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The Fruit Fly: A Model Organism to Study the Genetics of Alcohol Abuse and Addiction?

Minireview

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The social, economic, and personal costs of alcohol abuse and addiction are difficult to measure, but the extent of the problem can be illustrated with a simple statement: alcohol abuse and addiction are the leading causes of domestic violence and highway deaths. Social and political strategies, including better education, are key to a solution of this social problem. However, a better understanding of the physiological mechanisms, the molecular pathways, and the genetic components leading to abuse and addiction would pave the way to better drug design, and possibly help or even cure patients with these neurological diseases (Leshner, 1997; O'Brien, 1997).

Dopamine Secretion and Addiction

The past 20 years have seen a dramatic improvement of our understanding of the areas of the brain, and the molecular mechanisms that are involved in addiction. Many studies have implicated a vast array of proteins and signaling pathways in the acute and chronic responses to ethanol. One of the most important physical properties of ethanol is that it is soluble in both water and lipids and hence easily passes the blood-brain barrier to "hit" neurons in many areas of the brain. Most of the molecular targets of ethanol are membrane associated and include the N-methyl-D-aspartate (NMDA), γ-aminobutyric acid (GABA), and serotonin receptors, calcium and potassium channels, and adenosine transporters. In addition, a number of G protein–coupled receptors including dopamine, opioid, and adenosine receptors have been shown to be up- or down-regulated by ethanol in specific cultured cell lines (for review, see Diamond and Gordon, 1997).

Alcohol addiction, and probably most types of drug addiction, appear to share common mechanisms, an idea that is simply formulated as the "dopamine hypothesis." The hypothesis states that addictive drugs may activate certain areas of the brain known as the mesocorticolimbic dopamine system (MDS), leading to an increase in dopamine neurotransmitter release. The MDS comprises the ventral tegmental area and its targets, the nucleus accumbens and the amygdala. Elevation of dopamine in the nucleus accumbens probably provides a sense of well being, pleasure, or elation, and hence positive reinforcement. In the case of alcohol abuse or addiction, dopamine is almost certainly not the only neurotransmitter acting in the MDS or other areas of the brain. Indeed, neurotransmitter pathways using glutamate, serotonin, and GABA are also thought to be involved in the MDS (for review, see Tabakoff and Hoffmann, 1996; Koob and Le Moal, 1997).

Regulation of cAMP in the Acute and Chronic Response to Ethanol

A recurrent theme in different model cell culture systems subjected to ethanol treatment is the involvement of the cAMP pathway in both the acute and the chronic response. Acute exposure to ethanol has been found by most investigators in most cell culture systems to potentiate receptor-activated cAMP synthesis (Figure 1). In contrast, chronic exposure often causes a decrease in cAMP production. This decrease in cAMP signaling appears to be due to a desensitization of the Gs-coupled receptors or an increase in Gi protein levels or activity. Elevated levels of cAMP activate protein kinase A (PKA), which leads to phosphorylation of target proteins such as the cAMP response element binding protein (CREB) and possibly many other target proteins (Diamond and Gordon, 1997; Nestler and Aghajanian, 1997; Figure 1). Precisely how the activation of PKA causes its effects, which neurons are involved, which neurotransmitters play the most important roles in vivo, and which genes are involved remains to be elucidated.

