

Project 1: INIA - Robust Systems Genetics of Alcohol and Stress Effects on CNS

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1. SPECIFIC AIMS

Over the past four years we have collaborated with INIA investigators to study and test genetic and molecular causes of differential vulnerability to alcoholism. An important product has been the development of GeneNetwork/WebQTL (GN; Chesler et al., 2004a,c) and the publication of scientific findings related to complex interactions among gene variants and addictive behavior. We have generated a substantial number of resources, including genomic and SNP data sets for ~500 inbred strains of rodents (among them BXD, LXS mice, and HXB rats), and the generation of a greatly expanded panel of BXD strains (50+ new strains; Peirc et al., 2004). We have also built and continue to curate databases of neuropharmacological and ethanol-related trait data sets for major genetic reference panels (GRPs) of rodents—especially the BXD strains and HXB rats that we propose to use in this renewal.

We request continued support to create data sets, build software, and test hypotheses that will be of specific utility to NIAAA and INIA. The aims of Project 2 (Figure 1) have a simple structure: Aim 1: Acquire cross-validated array data for mouse and rat; Aim 2: Generate well-structured models that predict responses to ethanol and stress; and Aim 3: Test these models by working with members of INIA using, for example, repeated withdrawal as a treatment.

Aim 1. Data Generation.

We will generate data and produce cross-validated molecular networks with which to seed work in Aims 2 and 3. In **Aim 1A** we will acquire data on endogenous variation of mRNA expression in four forebrain regions: the dorsolateral bed nucleus of the stria terminalis (dBNST), the basolateral amygdala complex (BLA), nucleus accumbens (NAc), and medial prefrontal cortex (mPFC). We will acquire matched data from two GRPs and two species—BXD mouse recombinant inbred (RI) strains and HXB rat RI strains. We will study complementary lines that differ markedly in genetic structure—fully inbred RIs and non-inbred RI intercross hybrids (RIXs). **Aim 1A** includes a survey of a small number of classical strains—mouse and rat Diversity Panels (MDP and RDP). In **Aim 1B**, we will generate reference data sets on expression using identical methods across 48 or more CNS regions in two mouse and two rat strains—C57BL/6J, DBA/2J, SHR, and

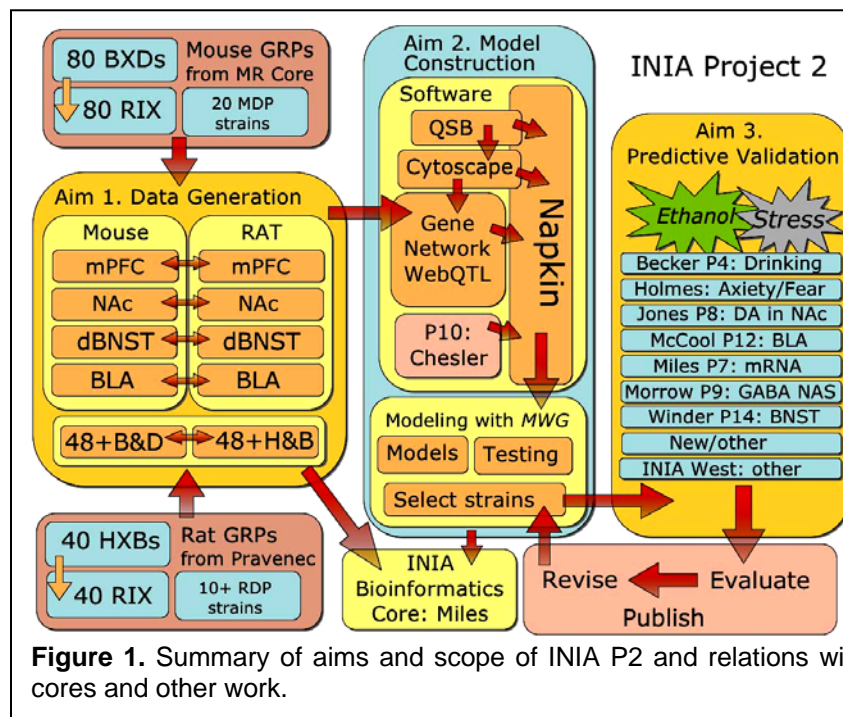


Figure 1. Summary of aims and scope of INIA P2 and relations with cores and other work.

