

## Issues in the Study of Alcohol Consumption in Genetically Modified Mice

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### Editorial

Genetic studies in humans have implicated a number of genes in alcoholism and the behavioral effects of ethanol. Genetically modified mice have been used to evaluate, under more controlled conditions than are possible in humans, whether modifications of these genes influence responses to ethanol or alcoholism-related phenotypes, in particular ethanol consumption. The genetic architectural of alcoholism, and drug abuse in general, appears to be both polygenic and heterogeneous [1]. Therefore, it is likely that variation in these genes contributes differentially to such phenotypes. A number of methods are used to assess differences in ethanol consumption in studies with genetically modified mice. However, when a particular gene is being investigated in such a context it is common only to examine a limited number of consumption conditions and to base evaluations of the role of that gene overall in ethanol consumption on that basis alone. Increasingly, research suggests that differences in ethanol consumption in genetically modified mice are not apparent under all experimental conditions. Thus, caution should be made when evaluating the results of such experiments. Looking forward, a broader range of conditions should be studied in order to prevent erroneous conclusions that a gene has no role in ethanol consumption.

The most commonly used method to examine ethanol consumption in rodents is a 2-bottle choice paradigm, in which animals are individually housed and provided two drinking bottles, one with a specific concentration of ethanol and one with plain tap water. Access is usually continuous and often only at a single concentration. The main problem with such an approach is that it fails to consider concentration dependent effects, that is, genotypic differences may only be seen at specific ethanol concentrations. Alternatively, a procedure may be used in which access is continuous, but multiple concentrations of ethanol are assessed, usually over a period of days beginning with the low concentration and proceeding to higher concentrations. Data from a study using this approach is presented in Figure 1, [2] which examined the effects of social isolation in Fawn Hooded and Wistar rats. It demonstrates one of the main caveats in using only a single concentration of ethanol in consumption studies. In most studies that use a single concentration the concentration used is 8-10% ethanol. As can be seen in Figure 1, if only this concentration had been used the conclusion would have been that neither strain (Fawn Hooded vs. Wistar) nor social isolation (Isolate versus social) had any effect on ethanol consumption. In fact there were substantial effects of both factors at higher and lower ethanol concentrations.

The earliest study of ethanol consumption in genetically modified mice was in serotonin 1B receptor (5-HT<sub>1B</sub>) knockout (KO) mice found increased consumption at all concentrations of ethanol (3-20%) in 5-HT<sub>1B</sub> KO mice, which but the effects were greater at higher concentrations as consumption increased [3]. A similar effect was found in prodynorphin KO mice [4]. A number of other studies in genetically modified mice have identified differences in ethanol preference or consumption only at certain ethanol concentrations, including dopamine D2 receptor (DRD2) KO mice [5],  $\mu$  opioid receptor KO mice [6], dopamine transporter KO mice [7], vesicular monoamine transporter 2 (VMAT2) KO mice [7], dopamine  $\beta$ -hydroxylase KO

mice [8], corticotrophin releasing factor receptor type 1 (CRF<sub>1</sub>) KO mice [9],  $\alpha$ -synuclein null mutant mice [10] and Cyclin D2 KO mice [11]. In most cases differences in ethanol consumption were more likely to be observed for higher ethanol concentrations, often above the concentration that is typically used in single concentration studies. Many differences in ethanol consumption in genetically modified mice are sex-dependent as well [6-8,12]. It must also be noted that many of these studies used limited ranges of ethanol concentrations so that the effect of concentration may in fact be under estimated.

Using operant ethanol self-administration, it was shown that although there were no differences for self-administration of 5% or 8% ethanol, consumption of 10% ethanol was increased in  $\delta$  opioid receptor (DOR) KO mice [13]. These differences emerged after an extended period of self-administration under varying conditions, including different ethanol concentrations in the presence or absence of saccharin. It could be that this extended access, and not concentration per se or concentration alone resulted in the observed differences. In the same mice there were no differences in 2-bottle choice consumption of the same concentration prior to operant testing, but increased consumption in DOR KO mice after extended operant self-administration of ethanol.

All of the studies discussed above used continuous access to ethanol. Other approaches use either limited access (access for a limited period of time each day) or intermittent access where ethanol is available only for certain days each week, but often for 24 hours. There are a number of variations on each of these paradigms. One variant of these procedures that has been popularized recently because it was suggested to induce a higher level of drinking, perhaps more similar to binge-drinking in humans, is called "drinking-in-the-dark" (DID). In this procedure ethanol access is limited to a 2 or 4-hour period of time at the beginning or shortly after the onset of the dark cycle [14]. The DID procedure can be conducted in a 2-choice fashion, similar to continuous access methods, or with only ethanol available, which has been suggested to be an important factor in the observation of differences in ethanol consumption in studies of corticotropin releasing factor type 1 receptor KO mice [15]. As with continuous access, some DID studies examine only single concentrations [16].

