 backcross to C57BL/6J, then crossed to 129/SvEvTac Maintained thereafter as HET × C57BL/6J	—	—	KO > WT (ethanol-activation, 1 and 1.25 g/kg)	—	KO < WT (ethanol-induced reduction in latency to move to center of open field) KO > WT (ethanol-induced reduction in open field center time and number of center entries)	—	—
	9	Male, female 129/Ola × C57BL/6, N6 backcross to C57BL/6J, then crossed to 129/SvEvTac Maintained thereafter as HET × C57BL/6J	—	—	—	KO < WT (LORR duration, BEC at recovery, 3.5 g/kg)	—	—	KO < WT [ethanol-enhanced $GABA_A$ inhibitory postsynaptic currents (IPSCs)] KO < WT (ethanol-induced increase in holding current; no ethanol effect in KO)
	15	Male, female 129/Ola × C57BL/6 Three backgrounds were used: F2 cross of KO × 129/SvJ; N6 backcross to C57BL/6J; F2 outcross to 129/SvEvTac	—	—	—	KO < WT (hypothermia, LORR duration, 3.8 g/kg, B6 × 129/Sv F2 background) KO < (HET = WT) (LORR duration, but not hypothermia, 3.8 g/kg, B6 × 129/SvEvTac F2 background) KO = HET = WT (hypothermia, LORR duration, C57BL/6J background)	—	KO < WT (chronic tolerance to hypothermia, LORR duration after 11 days liquid diet; no tolerance in KO on mixed backgrounds) KO = HET = WT (chronic tolerance to hypothermia, LORR duration on C57BL/6J background)	—

	16	Male, female F1 hybrid cross of B6.γPKC × S6.γPKC congenic strain heterozygotes	—	—	—	—	—	—	KO > WT (control levels of transthyretin expression in striatum, reversed by 11 days ethanol liquid diet; no ethanol effect in WT)
Fyn kinase	17	Male TT2 ES cells (C57BL/6 × CBA F1) to unknown background strain (possibly CD-1)	—	—	—	KO > HET (LORR duration, 2.5, 3.0, 3.5 g/kg)	—	KO < HET [acute tolerance to depressant effect of ethanol on NMDA-mediated excitatory postsynaptic potentials (EPSPs)]	KO = HET (clearance, 3.5 g/kg) KO < HET (enhancement of tyrosine phosphorylation by ethanol, 3.5 g/kg)
	18	Male, female AB1 ES cells 129/SvEvBrd to 129/Sv or C57BL/6; used B6129SF2/J littermate homozygotes as WT	KO < WT (intake, PR; male) KO = WT (intake, PR; female) 3%, 6%, 9%, 12%, 15% ethanol (4 days each) KO = WT (saccharin PR, quinine avoidance, total fluid intake; only male data reported)	—	KO = WT (increase in closed and total arm entries in elevated plus maze, 1.5 g/kg)	KO > WT (LORR duration, 3.8 but not 3.5 g/kg) KO = WT (ED_{50} to LORR) KO = WT (hypothermia, 3.8 g/kg, 0–240 minutes) KO = WT (dowel and fixed speed rotarod, 1.75 g/kg)	KO > WT (increase in % time in open arms of elevated plus maze and head dips, 1.5 g/kg) KO = WT (ethanol effects on closed arm entries in elevated plus maze; vertical activity; stretched attends; 1.5 g/kg)	KO < WT (acute functional tolerance, stationary dowel, 1.75 then 2.0 g/kg) KO = WT (acute functional tolerance, fixed-speed rotarod, 1.75 then 2.0 g/kg)	KO = WT (clearance, 3.8 g/kg)

Table 10 *Cont.*

Knockout	*Ref.*[a]	*Gender/background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Anxiety*	*Tolerance*	*Other*
	19	Male Two backgrounds: AB1 ES cells (129/SvEvBrd) to 129/Sv or C57BL/6 crossed with C57BL/6J; crosses with C57BL/6J or 129/SvJ generated homozygous KO or WT mice used	KO = WT (intake, PR) 3%, 6%, 10%, 20% ethanol (4 days each; effect on both backgrounds)	KO = WT (CPP, 2 g/kg; only tested 129/SvJ background)	—	KO > WT (LORR duration, 3.6 and 4 g/kg; effects on both backgrounds)	—	—	KO = WT (BEC at 15 and 90 min 4 g/kg; 129/SvJ background only)
Fyn kinase overexpression	20	Male, female 129/Sv × C57BL/6J hybrid	OX < WT (intake, PR) 3%, 6%, 9%, 12%, 15% ethanol (4 days each) OX = WT (saccharin PR, total fluid intake) OX < WT (quinine avoidance)	—	—	OX < WT (LORR duration, 3.8 but not 3.5 g/kg) OX = WT (BEC at righting, ED_{50} to LORR, 2.1–2.8 g/kg)	—	OX > WT (acute tolerance inferred from LORR data)	OX = WT (clearance of ethanol, dose not given)
cGMP-dependent protein kinase II	21	Male Two backgrounds: R1 ES cells to C57BL/6, to Sv/129 or N9 backcross to C57BL/6N	KO > WT (intake, PR; effect on both backgrounds) 5% ethanol (3 weeks) KO = WT (sucrose and saccharin PR; tested on C57BL/6N background only)	—	—	KO < WT (LORR duration, 4 g/kg; effect on both backgrounds)	—	—	KO = WT (clearance, 3 g/kg; effect on both backgrounds)

[a]Reference nos. 1, Thiele *et al.* (2000); 2, Ferraro *et al.* (2006); 3, Fee *et al.* (2006); 4, Fee *et al.* (2004); 5, Olive *et al.* (2000); 6, Choi *et al.* (2002); 7, Hodge *et al.* (1999); 8, Olive *et al.* (2005); 9, Proctor *et al.* (2003); 10, Wallace *et al.* (2006); 11, Bowers, Elliott & Wehner (2001); 12, Harris *et al.* (1995); 13, Bowers & Wehner (2001); 14, Hix *et al.* (2003); 15, Bowers *et al.* (1999); 16, Smith *et al.* (2006); 17, Miyakawa *et al.* (1997); 18, Boehm *et al.* (2003); 19, Yaka *et al.* (2003); 20, Boehm *et al.* (2004a); 21, Werner *et al.* (2004).

mice do not show ethanol enhancement of $GABA_A$ receptor function (Harris *et al.* 1995; Proctor *et al.* 2003), whereas deletion of PKCε augments the modulation of $GABA_A$ receptors by ethanol and by other positive allosteric modulators such as neurosteroids and benzodiazepines (Hodge *et al.* 1999; Proctor *et al.* 2003). Thus, the distinct changes in alcohol consumption produced by deletion of these two PKC isoforms would be consistent with ethanol activation of $GABA_A$ receptors playing a role in regulation of alcohol consumption.