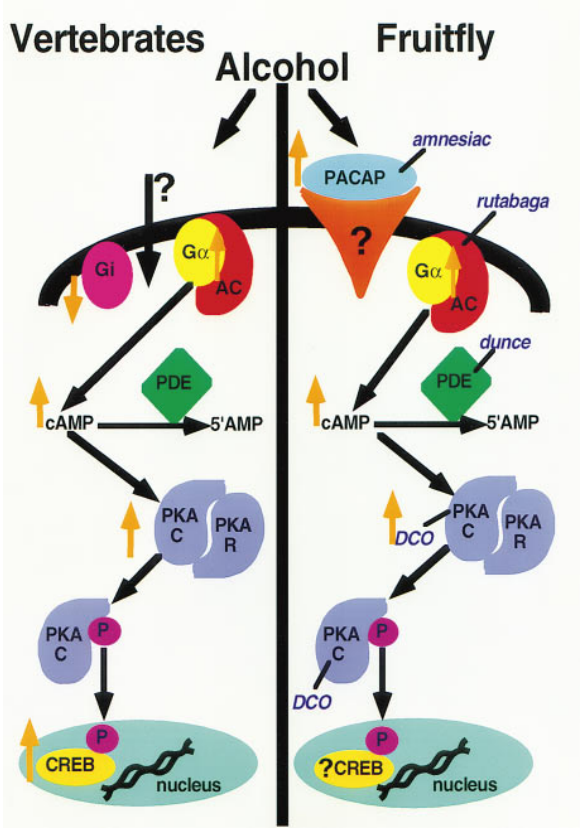

Figure 1. Signaling Components Affecting the cAMP Pathway

A comparison of the molecular components of the cAMP pathway that have been implicated in acute and chronic ethanol responses in vertebrate cultured neurons, and alcohol sensitivity in *Drosophila*. Acute alcohol exposure up-regulates the cAMP signaling pathway. However, not all cells may respond in a similar fashion. The genes studied by Moore et al. (1998) are highlighted in blue.

Is the dopaminergic pathway connected to short-term up-regulation of the cAMP pathway? Drinking alcohol has been shown to cause an increase in dopamine release in the nucleus accumbens, one of the targets of the ventral tegmental area. Dopamine receptors can act through a G protein–coupled system to activate adenylyl cyclase (AC) and hence cAMP synthesis. Since the nucleus accumbens is believed to play a role in motivational states (well-being, pleasure, etc.), it has been implicated in reinforcing the action of some drugs through cAMP metabolism (Diamond and Gordon, 1997; Nestler and Aghajanian, 1997). Addiction may result from a response in which the continuous stimulation of the cAMP pathway by ethanol is counteracted by a constitutive down-regulation of the pathway during chronic exposure. This down-regulation can then be partially compensated by ethanol, which would temporarily up-regulate or activate the pathway, thereby relieving the symptoms created by the down-regulation of the pathway. The opposite adaptation may occur in different neuronal subpopulations or with different drugs (Nestler and Aghajanian, 1997).

One of the major issues with the interpretations of the studies of ethanol-induced changes in cell culture systems is their relevance to the pathophysiological effects of alcoholism in humans. It has been argued that such in vitro observations have relevance because circulating lymphocytes of chronic alcoholics exhibit changes similar to those observed in some cell culture assays, e.g., reduced AC activity. Moreover, studies with alcohol-preferring rats and mice have in certain instances substantiated some of the in vitro observations (for review, see Diamond and Gordon, 1997). However, the direct in vivo relevance of many of the discussed observations remains to be established.

Individuals who are less sensitive at a young age to the effects of alcohol tend to be much more prone to alcoholism than those who are more sensitive (Schuckit, 1994). Moreover, lower or higher sensitivity to ethanol appears to be influenced genetically in humans and rodent model systems (Crabbe et al., 1994), and alcoholism seems to have an hereditary component in some families (Cloninger, 1987). Hence, it is now obvious that there is a genetic component in ethanol abuse and addiction, but the molecular mechanisms underlying the sensitivity to alcohol or alcoholism are unknown.

Ethanol Intoxication in **Drosophila** *Is Modulated through the cAMP Pathway*

In this issue of *Cell*, Moore et al. (1998) break new ground by providing compelling evidence that cAMP metabolism indeed plays an important role in vivo in the acute response to ethanol in fruit flies. They show that lack of the *amnesiac* gene, a previously isolated learning mutant, increases sensitivity to alcohol. In addition, the authors provide evidence that genetic and pharmacological manipulations of cAMP levels in vivo modify the sensitivity to ethanol.