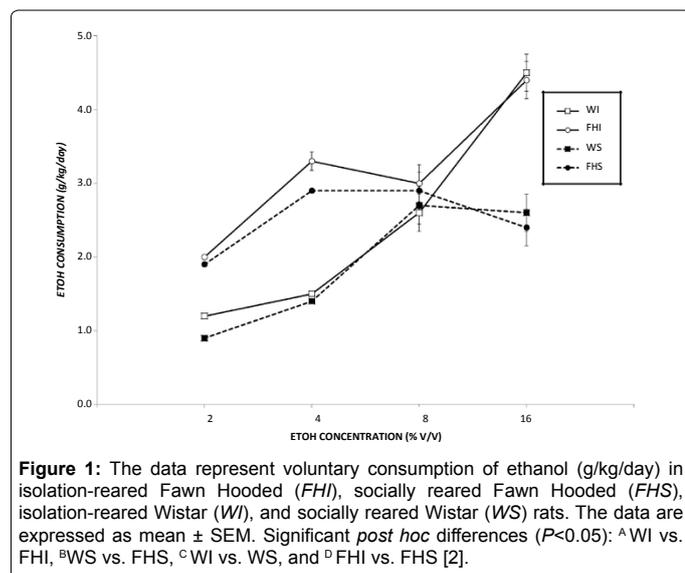
The idea behind the DID procedure is to produce a large amount of ethanol consumption over a short period. A variety of methods have been used to increase or escalate ethanol consumption that may be differentially involved in the influences of particular genes on alcoholism. Another factor is stress. Naturally there are a variety of

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in different ways. One such approach is the chronic mild stress (CMS) procedure that uses a regimen of repeated, varied and unpredictable stressors to induce depressive-like symptoms. This was used to examine ethanol consumption and preference in a 2-bottle choice procedure in DRD2 KO mice [17]. Only a single low (5%) concentration of ethanol was assessed, both with and without stress. Under non-stressed conditions mice with reduced DRD2 expression had reduced ethanol consumption. However, stress reduced ethanol consumption in WT mice, but increased ethanol consumption in DRD2 KO mice.

Other studies have more explicitly compared stress parameters. In WT mice a single forced swim stress did not impact consumption and preference for 10% ethanol but repeated forced swim stress did increase ethanol consumption and these effects persisted for a period of time after the stress exposure; these effects of stress were reduced in CRF<sub>1</sub> KO mice, and even more so in combined CRF<sub>1</sub>/CRF<sub>2</sub> KO mice [9]. Although note precisely stressful, a schedule-induced polydipsia procedure has also been used to increase ethanol intake, in which it was shown that DAT<sup>-/-</sup> mice have reduced consumption of 5% ethanol, while DAT<sup>+/-</sup> mice have increased consumption of ethanol compared to WT mice [18].

Continuous daily access to ethanol does not necessarily lead to increases in ethanol intake (e.g. escalation). Thus, although WT mice did not show escalation under such a regimen, escalation of ethanol intake was seen over just one week of continuous access to 10% ethanol in  $\alpha$ -synuclein null mutant mice [10]. Intermittent access paradigms more often lead to escalation of ethanol intake [19]. For instance, in the Pastor et al. [9] study, CRF<sub>1</sub> KO and WT mice were subjected to repeated cycles of alternating periods of 4 day of access to 10% ethanol and 4 day periods of no ethanol access. On the first day of the second period of ethanol access consumption and preference were increased, returning to previous levels over the next few days of continuous ethanol access. In our own studies, we have found that more consistent increases in ethanol intake (e.g. escalation) are achieved by intermittent periods of 24 hour access to 8% ethanol. Interestingly, the observation of escalation was highly dependent on the spacing of these periods of access – when ethanol was available 3x per week there was no escalation, but when ethanol was available 2x per week ethanol intake increased by as much as 100% in 3 weeks (Houston-Ludlam and Hall, unpublished observations).

The studies discussed above, which are just a sample of the ethanol consumption studies so far conducted in genetically modified mice, clearly demonstrate that examination of ethanol consumption under a variety of conditions is necessary before conclusions can be made regarding whether a particular gene may or, more importantly, may not be involved in responses to ethanol. Using a limited set of conditions may lead to Type II errors, the point being that it appears that many genetic effects do not contribute to ethanol responses under all circumstances, but rather do so under a more limited set of circumstances. As a final exemplar of this problem, a study of metabotropic glutamate receptor 5 KO mice examined five different ethanol consumption paradigms, finding no genotypic differences with three of those paradigms, but large reductions in ethanol consumption in two of those paradigms [20]. This idea fits with the highly polygenic and heterogeneous nature of the genetic contributions to alcoholism in humans. Furthermore, on this basis it may be possible to identify genetic contributions to therapeutic responses to alcoholism treatments based upon these underlying differences (e.g. pharmacogenomics).

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