Fyn kinase is a member of the Src family of tyrosine kinases (PKA and PKC are serine/threonine kinases). Deletion of Fyn kinase produces an increase in LORR duration, and this was attributed to a decreased development of acute functional tolerance, which is supported by data showing impaired tolerance to ethanol ataxia in a colony of Fyn mutant mice on a different background. Overexpression of Fyn in the forebrain reduces LORR duration, which nicely complements the data from null mutant mice. Of course, there are many potential targets for Fyn phosphorylation in brain, but there is evidence that the function of the NR2B subunit of the NMDA receptor is enhanced by Fyn phosphorylation, which may be important for the alcohol phenotypes seen in Fyn null mutant mice. Ethanol stimulates NR2B phosphorylation in the wildtype, but not Fyn null mutant mice, and ifenprodil, which selectively inhibits function of NMDA receptors with NR2B subunits, enhances alcohol's action in wildtype, but not Fyn null, mice (Yaka *et al.* 2003). Thus, the proposed interaction is that ethanol directly inhibits NMDA receptor function, but also indirectly activates Fyn, which phosphorylates NR2B and thereby enhances NMDA receptor function and opposes the action of ethanol. This would result in 'tolerance' to ethanol (i.e. resistance to its effects) at the receptor, and presumably behavioral, level.

GLUTAMATE (TABLE 11)

One metabotropic and four ionotropic glutamate receptors have been studied. Deletion of the GluR-C subunit of the AMPA receptor reduced cue-induced reinstatement of alcohol consumption. This effect was mimicked by administration of an AMPA antagonist. Surprisingly, the deletion did not alter operant or home cage alcohol consumption, only reinstatement, and the reinstatement effect was seen only on the first day. Similarly, deletion of the mGluR1 subunit did not affect ethanol preference or its elevation by the alcohol deprivation effect or a forced swim test. GluR1 knockouts were also not differentially sensitive to several sedative effects of ethanol, but were insensitive to ethanol hypothermia (Cowen *et al.* 2003). Knockout of mGluR4 also did not affect preference or sedative response to ethanol, but reduced alcohol stimulation of motor activity. Ethanol acutely inhibits NMDA receptor function, and this receptor is extensively studied in alcohol research, but it is the focus of only three null mutant studies. Two found no effect of deletion of the NR2A subunit on LORR or hypothermia. NR2A knockouts had equivalent intake of alcohol to wildtypes, but reduced conditioned place preference (Boyce-Rustay & Holmes 2005, 2006). Deletion of the epsilon 1 subunit did not affect LORR sensitivity, but abolished the development of tolerance to LORR with repeated injections (Sato *et al.* 2006). We included the clock gene *Per2* and the scaffolding protein Homer2 with glutamate genes because their effects are attributed to changes in glutamate systems (Spanagel *et al.* 2005). Homer2 null mutants do not accept high concentrations of ethanol and do not develop conditioned place preference or motor sensitization after repeated ethanol injection. Importantly, these changes were reversed by viral delivery of Homer2 into the nucleus accumbens. Mice lacking Homer2 are also deficient in plasma membrane NMDA receptors, and the importance of Homer2 in alcohol neuroplasticity may be due to changes to glutamate systems, but the Homer proteins are general scaffolding proteins and have many roles in cell function. *Per2* null mutants show increased ethanol consumption (two bottle choice) and worked harder to obtain 16% ethanol in an operant study. These effects are attributed to a hyperglutaminergic state caused by decreased levels of the glutamate transporter Eaat1.

NEUROPEPTIDE Y AND OTHER NEUROPEPTIDES (TABLE 12)

Neuropeptide Y (NPY) has long been known as a powerful orexigenic agent, and this appetite-stimulating effect is localized to hypothalamic structures such as the arcuate nucleus (Clark *et al.* 1984; Levine & Morley 1984). NPY also has powerful anxiolytic-like effects hypothesized to be mediated by NPY receptors in the amygdala (Heilig *et al.* 1993). In many cases, where pharmacological tools are available, the effects of NPY on behavioral responses to stressors are opposite to those of CRF. Both the orexigenic-like and anxiolytic-like effects of NPY could have an impact on ethanol drinking. There are five NPY receptor subtypes identified to date (Palmiter *et al.* 1998). The anxiolytic-like effects have been largely attributed to activation of the Y_1 receptor (Heilig *et al.* 1993). The orexigenic-like effects are largely attributed to the Y_5 receptor (Gerald *et al.* 1996; Pedrazzini *et al.* 1998). The Y_2 receptor is hypothesized to be presynaptic and as such could modulate NPY function indirectly (Wahlestedt, Yanaihara & Hakanson 1986).

NPY knockout mice on a segregating background of 129/SvEv × C57BL/6J showed increased ethanol intake

Table 11 Glutamate-related genes.

Knockout	*Ref.*[a]	*Gender/ background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Tolerance*	*Withdrawal*	*Other*
GluR-C	1	Male R1 ES cell through *cre* recombinase line of unspecified background, N6 backcross to C57BL/6N	KO = WT (intake, PR) 2%, 4%, 8%, 12%, 16% ethanol (several days each) KO < WT (alcohol deprivation effect at 1st reinstatement day after 6% ethanol for 14 weeks, then withdrawn for 3 weeks, then reinstated for 3 weeks; KO = WT days 2 and 3 of reinstatement)	KO = WT (operant self-administration; 10% ethanol, FR1, sucrose fading) KO < WT (light cue-induced reinstatement)	—	—	—	—	—
GluR1	2	Male 129X1/SvJ × C57BL/6N, then backcross to C57BL/6N	KO = WT (intake, PR) 2%, 4%, 8%, 12%, 16% ethanol (3–24 days each) KO = WT (alcohol deprivation effect reinstatement, 16%, 3 days) KO = WT (forced swim test-induced increase in intake)	—	KO = WT (ethanol-induced decrease in activity, 2 g/kg)	KO = WT (LORR onset, duration, 3.5 g/kg) KO = WT (rotarod ataxia, 2 g/kg) KO < WT (hypothermia, 2 g/kg)	KO = WT (tolerance to LORR duration, 3.5 g/kg, day 2)	—	—

mGluR4	3	Male, female 129/SvJ × CD-1 N6 backcross to C57BL/6N	KO = WT (intake, PR) 3%, 6%, 9%, 12% ethanol (4 days each) KO = WT (saccharin PR, quinine avoidance)	—	KO < WT (ethanol-induced activity, 1–2.5 g/kg; no increase in KO) KO > WT (baseline activity)	KO = WT (LORR duration, BEC at recovery, 3.6, 3.8 g/kg)	—	KO = WT (HIC after withdrawal from acute 4 g/kg)	—
NMDA NR2A	4	Male ES cells from CBA × C57BL/6J F1 to ICR, crossed with C57BL/6J, then N10 backcross to C57BL/6J	—	—	—	KO = HET = WT (LORR duration, 3 g/kg)	—	—	—
	5	Gender not specified ES cells from CBA × C57BL/6J F1 to ICR, crossed with C57BL/6J, then N10 backcross to C57BL/6J	KO = HET = WT (intake, PR) 3%, 5%, 7%, 9%, 11%, 13% ethanol (4–6 days each)	KO < HET < WT (CPP to 2 g/kg); KO > (HET = WT) (activity during test)	KO = HET = WT (activity increase, 1.5 and 2 g/kg, 10-min test) KO = HET = WT (activity increase on drug days in CPP test)	HET > (KO = WT) (rotarod ataxia, 2 g/kg, but not 1 or 2.5 g/kg) KO = HET = WT (LORR duration, 3 and 4 g/kg) KO = HET = WT (hypothermia, 3–4 g/kg) KO > (HET = WT) (falls from elevated plus maze after 1.5 g/kg ethanol; trends)	—	—	KO < (HET = WT) (BEC 30 min after 2 g/kg)
NMDAε1	6	Male ES cells from CBA × C57BL/6J F1 to ICR, crossed with C57BL/6	—	—	—	KO = WT (LORR duration, 3–4 g/kg)	KO < WT (tolerance to LORR, 4 daily 4 g/kg; no tolerance in KO)	—	KO = WT (clearance of 4th daily injection of 4 g/kg)

