

Project 6: Genomics Analysis of Social Stress and Individual Variation in Ethanol Drinking

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A. Specific Aims

Recent studies in our laboratory on ethanol 2-bottle choice drinking with C57BL/6 inbred mice have documented exceedingly consistent, substantial individual variation in daily drinking behavior. Preliminary data suggests that this individual variation in ethanol drinking is perhaps induced by environmental factors such as formation of social hierarchy. This variation in drinking is not seen for other tastants (quinine, saccharin) or total fluid intake. Initial microarray studies done on individual animals have identified expression patterns highly correlated with this drinking behavior. It is our hypothesis that stress (isolation, social hierarchy) has created long-lasting signaling alterations that influence drinking behavior in these genetically identical animals. This offers a unique opportunity to finely dissect molecular mechanisms underlying difference in drinking behavior generated by gene- environment interactions, particularly in regard to social interactions. The purpose of this project is to use behavioral assays, pharmacology, genetics and expression profiling to identify a network of brain region-specific genes functional in modifying individual variation in ethanol consumption. We predict our results will partially overlap with drinking behavior-related gene networks identified by “traditional” genetic approaches, as well as possibly identify novel modulators of drinking behavior not discernable in studies on populations.

Our specific aims:

Aim 1: Characterize behavioral conditions affecting individual differences in drinking behavior. Preliminary studies suggest that environmental factors such as formation of new social hierarchies (Prelim. Results, Fig. XXX) strongly influence individual behavior in 2-bottle choice drinking within an inbred mouse strain. Litter-effects (including maternal rearing) may also contribute significantly to the drinking behavior variation. This aim will characterize factors influencing individual variation in drinking behavior, providing behavioral models for behavioral, neurochemical and microarray studies in aims 2-3.

- a. Determine whether individual variation in drinking behavior correlates with litter of origin (ongoing in pilot).
- b. Determine duration of group housing needed to induce maximal variation in drinking behavior (ongoing in pilot).
- c. Determine whether social hierarchy dominance status correlates with drinking behavior (ongoing in pilot).
- d. Determine the persistence of group housing effect on drinking behavior variation.
- e. Determine whether repeated social defeat alters drinking behavior in C57BL/6 mice.

Aim 2: Determine whether individual variation in ethanol drinking behavior is reflected in behavioral or neurochemical measures of anxiety or ethanol anxiolysis.

- a. Determine whether differences in basal anxiety, immobilization-stress induced anxiety or ethanol-stimulated anxiolysis (ongoing in pilot) correlate with individual variation in drinking behavior.
- b. Determine whether neuroendocrine responses correlate with individual variation in drinking behavior. Basal and post-stimulation with ethanol, ACTH, CRF or dexamethasone levels of corticosterone and ACTH will be determined in littermate vs. group housed animals prior, during and after an ethanol drinking phase.
- c. Determine whether individual variation in drinking correlates with measures of helplessness in Porsalt swim test.

Aim 3: Identify expression patterns in brain regions involved in stress or drinking behavior that correlate with individual drinking behavior.

- a. Analysis comparing group housed vs. littermate housed animals prior, during and after ethanol drinking phase.
- b. Compare patterns following various durations of social environment (depending on results in Aim1).
- c. Characterize gene subnetworks from microarray studies by bioinformatic analysis (Bioinformatic Core) including clique analysis (M. Langston), ontological discovery analysis (E. Chesler), literature associations, pathway/network over-representation (INIA-WebGestalt) and linkage of basal expression with drinking behavior phenotypes in the WebQTL resource (collaboration with R. Williams).

Aim 4: Validate and characterize targeted gene expression patterns from microarray studies.

- a. Perform microarray studies on select subgroups of BXD recombinant inbred strains based on their possessing high vs. low ethanol drinking and determine covariance with patterns identified in Aim 3.
- b. Q-rtPCR, Western blot, in situ hybridization or immunocytochemistry validation and characterization of candidate genes from microarray studies.
- c. Using Q-rtPCR, determine if brain expression of candidate genes correlates with individual variation in drinking behavior in primates (collaboration with K. Grant) and rats (collaboration with Biggio).

Aim 5: Validate role of select candidate genes in drinking behavior by using existing genetic/pharmacological reagents or viral vector microinjections to alter function/expression of target genes.

- a. Propagate 5-10 genetrapp lines (Transgenic Core) for candidate genes validated in Aim 4 and test for drinking behavior and social stress responsiveness.
- b. Develop viral vectors for microinjection of candidate genes to alter expression in distinct brain regions.
- c. Have positive genes (genetrapp line or viral-injected) tested for more extensive responses to stress/ethanol (H. Becker, Behavioral Core).