

Project 7: Ethanol, Stress and Dopamine

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A. SPECIFIC AIMS

We propose to examine the impact of chronic ethanol exposure and withdrawal stress on the DA system of genetically defined mice. A repeated ethanol withdrawal paradigm developed within the INIA-Stress Consortium has been shown to increase drinking in C57Bl/6 (C57) mice, and we hypothesize that DA system function is reduced following this paradigm. Because of the central role that the DA system plays in the neurobiology of addiction, it is vital to understand the effects of ethanol exposure and withdrawal on the DA system. **Our general hypothesis is that low endogenous DA function in brain predisposes mice to high alcohol preference and consumption, and further that chronic ethanol exposure and withdrawal decreases DA function.**

We will use microdialysis in freely moving mice and cyclic voltammetry in brain slices to examine the function and dynamics of the DA system in detail. The brain areas to be examined were chosen by the consensus of INIA investigators who will interact with this project. They include the nucleus accumbens (NAc, core and shell regions), basolateral amygdala (BLA) and ventral tegmental area (VTA). These experiments will be performed first on inbred "alcohol-preferring" C57BL/6J (C57) mice and "alcohol-avoiding" DBA/2J (DBA) mice. We will then examine mice created or characterized by INIA consortium projects and cores with targeted deletions of genes, extreme gene expression patterns related to the DA system and extreme phenotypic responses to ethanol or stress effects.

Specific Aim 1: Determine the effects of repeated ethanol exposure and withdrawal (2 cycles) on drinking behavior and DA function in the brains of C57 and DBA mice. We postulate that C57 mice are hypo-dopaminergic relative to DBA mice, and this low endogenous DA function predisposes C57 mice to high alcohol preference and consumption. Since the effect of ethanol exposure and withdrawal is decrease DA system function, we anticipate that DBA mice will have low DA, and become more like C57 mice after ethanol exposure. This will be manifested in drinking patterns, D2 type DA autoreceptor sensitivity and DA response to acute ethanol challenge. Microdialysis will measure in vivo extracellular levels of DA in the NAc and voltammetry will be used to measure stimulated release, uptake and D2-type DA autoreceptor parameters in the NAc core and shell regions, BLA and VTA.

Specific Aim 2: Examine the responses of the 22TNJ mutant mouse strain generated by the INIA consortium (**Dr. Goldowitz's project**) to the two cycle ethanol exposure paradigm described above. The same brain regions and types of experiments described in Aim 1 will be used. We hypothesize that the 22TNJ mice already have low-functioning DA systems and will show blunted DA system changes in response to ethanol exposure and withdrawal relative to the control TNH mice.

Specific Aim 3: Examine the influence of an inducible 5-HT1A receptor knockout generated by **Dr. Delpire's Mouse Knockout Core** on DA system function at baseline and following two cycles of ethanol exposure and withdrawal. This knockout will be used as a high-anxiety mouse model. The same DA parameters will be measured in induced and non-induced 5-HT1A receptor knockout mice (5-HT1A KO) as in the inbred mouse strains in Specific Aim 1. Our hypothesis is that induced 5-HT1A KO mice are also a model of low DA system function and will show blunted DA changes.

Specific Aim 4: **A)** Test predictions of high or low DA system function based on mRNA expression levels of DA-related genes across recombinant inbred strains of mice (C57BL/6J X DBA/2J, or BXD) to be selected within **Dr. Robert Williams' project**. **B)** Examine the DA system in interesting ENU-mutated mice generated by **Dr. Dan Goldowitz's project** and screened for extreme ethanol or stress-related behaviors in the Behavioral Phenotyping Service Core under the direction of Dr. Howard Becker.