Table 11 *Cont.*

Knockout	*Ref.*[a]	*Gender/ background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Tolerance*	*Withdrawal*	*Other*
Homer2	7	Male, female 129X1/SvJ × C57BL/6J, F10-12	KO < WT (intake, PR; 12% ethanol) 3%, 6%, 12% ethanol (4 days each) KO = WT (saccharin PR, water intake)	KO shows conditioned place *aversion* to 3 g/kg; WT shows CPP to 2 and 3 (not 1) g/kg ethanol (biased CPP assay)	KO = WT (activity reduction to 3 g/kg; no change to 1 or 2 g/kg) WT but not KO shows sensitization of activity to 8th injection of 3 g/kg, 48 hrs apart, in CPP test; no change in KO (see tolerance)	KO > WT (LORR duration, 5 g/kg)	KO = WT (metabolic tolerance to 8th injection of 3 g/kg, 48 hrs apart, in CPP test) KO < WT (tolerance to activity reduction by 8th 3 g/kg dose; no tolerance in KO; see activity)	—	KO = WT (BEC 5, 15, 30 minutes after 3 g/kg)
Per2^{Brdm1}	8	Male Littermates from heterozygous crosses of 129/SvEv^{Brd} × C57BL/6-Tyr$^{c\text{-}Brd}$	KO > WT (intake, PR; 8–16% ethanol) 2%, 4%, 8%, 12%, 16% ethanol (3–8 days each) KO = WT (sucrose PR, quinine avoidance, food intake)	KO > WT (operant self-administration, FR1, 16% ethanol) KO > WT (break point in progressive-ratio)	—	—	—	—	KO = WT (clearance, 3.5 g/kg)

[a]Reference nos. 1, Sanchis-Segura *et al.* (2006); 2, Cowen *et al.* (2003), 3, Blednov *et al.* (2005); 4, Boyce-Rustay & Holmes (2005); 5, Boyce-Rustay & Holmes (2006); 6, Sato *et al.* (2006); 7, Szumlinski *et al.* (2005); 8, Spanagel *et al.* (2005).

Table 12 Neuropeptide Y (and other neuropeptides).

Knockout	*Ref.*[a]	*Gender/background*	*Preference*	*Activity*	*Sedation*	*Anxiety*	*Other*
NPY	1	Male F3-F5 hybrid 129/SvEv × C57BL/6J	KO > WT (intake, PR ≥ 6%) 3%, 6%, 10%, 20% ethanol (8 days each) KO = WT (sucrose PR, quinine avoidance, food intake, total fluid intake)	—	KO < WT (LORR duration, 4 g/kg)	—	KO = WT (BEC at 60 and 180 minutes, 4 g/kg)
	2	Male, female Two backgrounds Mixed 129/SvEv × C57BL/6J, and backcross of the hybrids to 129/SvEv	KO > WT (intake, PR, 20%) 3%, 6%, 10%, 20% ethanol (8 days each) Only males versus 129/SvEv were tested	KO > WT (increased activity after 1.5 g/kg; effect only in females on 129/SvEv, but in both sexes on 129 × B6 hybrid)	KO < WT (LORR duration after 3.5, but not 3 or 4 g/kg; only males versus 129 × B6 hybrid were tested) KO = WT (LORR duration after 2.5, 3 g/kg; only males versus 129/SvEv were tested)	—	KO = WT (BEC, 60 and 180 minutes, 3 g/kg; only males versus 129/SvEv were tested)
NPY overexpression	1	Male FVB background	OX < WT (intake, PR, ≥6%) 3%, 6%, 10%, 20% ethanol (8 days each) OX = WT (food intake; total fluid intake)	—	OX > WT (LORR duration, 4 g/kg)	—	OX = WT (BEC at 60 and 180 minutes, 4 g/kg)
NPY_1	3	Male, female HM1 ES cells (129/SvOlaHsd), N7 backcross to C57BL/6J	KO > WT (intake, PR, ≥6%) 3%, 6%, 10% ethanol (8 days each) KO = WT (sucrose intake, quinine avoidance, food intake, total fluid intake)	—	KO = WT (accelerating rotarod, 2.5 g/kg) KO < WT (LORR duration, 3.5, 4 g/kg; only males tested for both responses)	—	KO = WT (BEC at 60 and 180 minutes, 3.5 g/kg; only males tested)

Table 12 *Cont.*

Knockout	*Ref.*[a]	*Gender/background*	*Preference*	*Activity*	*Sedation*	*Anxiety*	*Other*
NPY_2	4	Male Two backgrounds Mixed 129/SvJ × BALB/cJ, N3 backcross to BALB/cJ	KO < WT (intake <10%, PR) 3%, 6%, 10% ethanol (8 days each) KO = WT (sucrose intake, quinine avoidance on mixed background) KO > WT (food intake, mixed background) KO = WT (intake, PR, on BALB/cJ)	—	KO = WT (LORR duration, 3, 3.5 g/kg, mixed background)	—	KO = WT (BEC at 60 and 180 minutes, 3 g/kg, mixed background)
NPY_5	2	Male AB1 ES cells to C57BL/6; studied on 129/SvEv background	KO = WT (intake, PR) 3%, 6%, 10%, 20% ethanol (8 days each)	KO = WT (increased activity after 1.5 g/kg)	KO > WT (LORR duration, 2.5, 3 g/kg)	—	KO < WT (BEC at 60 and 180 min, 3 g/kg)
Leptin (*ob*/*ob*) obese: leptin deficient	5	Male, female C57BL/6J-*Lep*^{ob} versus C57BL/6J	KO < WT (intake, PR) 3%, 6%, 9%, 12% ethanol (4 days each) KO < WT (saccharin PR, females only) KO > WT (quinine avoidance, males only) KO < WT (total fluid intake, female) (n.b., KO weighed 3 × as much as C57BL/6J WT)	—	—	—	KO < WT (peak BEC, 2.5, 3.5 g/kg) Complex effects of genotype × sex on clearance

Leptin (*db*/*db*) diabetic: leptin resistant	5	Male, female C57BL/6J-*m Lepr*^{db}/J versus C57BL/6J	KO < WT (intake, PR) 3%, 6%, 9%, 12% ethanol (4 days each) KO < WT (saccharin PR, females only) KO = WT (quinine avoidance, total fluid intake) KO > WT (total fluid intake, males only)	—	—	—	—
Melanocortin 3 receptor (*Mc3R*)	6	Male, female 129SvJ × C57BL/6J, N7 backcross to C57BL/6J	KO = WT (intake, PR) 3%, 6%, 10%, 20% ethanol (8 days each)	—	—	—	—
Cyclic AMP response element binding protein (CREB)	7	Male 129/SvJ × C57BL/6J were backcrossed to C57BL/6J, then crossed with 129/SvJ; tested mice were F2 littermate HET or WT	HET > WT (PR) 7%, 9%, 12% ethanol (3 days each) HET = WT (sucrose PR) HET = WT (body weight)	HET = WT (activity in elevated plus maze after 2 g/kg; no increase in activity with ethanol)	—	HET = WT (anxiolytic response to 2 g/kg in elevated plus maze)	HET = WT (BEC at 60 minutes, 2 g/kg)
Cholecystokinin-A receptor	8	Male 129/SvJ × C57BL/6J, > N7 backcross to C57BL/6	KO > WT (intake, PR, 6%, 20%) 3%, 6%, 10%, 20% ethanol (7 days each) KO = WT (saccharin PR, quinine avoidance) KO > WT (total fluid intake)	—	KO = WT (LORR duration, 4.5 g/kg; sensitivity assessed by rating scale)	—	—