To initiate a genetic approach, the Heberlein laboratory first investigated the behavior of fruit flies exposed to ethanol vapor. Ethanol vapor causes progressive behavioral changes within a 20 min timespan, e.g., hyperactivity, uncoordination, and sedation. To quantitate ethanol sensitivity, they used a simple but well-designed instrument, the inebriometer (see cover and Figure 2). First described by Cohan and Graf (1985), the inebriometer was used to measure ethanol resistance in natural *Drosophila* populations derived from different latitudes. In brief, the flies are transferred to a long vertical glass column through which ethanol vapor is pumped. As the flies become uncoordinated, they roll down a series of baffles, very much like proteins eluting from a sizing column (Figure 2). The mean elution times of various mutant fly strains permitted comparison of resistance to ethanol vapor, providing an assay for the isolation of ethanol-sensitive and -resistant mutants. It is worth noting that concentrations of ethanol that produce uncoordinated flies also affect human behavior. Inebriated flies contain as much as 50 mM ethanol, which corresponds to 0.2%, a concentration that seriously impairs most normal human behaviors (the legal limit for driving in many countries is at or below 0.1%).

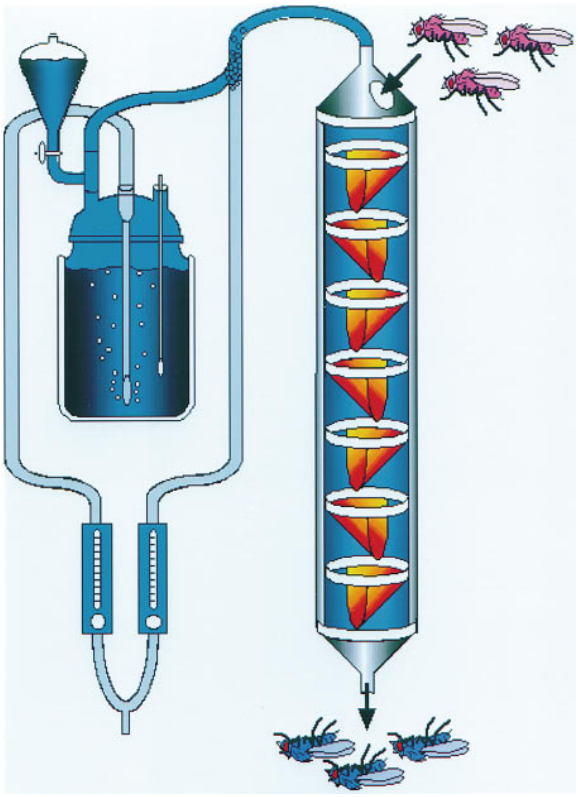

Figure 2. The Inebriometer

The apparatus in this cartoon consists of a tank filled with ethanol that is perfused with air. The ethanol vapor is mixed with air to obtain the desired concentration of ethanol vapor. This mixture of ethanol and air flows through a column with plastic baffles. Unanesthetized flies are poured into the top of the column. The flies can hold on to the baffles until they are too inebriated, upon which they fall down and elute out of the column. The mean elution time (MET) varies between 10 and 32 min, depending on the mutant strain or if the fly strain has been selected for ethanol resistance.

Moore et al. (1998) performed a P element–induced X chromosome mutagenesis screen for mutants that exhibit an abnormal mean elution profile from the inebriometer. They isolated various mutants, including a mutation that was more sensitive to ethanol and allelic to

a previously isolated learning mutant, *amnesiac* (Figure 1). The *amnesiac* gene was discovered in a screen designed to isolate olfactory learning mutants in *Drosophila*, and the gene was cloned by Feany and Quinn (1995). It was shown to encode a protein with homology to two mammalian neuropeptides: pituitary adenylyl cyclase activating peptide (PACAP) and growth hormone releasing hormone (GHRH), both of which are coupled positively with AC. It was therefore proposed that binding of the Amnesiac (PACAP) peptide to its cognate receptor would increase cAMP levels, possibly by activating the AC encoded by the *rutabaga* gene. *rutabaga* (AC) encodes a Ca^{2+}/calmodulin-dependent AC, which was also first identified as a learning mutant (for review, see Davis, 1996).

