Camouflage response in zebrafish
A model for genetic dissection of molecular and cellular circuitries underlying alcoholism

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Ethanol is a widely abused drug. Sensitivity to its acute effects has been strongly correlated with the risk of developing alcoholism in humans. Despite the importance of genetics in human alcoholism and alcohol-related diseases, the identification of genes has been difficult due to the complex nature of these disorders. We recently demonstrate that genetic screening using a simple and tractable ethanol-sensitive camouflage response in zebrafish can efficiently uncover genes that alter ethanol-modulated behaviors similarly observable in mammals. We isolated the fantasma (fan) mutant, and showed that it not only disrupts ethanol-modulated camouflage response, but also impairs behavioral sensitivity to ethanol. fan encodes the evolutionarily conserved adenyl cyclase 5 (AC5) that regulates the phosphorylation of extracellular-signal-regulated-kinase (ERK) in the brain. Pharmacological perturbation of the phosphorylation of ERK uncovered that it is a critical “gatekeeper” of behavioral sensitivity to ethanol. Therefore, ethanol-modulated camouflage response screen is a powerful system for molecular genetic dissection of neural circuits that are sensitive to ethanol. We propose that polymorphisms in ac5 or genes of the ERK signaling pathway may contribute to susceptibility of alcoholism and alcohol-related medical disorders in humans.

People drink ethanol, largely because at low concentrations it induces euphoria, relaxation, disinhibition, and reduces stress and anxiety. These “pleasurable” effects of ethanol are thought to be a major trigger for alcohol abuse ultimately progressing to alcoholism, a leading cause of morbidity and mortality worldwide.1,2 At high but still physiologically relevant concentrations, ethanol has neuro-depressive effects. Sensitivity to the acute intoxicating effects of ethanol is strongly correlated with the risk of developing alcoholism in humans.3 Moreover, it has been widely accepted that genetics plays important roles in the development of alcohol use and abuse.4,5 Despite the knowledge on clinical effects of ethanol, the understanding of molecular and cellular mechanisms underlying neural sensitivity to ethanol remains limited. Animal models from rodents to nematodes and behavioral assays of complex (e.g., self-administration) or simple (e.g., locomotor sensitivity) nature have been employed to study the mechanisms.8 In particular, unbiased forward genetic screens using locomotor behavior as a readout have been carried out in invertebrates such as Caenorhabditis elegans6 and Drosophila melanogaster.7 Although locomotor behavior is relatively simpler than some complex paradigms such as self-administration, it is still significantly complex, making it challenging for screening and subsequent identification of molecular and cellular mechanisms, particularly in vertebrates.

The Zebrafish is a vertebrate model organism that is amenable to molecular genetic analysis and has rather similar gene composition and organ systems to mammals.9-11 In an attempt to develop a zebrafish model for alcoholism, we noted that in addition to displaying behavioral

Key words: alcoholism, genes, neural circuits, zebrafish, camouflage response, adenyl cyclase, extracellular-regulated kinase (ERK), behavior, ethanol

Submitted: 07/10/09
Accepted: 07/10/09
Previously published online: www.landesbioscience.com/journals/cib/article/9488
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Addendum to: Peng J, Wagle M, Mueller T, Mathur P, Lockwood BL, Breitau S, et al. Ethanol-modulated camouflage response screen in zebrafish uncovers a novel role for cAMP and extracellular signal-regulated kinase signaling in behavioral sensitivity to ethanol. J Neurosci 2009; 29:8408-18; PMID: 19571131; DOI: 10.1523/JNEUROSCI.0714-09.2009.
When the phosphorylation of ERK was partially inhibited in wildtype zebrafish, it mimicked the reduction in sensitivity to stimulatory effects of ethanol observed in the fan mutant. Moreover, strong inhibition of phosphorylation of ERK rendered a stimulatory dose of ethanol sedating (Fig. 2). These results identify ERK signaling as a critical downstream effector of cAMP signaling in regulating behavioral sensitivity to ethanol.