Table 12 *Cont.*

Knockout	*Ref.*[a]	*Gender/background*	*Preference*	*Activity*	*Sedation*	*Anxiety*	*Other*
Cholecystokinin-B receptor	8	Male 129/SvJae × C57BL/6J, > N7 backcross to C57BL/6	KO = WT (intake, PR) 3%, 6%, 10%, 20% ethanol (7 days each) KO = WT (saccharin PR, quinine avoidance, total fluid intake)	—	KO ≤ WT (LORR duration, 4.5 g/kg; sensitivity assessed by rating scale)	—	—
Angiotensinogen	9	Gender not specified, CBA/J × C57BL/6J F1 ES cells × ICR, mixed background studied	KO < WT (intake, PR) 10% ethanol (20 days) KO > WT (total fluid intake)	—	—	—	—
Angiotensinogen overexpression (rat gene)	9	Gender not specified, on NMRI strain	OX > WT (intake, PR) 10% ethanol (32 days) OX = WT (sucrose PR, quinine avoidance)	OX > WT (activity decrease, 2.5 g/kg)	OX > WT (LORR duration, 4 g/kg)	—	OX = WT (BEC at 120 and 210 minutes, 3.5 g/kg)
Angiotensin receptor-1 A	10	Gender not specified ES cell line BK4 to C57BL/6J	KO < WT (PR) 10% ethanol (15 48-hr drinking periods)	—	—	—	—
Angiotensin receptor-2	10	Gender not specified ES cell line 14–1 to C57BL/6J, F2 hemizygotes tested	KO = WT (PR) 10% ethanol (15 48-hr drinking periods)	—	—	—	—
Bradykinin B2 receptor	10	Gender not specified 129/SvEv × C57BL/6J, N7 backcross to C57BL/6J	KO = WT (PR) 10% ethanol (15 48-hr drinking periods)	—	—	—	—

[a]Reference nos. 1, Thiele *et al.* (1998); 2, Thiele *et al.* (2000); 3, Thiele *et al.* (2002); 4, Thiele *et al.* (2004); 5, Blednov *et al.* (2004); 6, Navarro *et al.* (2005); 7, Pandey *et al.* (2004); 8, Miyasaka *et al.* (2005); 9, Maul *et al.* (2001); 10, Maul *et al.* (2005).

in a two-bottle preference test across a concentration range of 3–20%, but no differences were observed in sucrose preference, quinine avoidance, food intake, or total fluid intake (Thiele *et al.* 1998, 2000). Some differences were observed with background strain, as mice from this stock backcrossed to an inbred 129/SvEv background only showed increases in ethanol consumption at the highest concentration (20%) (Thiele *et al.* 2000). The NPY knockouts on the hybrid background showed a decreased sensitivity to the sedative effects of ethanol as measured by LORR and an increased locomotor response to ethanol, but there were no differences in sedative or stimulant effects of ethanol in the NPY knockouts on the inbred 129/SvEv background, suggesting important genetic background effects (Thiele *et al.* 1998, 2000). NPY-overexpressing mice showed largely the opposite effects, with transgenic mice showing decreased ethanol intake in a two-bottle preference test and increased sensitivity to the sedative effects of ethanol as measured by LORR (Thiele *et al.* 1998). No differences were observed in food intake or total fluid intake. The overexpression line was on a different background strain, FVB. Thus, NPY knockout mice show increased intake of ethanol and decreased sensitivity to the sedative effects of NPY. NPY transgenic overexpressing mice show the opposite effects, but these comparisons must be qualified by unknown dependence on background genetic effects.

Y_1 receptor knockout mice backcrossed to C57BL/6J showed increased ethanol preference in a two-bottle choice test for higher ethanol concentrations. They showed mixed sensitivity to ethanol's sedative effects: a decreased sensitivity on the LORR test, but no difference in ataxia on a rotarod after ethanol (Thiele, Koh & Pedrazzini 2002). Y_2 knockouts on a mixed 129/SvJ × BALB/cJ background showed decreased ethanol intake at low doses of ethanol (3% and 6%) in a two-bottle preference test, but when backcrossed to BALB/cJ showed normal ethanol intake (Thiele, Naveilhan & Ernfors 2004). There were no differences in sucrose preference, quinine avoidance, food intake, total fluid intake, or ethanol-induced sedation as measured by LORR in either of these knockouts. Y_5 knockouts from a 129/C57BL/6J mixture on an inbred 129/SvEv background showed no differences in voluntary ethanol consumption in a two-bottle preference test and no difference in locomotor stimulation produced by ethanol; however, they did show increased sleep time in the LORR test (Thiele *et al.* 2000).

Together these results show that NPY can modulate ethanol preference largely through the Y_1 receptor with potential modulation via the presynaptic Y_2 receptor. Decreased NPY function results in increased ethanol consumption, and increased NPY function results in decreased ethanol consumption—an effect opposite to that observed with CRF_1 knockout in dependent animals (see *CRF* section above). However, caution must be invoked here, as there are powerful background effects that interact with the NPY gene that may mask a given phenotype, suggesting that any negative results with NPY knockouts should be revisited with different background strains. Interestingly, CREB gene transcription factor-haplodeficient mice had a higher consumption of ethanol and a higher preference for ethanol (Pandey *et al.* 2004). The CREB-haplodeficient mice derived from the 129/SvJ strain and then backcrossed to C57BL/6 showed almost twice as much intake in a two-bottle preference test (Pandey *et al.* 2004). These results support neuropharmacological evidence linking decreases in CREB with increased drinking behavior. Chronic voluntary ethanol exposure decreases CREB phosphorylation in the amygdala, which reduces the expression of NPY, is hypothesized to lead to increased anxiety-like behavior and subsequently increased ethanol intake (Pandey 2003; Pandey, Roy & Zhang 2003).

The renin angiotensin system has long been known to control fluid balance and thirst (Mann *et al.* 1987) and was hypothesized to be involved in alcohol consumption and preference. A series of studies showed that angiotensin II injected systemically and intracerebroventricularly increased ethanol intake in rats (Grupp *et al.* 1988; Fitts 1993) and mice (Weisinger *et al.* 1999). However, the influence of angiotensin II to stimulate fluid intake in general clouded the interpretation of these data (Grupp 1993). Molecular genetic approaches to activation and inactivation of the renin angiotensin system have reawakened interest in the potential role of this system in regulating ethanol consumption and preference. Transgenic rats that express an antisense RNA against angiotensinogen, with consequently reduced angiotensin II levels exclusively in the central nervous system, consumed less ethanol than wildtype controls. An inhibitor of angiotensin-converting enzyme significantly reduced ethanol consumption and preference in wildtype but not transgenic rats (Maul *et al.* 2005). Together these results suggest that decreasing brain availability of angiotensin II decreased ethanol consumption and preference. In support of this hypothesis, control mice not expressing the angiotensinogen transgene showed lower ethanol consumption and preference (Maul *et al.* 2001), as did knockouts of the type 1A angiotensin II receptor (Maul *et al.* 2005; 129 × C57BL/6 F1 and C57BL/6 backgrounds, respectively; Ichiki *et al.* 1995; Ito *et al.* 1995). However, type 2 receptor knockouts showed normal preference (Maul *et al.* 2005). While intriguing, the absolute values of ethanol consumption were low in these studies where reported (<2.6 g/kg in rats at 7.5% and <2 g/kg in mice) calling into question whether pharmacologically relevant amounts of ethanol were ingested and how specific these effects are *vis-à-vis* overall fluid consumption.