Interestingly, Moore et al. (1998) demonstrate that loss-of-function mutations in *rutabaga* (AC) and *DCO*, which encodes a catalytic subunit of the cAMP-dependent protein kinase (PKA-C) (Lane and Kalderon, 1993), increase the sensitivity to alcohol. This increase in ethanol sensitivity is similar to that observed in *amnesiac* (PACAP) mutants. In contrast, flies lacking the cAMP-specific phosphodiesterase (*dunce*), exhibit elevated levels of cAMP (Davis, 1996) but show ethanol sensitivities similar to wild-type control flies. Hence, *dunce* (PDE) flies appear to be desensitized to up-regulation of cAMP. These data suggest that the inability to transiently up-regulate the cAMP signal causes an increased sensitivity to ethanol. These observations are not inconsistent with the data gathered from cell culture experiments, which suggest that a surge in cAMP occurs upon an acute ethanol challenge. Hence, it is possible that the inability to up-regulate cAMP in vivo may lead to increased sensitivity.

To provide further evidence that cAMP is involved in ethanol sensitivity, the authors blocked the sensitivity associated with *amnesiac* (PACAP) mutants by feeding the flies forskolin, an AC activator. Similarly, forskolin blocks the sensitivity associated with the *rutabaga* (AC) mutation, suggesting that ACs other than that encoded by *rutabaga* (AC) may play a role as well. Finally, blocking PKA-C activity by feeding wild-type fruit flies a drug increased the sensitivity to ethanol. All these data suggest that the ability to activate PKA is critical to ethanol resistance. Unfortunately, the story is not this simple!

The complexity of the regulation of cAMP levels is revealed by two independent observations that do not fit the simple model described above. Yet, both of these observations are internally consistent. First, although single mutations in either *amnesiac* (PACAP) or *rutabaga* (AC) increase the sensitivity to ethanol, double mutants that lack both *amnesiac* (PACAP) and *rutabaga* (AC) are not significantly different from wild-type controls. If the inability to transmit a cAMP signal causes increased sensitivity to ethanol, these mutants should certainly be as sensitive as those that lack *amnesiac* (PACAP) or *rutabaga* (AC). This observation would certainly argue against a simple model in which cAMP up-regulation determines ethanol sensitivity. Second, blocking PKA activity in *amnesiac* (PACAP) flies partially reverted the increased ethanol sensitivity associated with loss of *amnesiac* (PACAP). Again, affecting two positive elements in the signaling pathway suppresses the phenotype caused by each separate alteration. A simple explanation is that elevation of cAMP is not sufficient, but that a cAMP signal must be transmitted. This hypothesis is somewhat supported by the observation that *dunce* (PDE) mutants (which have elevated levels of cAMP), as well as double mutants for *dunce* (PDE) and *amnesiac* (PACAP), do not exhibit an altered sensitivity to ethanol when compared to wild-type controls. Alternatively, it is possible that an even more complex network regulating the various proteins involved in the cAMP pathway underlies the ethanol-sensitive phenotype. A detailed understanding of this regulatory network will probably be required before we can interpret these data in a meaningful way. If the cAMP signaling network is as intricate as that observed in the social amoeba *Dictyostelium* (for review, see Loomis, 1998), many important regulatory steps could interfere with the resistance to ethanol. Given the wealth of mutants and the detailed knowledge of cAMP signaling in *Dictyostelium*, it may be worth studying the sensitivity and adaptation to ethanol in this species as well.

Implications for Further Studies

It is interesting to compare the observations described by Moore et al. (1998) with our current knowledge of cAMP pathways and addiction. It is striking that all the genes thought to be required for the modulatory up-regulation of the cAMP pathway, *amnesiac* (PACAP), *rutabaga* (AC), and *DCO* (PKA-C) were previously implicated in learning and memory. The proteins encoded by *rutabaga* (AC) and *DCO* (PKA-C) are known to be expressed at significantly higher levels in the mushroom bodies of the flies than in other brain cells. These organized neuronal structures are the centers for olfactory learning and memory (Davis, 1996) but do not seem to be the locus of increased sensitivity to ethanol. Indeed, flies that lack mushroom bodies do not display an increased sensitivity to ethanol (Moore et al., 1998). Hence, other neurons or cells of the fly are probably involved in the acute response to ethanol. Since many of the genes involved in cAMP regulation are expressed in many tissues, those that may be expressed in specific cells of the nervous system, e.g., *amnesiac* (PACAP), may provide very useful information about the site of action of the cAMP pathway.

