Network: Systematic Gene Identification and Functional Analyses in Common CNS Disorders

Project: Conditional Genetic Manipulation of Candidate Genes Involved in Alcohol Drinking, Stress- induced Alcohol Drinking and Relapse: Role of L-type Voltage-gated Calcium Channels

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Introduction

Drug and alcohol abuse is a major burden to our society. Epidemiological studies indicate that the risk factors of an individual to a drug addiction are about 40-60% determined by genetic factors. Therefore, the identification of vulnerability genes and key regulatory pathways involved in the different phases of alcohol addiction would lead to a better understanding of the disease and to potential cures.

Animal models provide unique opportunity to study the role of gene expression in different phases of alcoholism. Established rat drinking paradigms model different phases of human alcohol dependence such as acquisition and maintenance of alcohol drinking, compulsive alcohol-seeking behavior and relapse. To identify genes with altered expression in different phases of addiction, expression profiling studies are being performed worldwide. To further study and validate the causal role of individual genes in these processes, transgenic rats that allow both the spatial (brain region affected) and the temporal (phase of alcohol dependence) regulation of the expression of the candidate gene are necessary.

We have therefore decided to develop rat transgenics, based on the tetracycline inducible tet- system, which allows inducible and dose dependent regulation of gene expression by feeding animals doxycycline. We focus primarily on the role of L-type voltage-gated calcium channels (LVGCCs) in alcohol drinking and relapse behavior. LVGCCs together with NMDA and AMPA glutamate receptors are major gates of Ca^{2+} influx upon neuronal stimulation and key molecules in calcium-mediated synaptic plasticity. Blockade of LVGCCs by the dihydropyridine (DHP) antagonists attenuates the reinforcing stimulus effect of ethanol and reduces alcohol intake in free choice drinking. Prolonged ethanol administration up-regulates LVGCCs expression in the rat brain. In parallel, chronic administration of DHP antagonists prevents this up-regulation and reduces ethanol tolerance and ethanol withdrawal syndrome. Thus, up-regulation of LVGCCs expression may be critical for alcohol-related plastic changes in the brain and potential target for pharmacological intervention. Chronic administration of DHP LVGCCs antagonists produces large side effects, mainly by lowering blood pressure. To test the causal role of LVGCCs up-regulation in alcohol phenotypes, we therefore genetically and conditionally manipulate the expression of $Ca_V1.3$ α subunit in the rat forebrain in different phases of alcohol dependence.

Project Status

We have created several rat (SD) lines with the tTA activator expression targeted to the rat forebrain. Using large BAC with the CAMKII regulatory sequences, we target tetracycline activator, and thus doxycycline regulated transgene expression to the forebrain. By breeding the tTA rats with rats carrying the $Ca_V1.3$ α subunit under tetO control LVGCC expression can be switched on or off during the drinking experiment.

To generate rats with tightly controlled transgene expression from the tetO regulatory sequences we have optimized DNA transgenic vectors. The expression of transgenes from the tetO sequence in rat is regulated by the tTA activator, but is also dependent on its (random) integration in the genome of the transgenic animal. We have modified previously identified locus LC1, cloned in BAC, which allows tight control of tetO driven transgenes in mice. In rats, candidate genes expressed from such tightly controlled locus should allow position independent regulation of transgene expression.

To test the LC1-BAC technology and to optimize the doxycycline administration for regulating transgene expression, we have first generated two rat transgenic reporter lines with bicistronic luciferase and GFP reporter genes regulated by a single tetO sequence. Preliminary data indicate that the random integrated LC1-BAC in transgenic rats allows tight control of reporter expression comparable to the control in mice.

The doxycycline administration in drinking water is an efficient way of assuring steady concentrations of doxycycline to the brain of transgenic animals. Unfortunately, the low blood-brain permeability of doxycycline currently limits the use of the inducible rtTA in the brain. We have therefore tested novel tetracycline derivatives and administration protocols to enhance doxycycline concentration in the brain. Our data indicate that at least fifty fold enhancement can be achieved in mice. We hope that similar enhancement can be achieved in transgenic rats as well.

Finally, we have cloned the $Ca_V1.3$ α LVGCC subunit into the optimized LC1-BAC and performed pronuclear injections. The transgenic animals are currently analyzed.

Outlook

We have generated transgenic rats wit components of the tetracycline-inducible system. We hope that by establishing efficient transgenic technology for generation of transgenic rats with tightly regulated, tissue specific expression of target genes we will make the unique rat physiological model accessible for genetic manipulations and thus available for neurobiological, behavioral, pharmacological and genetic studies of the role of genes in drug abuse and alcoholism.