The roles of three additional neuropeptide systems related at least indirectly to appetite regulation have been explored using molecular genetic approaches. There is some evidence suggestive of a role for cholecystokinin (CCK) in the control of appetite, as well as anxiety-like responses (Ravard & Dourish 1990). Leptin is an adipocyte-derived energy-modulating cytokine. It suppresses NPY activity in the arcuate nucleus during periods of high food intake, which tends to restore energy balance (Chehab 2000). Mice with knockout of the CCK-A receptor on a background backcrossed to C57BL/6J showed increased ethanol consumption but no significant change in preference compared with wildtype and knockout of the CCK-B receptor (Miyasaka *et al.* 2005). The increased consumption was dramatic, reaching almost 20 g/kg/day, with no changes observed in saccharin preference or quinine avoidance. Knockout of the CCK-A receptor has been linked to increased anxiety-like behaviors in the elevated plus maze (Miyasaka *et al.* 2002), another example of where increased anxiety-like responses are associated with increased ethanol intake in knockout mice. Mice with knockout of the melanocortin-3 receptor did not differ in their response to ethanol in a two-bottle preference test or in decreased intake of ethanol and food intake produced by a non-selective melanocortin receptor agonist (Navarro *et al.* 2005). However, a selective melanocortin-4 agonist decreased ethanol intake, and a non-selective melanocortin antagonist decreased ethanol intake in C57BL/6J mice (Navarro *et al.* 2005). These results suggested that the decreased ethanol intake and food intake produced by melanocortin activation is mediated via the melanocortin-4 receptor. Both *ob*/*ob* mice that lack leptin and *db*/*db* mice that lack the leptin receptor showed a significant decrease in ethanol intake and ethanol preference in a two-bottle choice test (Blednov, Walker & Harris 2004). However, the decrease in ethanol consumption in the leptin-deficient mice could not be reversed by administration of leptin, leading the authors to conclude that the decrease in ethanol intake may be due to compensatory developmental changes in other systems.

In summary, neuromodulators that modify food intake also modify ethanol intake and may represent an overlap in the neurobiological substrates for the appetitive nature of both behaviors either at the motivational or at consummatory level.

CHEMOKINES (TABLE 13)

Deletion of chemokines (Ccl2 or Ccl3) or chemokine receptors (Ccr2 but not Ccr5) leads to a substantial reduction of alcohol consumption and preference at higher concentrations. Increased aversion to alcohol could explain these reductions, and all three mutants (Ccl2, Ccl3, Ccr2) demonstrated stronger conditioned taste aversion to ethanol than control mice. Reduced preference for alcohol is also genetically linked to increased susceptibility to ethanol withdrawal symptoms (Metten *et al.* 1998). However, none of the four mutants showed severity of acute ethanol-induced withdrawal that differed from wildtype mice. The similarity between alcohol phenotypes of Ccl2 and Ccr2 null mutant mice is consistent with the finding that Ccl2 is one of the major ligands for the Ccr2 receptor (Murphy *et al.* 2000). Chemokines are produced both in the brain and in cells of the immune system, and it is not clear which of these sources is important for maintaining alcohol consumption. In considering mechanisms of action of chemokines to reduce drinking, it may be important to understand the relationship between chemokines and cytokines. Exposure to pathogens results in behavioral and physiological responses, such as fever, disruption of feeding and drinking, and reduced social and exploratory behaviors known as 'sickness behavior' (Kent *et al.* 1992). Many of these responses are produced by cytokines released by activated immune cells (Kent *et al.* 1992). Cytokines may convey peripheral information to the central nervous system. The human hangover syndrome has some similarities with described 'sickness behavior', and an increased level of cytokines was found in human volunteers during the hangover state (Kim *et al.* 2003). Proinflammatory cytokines are increased after acute consumption of alcohol in humans (Volpato *et al.* 2004) as well as after chronic ethanol consumption in rats (Valles *et al.* 2003) and mice (Romics *et al.* 2004). Finally, craving for alcohol in human alcoholics has been linked to the production of proinflammatory cytokines (Kiefer *et al.* 2002). A link between cytokines and chemokines is suggested by reports that a decrease in either chemokines or chemokine receptors can change levels of cytokines. Specifically, reduction of Ccl2 or Ccr2 resulted in increased cytokine production by lung leukocytes (Traynor *et al.* 2002), and increased cytokine production was shown also in Ccr5 knockout mice (Andres *et al.* 2000). Thus, increased levels of cytokines or increased sensitivity to cytokines in chemokine mutant mice might lead to increased conditioned taste aversion to ethanol and therefore to decreased ethanol intake.

OTHER GENE TARGETS (TABLES 14–16)

Several other genes have been targeted for their potential roles in modulating ethanol sensitivity. These targets did not fit easily into the groups or tables already discussed above. Results from these studies are summarized in Table 14 (Growth factors), Table 15 (Enzymes) and Table 16 (Miscellaneous genes), but are not specifically discussed herein.

Table 13 Chemokine-related genes.

Knockout	*Ref.*[a]	*Gender/ background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Anxiety*	*Tolerance*	*Withdrawal*	*Other*
Chemokine receptor-2 (*Ccr2*)	1	Male, female 129/Ola × C57BL/6J hybrid, > N10 to C57BL/6	KO < WT (intake, PR) 3%, 6%, 9%, 12%, 15% ethanol (4 days each) KO = WT (saccharin PR, quinine avoidance) KO > WT (total fluid intake)	KO > WT (CTA, 2.5 g/kg)	—	KO = WT (LORR duration; 3.8 g/kg)	—	—	KO = WT (acute withdrawal severity, 4 g/kg)	KO = WT (clearance, 4 g/kg, data not shown)
Chemokine receptor-5 (*Ccr5*)	1	Male, female 129/Ola × C57BL/6J hybrid, > N10 to C57BL/6	KO = WT (intake, PR, males) KO > WT (intake, not PR, females) 3%, 6%, 9%, 12%, 15% ethanol (4 days each) KO = WT (saccharin PR, quinine avoidance) KO > WT (total fluid intake)	KO > WT (CTA, effect in females only, 2.5 g/kg)	—	KO = WT (LORR duration, 3.8 g/kg)	—	—	KO = WT (acute withdrawal severity, 4 g/kg)	KO = WT (clearance, 4 g/kg, data not shown)
Chemokine ligand-2 (*Ccl2*)	1	Male, female 129/SvJ × C57BL/6J hybrid, > N10 to C57BL/6	KO = WT (intake, PR, males) KO < WT (intake, PR, females) 3%, 6%, 9%, 12%, 15% ethanol (4 days each) KO = WT (saccharin PR, quinine avoidance) KO > WT (total fluid intake; effect in males only)	KO > WT (CTA, 2.5 g/kg)	—	KO > WT (LORR duration, 3.8 g/kg, bigger difference in females)	—	—	KO = WT (acute withdrawal severity, 4 g/kg)	KO = WT (clearance, 4 g/kg, data not shown)