A PACAP-like neuropeptide has been shown to be involved in synaptic transmission in *Drosophila*. This peptide coactivates the *Ras/Raf* and *rutabaga* (AC) pathways, suggesting that upon activation of a G protein–coupled receptor, two pathways are activated. The pathways then converge to modulate K^+ channel activity, leading to an efflux of K^+ (Zhong, 1995). Since *amnesiac* encodes a PACAP-like peptide, it will be of interest to determine if the gene product of *amnesiac* activates these two pathways, and if the *Ras/Raf* pathway plays a role in ethanol sensitivity as well. If *amnesiac* (PACAP) is indeed involved in regulating K^+ efflux in specific neuronal populations, it may play a key neuromodulatory role.

The cAMP pathway has been implicated in forms of addiction other than alcoholism. Chronic administration of opiates leads to up-regulation in the cAMP pathway in cultured cells, whereas acute exposure inhibits the cAMP pathway (Nestler and Aghajanian, 1997). Hence, the same pathway seems to respond differently to chronic administration of opiates and alcohol. McClung

and Hirsh (1998) have recently initiated studies to determine the effects of cocaine in flies. Cocaine administered in volatile form induces multiple responses that resemble the behaviors seen in rodents, including sensitization. These authors also propose to initiate genetic screens to isolate mutants with altered responses to cocaine. It will be interesting to see how all these fly mutants respond to different addictive drugs, and if common pathways are involved.

While the study by Moore et al. (1998) focuses on the acute responses to ethanol, it is likely that chronic exposure to lower levels of ethanol, or intermittent exposure to acute doses of ethanol, induces pathophysiological changes that can also be measured in flies. In vertebrates, chronic exposure of cells to ethanol causes the opposite effect as the acute response: i.e., a downregulation of the cAMP pathway. If so, the same genes that affect the cAMP pathway in the acute response may play an important role in chronic responses, and the inability to modulate the cAMP levels in the mutants may affect various behaviors. For example, *dunce* (PDE) females have been shown to cause sexual hyperactivity in males, and *dunce* (PDE) flies exhibit reduced longevity in both sexes (Bellen and Kiger, 1987). In addition, one would predict that *dunce* (PDE) flies would be more resistant than other mutants to chronic exposure to ethanol as their cAMP levels may only be partially downregulated. It will thus be of interest to compare some of the behavioral phenotypes displayed by the cAMP mutants upon chronic exposure to ethanol.

Finally, this study provides an important glimpse at the potential genetic factors that may be implicated in alcoholism. Previous studies with wild-type fruit flies had shown that using the inebriometer, one can select for flies that are much more resistant to ethanol knockdown, e.g., from a mean elution time (MET) of 12.6 min in generation 1, to a MET of 34.6 min in the 20th generation, an increase of a factor of 2.7 (Cohan and Hoffmann, 1986). Hence, it is likely that multiple genetic components underlie the sensitivity to ethanol. Similarly, humans are known to have very different abilities to resist ethanol, and as pointed out earlier, those who are more resistant are much more likely to become alcoholics (Schuckit, 1994). In addition, alcoholism clearly has a hereditary component, but despite compelling evidence that genetic factors are involved in alcoholism, no genes responsible for this disease have been identified. Given the data presented by Moore et al. (1998), it will be interesting to sequence the genes encoding some of the ACs, PKAs, and PDEs in selected flies and specific humans, and to determine if polymorphisms exist that may correlate with the predisposition to become more resistant to ethanol intoxication or alcoholism.

In summary, the most exciting feature of the study by Moore et al. (1998) is that it lays the foundation for a genetic approach to dissect the acute, and possibly the chronic, effects of ethanol in vivo. The fruit fly may pave the way for an in-depth study of many of the genes involved in acute and chronic effects of ethanol, as well as other drugs like cocaine (McClung and Hirsh, 1998). These studies are therefore likely to foster many other studies in other model organisms that are amenable to genetic analysis as well as studies with cultured cells. These novel data should increase our understanding of the molecular mechanisms causing drug abuse and addiction in the near future.

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