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Molecular and genetic approaches in the study of neurobiology

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The enormous advances in molecular biology have added a new perspective to the genetic analysis of nervous system function, the generation of mouse lines with selective modification of genes suspected to play a role in nervous system function or development. This lecture will review established techniques that are widely used in neurobiology, and close with an outlook on newer techniques that are about to be introduced and may dominate the field in the years to come.

Insertion of DNA-constructs at random position by pronuclear injection in oocytes and targeted modification of specific loci by homologous recombination in embryonic stem cells were introduced more than a decade ago and are now well established techniques. Pronuclear injection is used to overexpress endogenous genes, to introduce disease-associated alleles of human genes, or to express dominant negative protein variants. Homologous recombination in embryonic stem cells is most often used to delete genes (knock out), but may also serve to introduce alleles with modified function (knock in). Homologous recombination in embryonic stem cells produces constitutive mutations, permitting neither spatial nor temporal control of the altered gene expression. This disadvantage can be circumvented by the more recently introduced technique of conditional gene knockouts that exploit the phage-derived Cre-loxP recombination system. In one mouse line, the target gene is flanked by loxP recognition sequences using homologous recombination. The Cre recombinase gene is combined with a promoter that directs expression to the tissues of choice and is introduced into a second mouse line by pronuclear injection. The two lines are crossed to create a double mutant line where the target gene will be permanently removed only in those cells that express the Cre recombinase. The aim of more recent developments using viral gene transfer, drug controlled transactivator systems, or RNA interference is to achieve even more precise spatial control over changes of gene expression and to create reversible mutations than can be turned on or off at will.