Table 13 *Cont.*

Knockout	*Ref.*[a]	*Gender/ background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Anxiety*	*Tolerance*	*Withdrawal*	*Other*
Chemokine ligand-3 (*Ccl3*)	1	Male, female Background not specified, > N10 to C57BL/6	KO < WT (intake, PR) 3%, 6%, 9%, 12%, 15% ethanol (4 days each) KO = WT (saccharin PR, quinine avoidance, total fluid intake)	KO > WT (CTA, 2.5 g/kg)	—	KO > WT (LORR duration, 3.8 g/kg, bigger difference in females)	—	—	KO = WT (acute withdrawal severity, 4 g/kg)	KO = WT (clearance, 4 g/kg, data not shown)
Chemokine ligand-2/ Chemokine receptor-2 double KO	1	Male, female Background from intercross of single mutant strains, > N10 to C57BL/6	KO (double) < KO (*Ccl2*) (intake, PR, not compared with WT) 3%, 6%, 9%, 12%, 15% ethanol (4 days each) KO (double) < KO (*Ccr2*) (intake, not PR, effect in males only; not compared to WT) KO (double) < KO (*Ccl2*) <KO (*Ccr2*) (total fluid intake; not compared with WT)	—	—	—	—	—	—	—

[a]Reference no. 1, Blednov *et al.* (2005).

Table 14 Growth factors.

Knockout	*Ref.*[a]	*Gender/background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Tolerance*	*Other*
Brain-derived neurotrophic factor (BDNF)	1	Male J129ftm1Jae × C57BL/6 hybrid	HET > WT (intake) 20% ethanol (4 days after 2-week exposure to 20% ethanol versus water and 2-week ethanol deprivation; only mice with PR > 40% reported)	HET > WT (CPP, 2 g/kg)	HET > WT (ethanol activation in open field, 2 g/kg) HET > WT (sensitization, 2 g/kg test dose after initial 2 g/kg test then 10 2.5 g/kg daily injections)	—	—	—
	2	Male J129ftm1Jae × C57BL/6 hybrid Also studied C57BL/6J	HET = WT (intake, 18 hours, last day of 4-week 10% ethanol versus water)	—	—	—	—	—
Transforming growth factor alpha (TGF-α) overexpression	3	Male Human TGF-α on CD-1 background	OX > WT (PR) 5% ethanol (7 days) OX = WT (total fluid intake)	—	—	—	—	OX < WT (ethanol-induced changes in resident-intruder aggression, 0.6, 1.2, 2.0 g/kg; WT showed increase at 0.6, decrease at 2.0; no response to ethanol in OX) OX = WT (no ethanol-induced increases in social interaction)

Table 14 *Cont.*

Knockout	*Ref.*[a]	*Gender/background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Tolerance*	*Other*
Insulin-like growth factor I (IGF-I) overexpression	4	Gender not specified, ≥F5, C57BL/6J background × unknown founder strain (different from founder for IGF-bp1 OX) 'WT' = 50/50 mixture of controls for IGF-1 and IGF-bp1 preparations	—	—	—	OX = WT (ataxia, fixed speed rotarod, 1 and 2 g/kg) OX = WT (hypothermia, 3 g/kg) OX < WT (LORR duration, 3 g/kg)	OX < WT (LORR tolerance, initial 3 g/kg test, seven daily 1.5 g/kg doses, then retest with 3 g/kg) OX = WT (hypothermia tolerance and BEC at end of tolerance test)	OX = WT (clearance, 2 g/kg)
IGF binding protein-I overexpression (IGF-bpI)	3	Gender not specified, ≥F5, C57BL/6J background × unknown founder strain (different from founder for IGF-I OX) 'WT' = 50/50 mixture of controls for IGF-I and IGFbp-I preparations	—	—	—	OX = WT (ataxia, fixed speed rotarod, 1 and 2 g/kg) OX = WT (hypothermia, 3 g/kg) OX > WT (LORR duration, 3 g/kg)	OX > WT (LORR tolerance, initial 3 g/kg test, 7 daily 1.5 g/kg doses, then retest with 3 g/kg) OX = WT (hypothermia tolerance and BEC at end of tolerance test)	OX = WT (clearance, 2 g/kg)

[a]Reference nos. 1, McGough *et al.* (2004); 2, Jeanblanc *et al.* (2006); 3, Hilakivi-Clarke & Goldberg (1995); 4, Pucilowski, Ayensu & D'Ercole (1996).

Table 15 Enzymes.

Gene	*Ref.*[a]	*Gender/background*	*Preference*	*Sedation*	*Tolerance*	*Withdrawal*	*Other*
Cytochrome P450 2e1 (Cyp2e1)	1	Male, female 129/Sv × C57BL/6N background	—	KO > WT (LORR duration, 3, 4 and 5 g/kg, male; trend in female)	—	—	KO = WT (clearance, 2, 3, 4, and 5 g/kg) KO < WT (blood acetaldehyde levels, females only)
Acatalasemic (C^{sb}/C^{sb})	1	Male, female Spontaneous mutation on unknown background, maintained on C3H/HeJ background	—	KO > WT (LORR duration, 3, 4 and 5 g/kg)	—	—	KO = WT (clearance, 2, 3, 4, and 5 g/kg)
CYP2E1/ Acatalasemic *Cyp2E1* -/-/C^{sb}/C^{sb} double knockouts	1	Male, female 129/Sv × C3H/HeJ F3 hybrid	—	Double KO > WT (LORR duration, 3, 4 and 5 g/kg)	—	—	Double KO = WT (clearance, 2, 3, 4, and 5 g/kg)
Neutral endopeptidase (EC 3.4.24.11, NEP)	2	Male J1 ES cells to 129/Sv × C57BL/6J hybrid	KO > WT (intake, PR) 10% ethanol (4 weeks) KO = WT (total fluid intake) KO > WT (food intake)	—	—	—	KO < WT (increase in angiotensin-converting enzyme activity in tegmentum/colliculi after 4 week ethanol drinking; no effect in KO; no genetic difference in olfactory bulb and cortex increases)
Monoamine oxidase-A	3	Male Tg8 transgenic KO line on C3H/HeJ background	KO = WT (ml consumption) 10% ethanol (free choice first 2 hrs and all 24 hrs after 24-hr water deprivation)	KO < WT (hypothermia, 3 g/kg) KO < WT (LORR duration, latency, 3 g/kg)	—	—	—

Table 15 *Cont.*

Gene	*Ref.*[a]	*Gender/background*	*Preference*	*Sedation*	*Tolerance*	*Withdrawal*	*Other*
	4	Male Tg8 transgenic KO line on C3H/HeJ background	—	KO < WT (hypothermia, 5 g/kg) KO < WT (LORR duration, 5 g/kg)	KO = WT (no tolerance to hypothermia, seven daily injections of 5 g/kg or 30-day access to oral 10% ethanol) KO < WT (tolerance to LORR after 30-day access to oral 10% ethanol in WT, but *increased* sensitivity in KO) KO < WT (tolerance to LORR after seven daily injections, but possible floor effect)	—	—
nNOS	5	Gender not specified 129X1/SvJ, N3 backcross to C57BL/6J	KO > WT (intake, ≥8%) 2%, 4%, 8%, 12% and 16% ethanol (number of days varied across concentration) KO > WT (sucrose PR) KO = WT (quinine avoidance)	KO < WT (LORR duration, 2.5 and 4.5 g/kg) KO < WT (hypothermia, 3.5 g/kg)	KO < WT (hypothermia tolerance, 3.5 g/kg, 24 hours apart; no tolerance in KO)	—	KO = WT (clearance, 3.5 g/kg)
Aldehyde dehydrogenase 2 (Aldh2)	6	Male E14 or D3 ES cells to C57BL/6J, N7-8 backcross to C57BL/6	KO < WT (intake, PR; intakes very low in both) 3% ethanol (8 days)	—	—	—	KO = WT (acetaldehyde levels in brain after drinking; KO > WT if expressed as per unit ethanol intake)
	7	Male E14 or D3 ES cells to C57BL/6J, > N8 backcross to C57BL/6	—	—	—	—	KO < WT (induction of hepatic cytochrome P450 enzymes by ethanol, 2 g/kg; expression and protein levels)