While previous studies have shown a role of cAMP signaling in suppressing behavioral sensitivity to ethanol,7,18 our findings reveal a novel, isoform-specific role of AC signaling in promoting ethanol sensitivity, and suggest that behavioral sensitivity to ethanol is not simply correlated with cellular cAMP levels. This conclusion is particularly important when considering the use of pharmacotherapy to target cAMP signaling to thereby modulate behavioral sensitivity to ethanol, as it uncovers the in vivo complexity of the involvement of cAMP signaling in ethanol sensitivity to ethanol observed in mammals.12-14 zebrafish exhibited an altered camouflage response upon acute exposure to ethanol (Fig. 1). Camouflage response does not occur in humans but is a fascinating property of many aquatic and terrestrial animals and is critical for their foraging, anti-predator behavior and social communication. Camouflage largely manifests as a cellular behavior composed of movement of organelles named melanosomes in neural crest-derived melanocytes in response to environmental signals.15,16 Pharmacological studies, mainly carried out in culture systems, have implicated the involvement of sophisticated neuro- and endocrine-systems [e.g., hypothalamus, pituitary, GABA, dopamine, neuropeptide Y, corticotropin releasing hormone (CRH), and serotonin] in regulating this process.15,17 However, genes and neural circuits that regulate the camouflage response in vivo are poorly understood.

We demonstrated that ethanol modulates the camouflage response by stimulating the dispersal of melanosomes in melanocytes (Fig. 1). A genetic screen using the ethanol-modulated camouflage response assay led to the isolation of the fantasma (fan) mutant, which failed to disperse melanosomes resulting in a “ghost”-like light appearance in the presence of ethanol. While the fan mutants displayed overall normal morphology and neuronal patterning and are adult viable and fertile, behavioral characterizations revealed that the mutant animals displayed reduced sensitivity to ethanol’s stimulatory activity, and recovered faster from ethanol-induced sedation. These observations indicate that ethanol-modulated camouflage response screen can recover mutants that also display other behavioral sensitivity to ethanol.

When the phosphorylation of ERK was partially inhibited in wildtype zebrafish, it mimicked the reduction in sensitivity to stimulatory effects of ethanol observed in the fan mutant. Moreover, strong inhibition of phosphorylation of ERK rendered a stimulatory dose of ethanol sedating (Fig. 2). These results identify ERK signaling as a critical downstream effector of cAMP signaling in regulating behavioral sensitivity to ethanol.

Through positional cloning, we showed that the fan mutant disrupts the evolutionarily conserved adenylyl cyclase 5, which is expressed in both the brain and melanocytes. For ethanol-modulated camouflage response, Fan/AC5 was needed in melanocytes to promote melanosome dispersal. For behavioral sensitivity to ethanol, Fan/AC5 was required to promote the phosphorylation of extra-cellular signal regulated kinase (ERK) in the brain.
sensitivity. It also highlights the importance of understanding molecular signaling mechanisms in the context of specific neural circuits and genetic pathways.

It is intriguing that decreasing the phosphorylation of ERK did not affect basal activity but reduced the stimulatory effect and enhanced the neuro-depressive effect of ethanol. How does phospho-ERK act as a "gatekeeper" to distinct behavioral responses to ethanol? In order to answer this question, it is critical to know which cell types and what target molecules the ERK signaling pathway acts upon in regulating behavioral sensitivity to ethanol.

The amenability to molecular genetic, cellular and pharmacological experiments makes the camouflage response in zebrafish a powerful model system for understanding the development and function of a neural circuit at both molecular and cellular levels. Because of involvement of the hypothalamus, pituitary, and various neurotransmitter/neuropeptide systems, understanding of the camouflage circuit may shed important light on more complex emotion- and motivation-related behaviors that engage similar genes or neural systems. Moreover, its sensitivity to ethanol and possibly other psychoactive drugs provide a salient venue to ask how these substances act at molecular and cellular levels.

Acknowledgements

This work was supported by NIH Grant AA016021, David and Lucile Packard Science and Engineering fellowship, and award from the Sandler Family Foundation. We thank Peter Lu for composing the image used in Figure 1.

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