BN—, and both sexes. Data will be made publicly available on GN after quality control.¹

Aim 2. Model Construction: We will assemble statistical models and hypotheses of putative causal relations between gene expression differences, synaptic function, and behavioral responses to ethanol and stress. Models will be compared and cross-validated using data sets generated in Aims 1 and (recursively) in Aim 3. In **Aim 2A**, we will write, test, and distribute an open-source program called *Napkin* (Network Application and Knowledge Integrator) that we will use to draw process diagrams and networks. *Napkin* will be based on the current QSB Java program, but will take advantage of Cytoscape and GeneNetwork code. *Napkin*'s graphical user interface (GUI) will make it possible to construct modular and nested models of networks, including data from Aim 1 on transcript and neurotransmitter systems in interconnected CNS regions. Users will test relations among components and use *Napkin* to identify interesting patterns and strains and to generate predictions of effects and responses of different strains to treatment

In **Aim 2B**, we will use GeneNetwork and *Napkin* to test models in collaboration with the INIA *Models Work Group* (MWG). This group consists of INIA collaborators (Aim 3) and our core informatics group (RWW, LL, DCA, and JLP). Every three months the *MWG* core group will use a number of INIA resources and statistical packages to produce and distribute reports that include testable models based on the cross-validated array data and on many external data sets and publications in the ODS (Project 9). Reports will be posted on the INIA site for critique and extension. Models will be tested in **Aim 3**.

Aim 3. Predictive Validation. In Aim 3 we test hypotheses generated in Aim 2B. Sets of isogenic RI and RIX animals will be provided by the Mouse Resources Core (MRC) to INIA members. The question we intend to answer is: Can we predict responses to ethanol and stress in untested but fully genotyped lines? We will have the benefit of excellent sequence data and detailed transcriptome data for key CNS regions. We also have the advantage of replication; and in the case of the BXD strains, extensive supporting data from many other investigators. Is this information sufficient to predict patterns of use, addiction, withdrawal severity, interactions with stressors, and other behavioral and physiological properties in untested progeny? All experiments will rely on INIA SOPs (in some cases, repeated withdrawal) and be carried out by members of the *MWG* who are skilled experimentalists. Each test will typically involve an analysis of sets of up to 10 animals from 6 to 20 carefully selected RI or RIX lines. We currently have solid plans for several experimental tests. Several tests are actually already in progress with A Holmes and MF Miles. Other INIA collaborations that are part of **Aim 3** are described briefly in Section 4 to provide the Panel with examples of how tests will be implemented.

Completion of the aims will lead to (i) massive and robust public data sets on genetic variation in expression in key forebrain regions in mouse and rat and a long-needed survey of expression in 48 or more CNS regions of major rat and mouse strains; (ii) well

¹ **ABBREVIATIONS (uncommon or non-standard):** BXD: panel of mouse strains; GUI: graphic user interface; GN: GeneNetwork.org; GRP: genetic reference panel; HXB: panel of rat strains; LXS: panel of mouse strains; MBL: Mouse Brain Library; MDP: mouse diversity panel, MWG: Models Work Group; MRC: Mouse Resource Core of INIA; QSB: JAVA program for QTL/SNP/Bayesian network analysis; RDP: rat diversity panel, RI: recombinant inbred; RIX: recombinant inbred intercross; WebQTL: module of GeneNetwork

integrated statistical tools and source code with which a community of neuroscientists can accurately describe molecular, synaptic, and neuronal networks involved in addictive behaviors; (iii) rigorous experimental tests of predictions of physiological and behavioral responses in isogenic rodent lines for which we have superb control over the environment and relatively complete data on genomic structure and gene expression. All resources we generate in this project will be linked and integrated with those generated by other INIA project, particularly the Bioinformatics Core and Project 10 (Chesler).