[a]Reference nos. 1, Vasiliou *et al.* (2006); 2, Siems *et al.* (2000); 3, Popova *et al.* (2000); 4, Ivanova & Popova (2002); 5, Spanagel *et al.* (2002); 6, Isse *et al.* (2002); 7, Kim *et al.* (2005).

Table 16 Miscellaneous genes.

Knockout	*Ref.*[a]	*Gender/background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Tolerance*	*Other*
FosB	1	Male, female J1 ES cells, 129/Sv× BALB/c hybrid	KO = WT (intake, PR) 2%, 4%, 8% ethanol (2–8 days each) KO > WT (saccharin PR) KO = WT (total fluid intake, quinine avoidance)	—	—	—	—	—
m-neu1	2	Male JM1 ES cells, 129/Sv× C57BL/6 hybrid	—	—	—	KO > WT (rotarod ataxia, 2 g/kg)	—	—
Norepinephrine-deficient (*Dbh*)	3	Male, female 129/SvEv× C57BL/6J hybrid	KO < HET (intake, PR, tested only in males) 3%, 6%, 10% ethanol (8 days each) KO = HET (sucrose PR, quinine avoidance) KO > HET (food intake, water intake)	KO = HET (ethanol-induced CTA for saccharin, 2 g/kg) KO < HET (rate of extinction of ethanol-induced CTA) KO = HET (LiCl-induced CTA)	—	KO > HET (hypothermia, 3 g/kg, when ambient temperature = 22°C, but not 30°C) KO > HET (LORR duration, 3 g/kg)	—	KO = HET (clearance, 3 g/kg, at 30°C) KO < HET (clearance, 3 g/kg, at 22°C)

Table 16 *Cont.*

Knockout	*Ref.*[a]	*Gender/background*	*Preference*	*Other ethanol reward*	*Activity*	*Sedation*	*Tolerance*	*Other*
Uncoupling protein 2 (UCP2) overexpression	4	Female Human UCP2 gene on C57BL/6J background	—	—	—	OX = WT (LORR duration, 3.5 g/kg)	OX > WT (LORR tolerance day 2, but similar thereafter, five daily 3.5 g/kg injections) OX = WT (tolerance to ethanol on hotplate test, day 1 versus day 5)	OX = WT (BEC at 1 hour, 3.5 g/kg) OX = WT (ethanol analgesia on hotplate, 2 hours after 3.5 g/kg)
Uncoupling protein 2 (UCP2) knockout	4	Male CF-1 background	—	—	—	KO > WT (LORR duration, 3.5 g/kg)	KO = WT (LORR tolerance, five daily 3.5 g/kg injections)	KO = WT (BEC at 1 hour after 3.5 g/kg) KO = WT (ethanol analgesia on hot plate, 2 hours after 3.5 g/kg)
Apolipoprotein-E	5	Male, female E14TG2a ES cells to C57BL/6J background	KO = WT (intake, PR) 3%, 6%, 10% ethanol (4–6 days each) KO = WT (total fluid intake)	KO > WT (CPP, 2 g/kg)	KO < WT (ethanol-induced stimulation in CPP, female only, 2 g/kg)	—	—	KO < WT (BEC 30 min, not 60 min after 2 g/kg)
Metallothionein I/II	6	Male, female 129S7/SvEvBrd vs 129S1/SvImJ (WT)	KO > WT (PR) 10% ethanol (14 days; only males significant, but females tended to differ) KO = WT (total fluid intake)	—	—	—	—	—

[a]Reference nos. 1, Korkosz *et al.* (2004); 2, Ruan *et al.* (2001); 3, Weinshenker *et al.* (2000); 4, Horvath *et al.* (2002); 5, Bechtholt *et al.* (2004); 6, Loney, Uddin & Singh (2006).

COMPARISONS WITH HUMAN CANDIDATE GENES

Making a connection between alcohol phenotypes produced by human genetic diversity and engineered genetics in mice is, of course, an important goal, but it is more of a future goal than a current realization. In part, this is because we lack precise knowledge about the human polymorphisms that are important for alcoholism or other alcohol-related phenotypes in humans (with the exception of the alcohol and aldehyde metabolizing enzymes). For example, several studies link genes coding for $GABA_A$ receptors with human alcoholism (Lappalainen *et al.* 2005; Radel *et al.* 2005), but in most cases, the exact polymorphism is not known, and it is not clear if we would expect an increase or decrease in $GABA_A$ receptor number or function to be associated with alcoholism risk. There is a specific amino acid polymorphism (serine to proline at position 385) in the α6 subunit which is associated with a low response to alcohol in rats, but deletion of the α6 subunit in mice does not appear to alter alcohol sensitivity. A recent study reports an association between haplotypes of the human CRHR1 receptor and binge drinking in both adolescents and adults (Treutlein *et al.* 2006), but an effect on drinking was only seen in CRF_1, receptor null mutants in response to stress (Sillaber *et al.* 2002). Linking human and mouse genetics will require detection of human polymorphisms that alter the promoter or protein coding regions of specific genes and engineering of similar polymorphisms into mutant mice. This has been accomplished in other areas of neurobiology, such as Alzheimer's disease research (German & Eisch 2004).

DISCUSSION AND CONCLUSIONS

There can be no question that studies with genetically engineered mice have yielded much data regarding ethanol's effects. Yet, it is a fair question whether these data have advanced our understanding of how ethanol affects the brain in ways that are unique, rather than simply reinforcing information from pharmacological studies without a genetic focus. Are there any genes whose effects seem especially prominent or pronounced? Do any manipulated genes or groups of genes indicate the potential importance of heretofore novel neural pathways? For the most part, our tables were organized around candidate neurotransmitters, pathways, signaling cascades, and the like. One could reasonably expect that the volume of work devoted to certain of these candidate systems should offer a strong background on which to reveal genetic modulatory effects.

GABA has been known to be an important target for ethanol for many years. Numerous studies with $GABA_A$ receptor subunit deletion and overexpression mutants have confirmed this, but have not quite pinpointed any particular subunit as crucial. Consideration of the synaptic versus extrasynaptic roles that GABA plays in the brain did not prove to be a strikingly useful organizing principle for interpreting results; however, it is possible and even likely that both synaptic and extrasynaptic $GABA_A$ receptors, composed of multiple subunits, are important for alcohol action. It is an accepted fact that dopaminergic systems are one important contributor to motivated behaviors, whether they are reflecting drug reward or motor responses to incentives. Studies of ethanol intake in mice with mutated dopaminergic receptor or transporter genes only modestly illuminated the involvement of specific dopamine-related proteins in alcohol-related effects on the brain. Similarly, CRF and opioid pathways are important modulators of alcohol and drug responses, an assertion supported by results from the mutant studies. However, the results also indicate that the role of a single protein must be evaluated in the context of a complex system; thus, effects have depended upon the background level of activation of the stress axis in some cases and on genetic background in others.

