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# Genetic Approaches to Understanding Brain Functions

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A major aim of modern biology is to understand how normal gene activities give rise to the structure, function, and behavior of complex organisms. Such knowledge will eventually lead us to understanding the mechanisms of diseases. Current technologies of gene manipulations fortunately allow making a specific change in a given gene *in vivo*. A gene targeting experiment begins with the generation of targeting vector to modify a cloned gene (usually to stop the function of the gene) by using the recombinant DNA technologies. Next, the targeting vector is introduced into mouse embryonic stem cells, which are totipotent and can be used to generate live animals. Mutant mice generated through gene targeting therefore allow in mammals to study the function of a specific gene in the context of a whole organism. These approaches have been successfully applied to studies of diverse questions in biomedical sciences, including cancer, development, immunology, and lately brain functions and dysfunctions. Here, the practical aspects of gene-targeting experiments will be briefly discussed with some examples from my own groups. I will particularly talk about works on the calcium channel gene mutations related to the sensory gating, consciousness, and absence seizures.

Voltage-dependent Ca2+ channels are involved in diverse cellular functions. There are multiple types of Ca2+ channels encoded by different genetic loci. Furthermore, alternative splicing of transcripts from each locus produce a vast array of different channel products, which suggest a level of complexity in the regulation of Ca2+ channel functions. Today I will focus my talk on two of these Ca2+ channel types, a1A and a1G, and their roles in the pathogenesis of absence seizures.

Complex interactions among multiple components contribute to the genesis of absence seizures, which are characterized by a brief loss of consciousness associated with an EEG recording of bilaterally synchronous spike-and-wave discharges (SWDs). We tried to define the role of calcium channels in the SWDs seizures by utilizing knockout mice for different calcium channel genes. First, we found that knockout mice for the a1A subunit of high-voltage-activated (HVA) Ca2+ channels (a1A-/-)

show 3-Hz SWDs with behavioral absence seizures. In an effort to identify the cellular mechanisms underlying the absence seizures, we examined the thalamic relay neurons by electrophysiological means. Whole-cell patch clamp analysis showed that low-voltage-activated (LVA) T-type calcium currents were increased whereas HVA currents were reduced in the mutant neurons, suggesting a role of T-type calcium channels in the genesis of absence seizures. To test this question, we generated a knockout of the a1G subunit of T-type channel (a1G-/-). The a1G-/- mice were resistant to the generation of SWDs in response to GABAB receptor activation. To examine gene interactions between the two isotypes, double mutants for the two channels were obtained. EEG analysis showed that SWDs of a1A-/- disappeared in the double mutants, indicating a genetic suppression of a1A-/- absence seizures by a1G-/-. These results indicate that the increase of T-type calcium currents in the thalamocortical neurons is the primary pathogenic component in the genesis of absence epilepsy in vivo, and provide insights into possible treatment strategies for absence epilepsy.