One possible explanation for apparently inconsistent findings is that we are seeking consistency of effects at an inappropriate level of analysis. GABA, dopamine, CRF and endogenous opioids are all crucial for many primary brain-related functions subserving motivation, arousal, activation, memory, learning and reward. The insult introduced by deleting the function of a major receptor in these systems may be so threatening as to demand massive and widespread functional compensation, obscuring any attempt to link a specific gene deletion with a specific response, and could be the basis for many of the significant findings reported in the tables. Deletion of a gene of more restricted relevance to brain function might offer a cleaner experiment. More interpretable studies targeting the same genes could be envisioned using knock-in mutations, conditionally expressed gene deletions, or RNA silencing methods. These preparations are currently sparse, but the few examples reported are encouraging. For example, the conditional knockout of PKCε allowed the investigators to turn ethanol preference and sensitivity on and off at will, suggesting that the kinase indeed has a role in ethanol responses (Choi *et al.* 2002).

We should also consider the notion that we are examining a collection of phenotypes that is too limited. In the late 1970s, Gerald McClearn selectively bred mice to be highly sensitive or resistant [Long Sleep (LS) and Short Sleep (SS)] to the effects of ethanol to induce LORR (McClearn & Rodgers 1959). Hundreds of papers have been published since then studying these genetically divergent lines of mice (Phillips & Crabbe 1991), and they have been used in sophisticated studies to identify the

genomic location of several genes influencing this trait (Bennett *et al.* 2002; MacLaren *et al.* 2006). Yet, the physiological effects of ethanol that lead to LORR remain only partly understood, and the relevance of this extreme ethanol-sedation trait to human alcoholism has not been established. Further, the generality of genetic differences in sensitivity to the LORR to other traits reflecting ethanol's sedative effects (e.g. hypothermia, ataxia-like responses) is weak. Finally, LORR by definition reflects a combination of initial brain sensitivity and the rapid development of acute functional tolerance, which are to a major extent distinct genetic phenomena (Ponomarev & Crabbe 2004). Low response to alcohol has been used as a predictor of later human risk for alcohol dependence (Schuckit *et al.* 2004). We looked for correspondences between the effects of single gene manipulations on preference and sedation. Nineteen of the 48 genes studied for both traits (40%) had opposite effects, in agreement with Schuckit *et al.* (2004), while 12% augmented both traits, and 23 (48%) had equivocal effects.

The other trait studied by nearly every group is two-bottle preference for ethanol solutions. This choice, too, is historically based, since the initial demonstrations that rats could be bred to differ in preference in the late 1940s (Mardones & Segovia-Riquelme 1983). Many hundreds of studies have been performed since, with multiple lines of rats and mice selected to differ in two-bottle preference for ethanol (McBride & Li 1998), and C57BL/6J mice have been known to be the most highly preferring inbred strain since the first reported experiments with mice (McClearn & Rodgers 1959). Nonetheless, under most circumstances (and in almost all of the experiments reported in this review), neither mice nor rats will self-administer enough ethanol to achieve sustained intoxicating blood alcohol levels (Dole & Gentry 1984). A number of newer methods (or renewed application of older methods) have shown that substantial voluntary intakes of ethanol, resulting in intoxicating blood ethanol levels, can be achieved without drastic (or even any) food or water restriction, in both mice and rats. It would be of interest to see whether any of the mutants studied here differed in self-intoxication, rather than in a tendency to self-administer relatively high levels of ethanol without intoxication. For discussions of some of these alternative methods, see Tomie *et al.* (2002), Tordoff & Bachmanov (2003), Middaugh *et al.* (2003), Mittleman, Van Brunt & Matthews (2003), Bell *et al.* (2004a,b), O'Dell *et al.* (2004), Lopez & Becker (2005), Sharpe *et al.* (2005), Finn *et al.* (2005), Rhodes *et al.* (2005) and Fidler, Clews & Cunningham (2006). While it is worth considering whether two-bottle preference drinking should remain the standard behavioral assay for studying genetic proclivity toward ethanol intake, the fact remains that we know a great deal about this phenotype, and relatively little about some of the newer, alternative methods. Evidence to date suggests some commonality of genetic influence on two-bottle preference and higher-intake phenotypes, which encourages us to explore the latter further. Because of the well-established negative genetic correlation between drinking and withdrawal traits in mice (Metten *et al.* 1998), we also examined results for 16 genes studied for both traits. Two genes showed the predicted opposite relationship, but 3 had the same effect, and most (11) had equivocal effects. Neither this result nor the comparison of preference versus sedation mentioned above is either surprising or definitive for preference and sedative responses are both known to be influenced by numerous genes, some shared and some not shared.

The data summarized here support the use of null mutant mice to confirm prior evidence of the involvement of specific neurobiological pathways in several effects of ethanol. However, there is a need for wider use of sophisticated methods that afford control over anatomical and temporal factors. Further, focusing on the effects of single genes without consideration of genetic variation at other loci is not likely to provide a very complete genetic analysis of the complex traits under investigation. While studies that take these complexities into account are technically feasible, they are not often undertaken. An elegant example of attention to genetic background is the explorations of GSα subunit mutants on three genetic backgrounds (Wand *et al.* 2001). This study also yielded consistent results on preference and LORR. By studying ethanol responses in more depth, a more firm link can be established between gene deletion and behavior. A nice example of a study that went into great phenotypic depth is the study of PKCε by Wallace *et al.* (2006). These investigators carefully explored different forms of ethanol tolerance to multiple sedative responses and were rewarded with a consistent pattern of responses they also could link to *in vitro* GABA sensitivity.

Although these integrative approaches appear logical, it is unlikely that we will succeed in rebuilding complex traits simply from a collection of parts. However, the combined information from null mutants, pharmacological studies, gene expression analyses, and studies using other genetic methods (e.g. selected lines, RNA interference) may get us closer to our target (Belknap *et al.* 2001). More studies should attempt to verify the effects seen in genetically engineered mutants using selective small molecule inhibitors (previously known as drugs). An example can be drawn from studies targeting the cannabinoid CB_1 receptor. The decreased self-administration generally seen in CB_1 knockouts (Table 5) is mirrored by the reduction of drinking in multiple experiments using CB_1 antagonists (Colombo *et al.* 2004; Economidou *et al.* 2006; Gessa *et al.* 2005). Knocking out the FAAH gene, which

increases endogenous cannabinoids, elevated ethanol consumption (Basavarajappa *et al*. 2006), and recent studies with AA rats, selectively bred to prefer 10% ethanol over water, have revealed decreased expression of FAAH in prefrontal cortex accompanied by down-regulation of CB_1 receptor binding (Hansson *et al*. 2006). Injection of a competitive FAAH inhibitor into rat prefrontal cortex increased alcohol self-administration in this study. This combination of gene deletion in multiple genes with downstream effects on cannabinoid signaling, coupled with antagonist administration in pharmacological experiments, has produced a consistent set of results on alcohol self-administration, and the cannabinoid antagonist, rimonabant, is currently being investigated in clinical trials. We are sanguine about the value of future studies with gene targeting as they are increasingly incorporated into a literature using complementary methods.

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