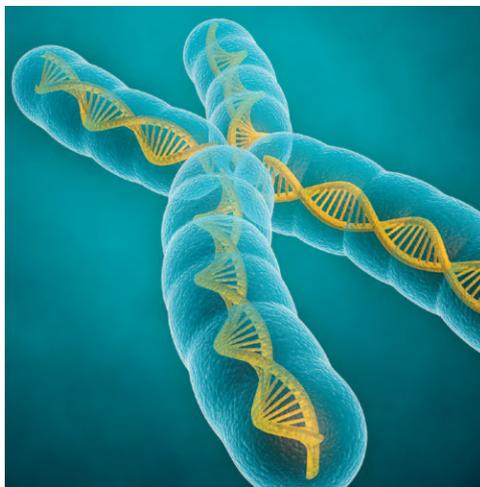


The Expanding Genetic Toolkit for Exploring Mechanisms of General Anesthesia

SERIOUS scientific efforts aimed at understanding how general anesthetics produce their powerful effects began soon after the historic demonstration of ether anesthesia on October 16, 1846 in Boston, Massachusetts. Progress was slow for most of the intervening 166 years, but has dramatically advanced during the last three decades, aided by new ideas and the revolutionary techniques of molecular biology. One result of this progress is the report by Zhou *et al.*¹ in this month's ANESTHESIOLOGY, describing studies on ketamine in conditional knockout animals. Here, I briefly sketch how genetic techniques have spurred our understanding of general anesthetic mechanisms.

The basic processes underlying all genetic manipulations include cloning, amplification, and editing of DNA, and reprogramming of targeted cells with this exogenous genetic material (transgenes), using delivery vectors such as plasmids and viruses. In the 1980s, a time when many laboratories were first adopting basic molecular biology techniques, scientists interested in molecular targets of anesthetic drugs were shifting their attention away from lipids and toward proteins.² The search for targets largely focused on ion channel proteins that control the activity of electrically excitable cells in muscle and the nervous system. Traditional pharmacological studies in native cells and tissues required a cocktail of inhibitors to isolate the activity of one channel among a plethora of others. Using molecular tools, scientists could heterologously express genes encoding ion channels or mixtures of their subunits, in electrically quiet cells. This approach facilitated the testing of many potential general anesthetic target channels, including many new receptor subtypes that were also discovered through genetic sequencing.

From these studies we learned that some anesthetics act more selectively than others and that different anesthetics



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affect different sets of ion channels.³ For example, etomidate specifically and stereoselectively modulates γ -aminobutyric acid type A (GABA_A) receptors containing β 2 or β 3 (but not β 1) subunits.⁴ Ketamine was found to have no effect on GABA_A receptors, while stereoselectively inhibiting sensitive glutamate receptors, neuronal nicotinic acetylcholine receptors, and later, hyperpolarization-dependent cyclic nucleotide-gated (HCN) potassium channels.⁵ Notably, volatile inhaled anesthetics, which display low potency and weak or no stereoselectivity, promiscuously affect a wide variety of ion channel targets.

Genetic approaches also enable scientists to replace native genes in germ cell lines. This results in transgenic animals for testing a potential target's role in anesthetic actions. In mice, both knockin and knockout transgenic approaches have been applied to these ends. The most specific inferences are based on knockin studies, wherein the target gene product, such as a critical ion channel subunit, is mutated in a way that confers known molecular effects. A remarkably successful example of these approaches is the GABA_A β 3N265M transgenic mouse line.⁶ The β 3N265M mutation reduces sensitivity to etomidate, propofol, and other anesthetics in mammalian receptors, without affecting responses to GABA.⁷ Knockin mice harboring β 3N265M mutations require dramatically increased doses of etomidate, propofol, and pentobarbital to achieve a standard sedative-hypnotic effect, loss of righting reflexes, yet are otherwise indistinguishable from wild-type animals. Unlike knockins with specific mutations, global knockout animals incorporate transgenes that totally eliminate functional expression of target proteins by deletion or severe truncation of the normal gene. In the absence of a suitable mutation for a knockin, Chen *et al.*⁵ created HCN1 global knockout (HCN1^{-/-}) mice. The HCN1^{-/-} mice required

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- ◆ This Editorial View accompanies the following article: Zhou C, Douglas JE, Kumar NN, Shu S, Bayliss DA, Chen X: Forebrain HCN1 channels contribute to hypnotic actions of ketamine. ANESTHESIOLOGY 2013; 118:785–95.

approximately twice the IV ketamine dosage that produced loss of righting reflexes in wild-type littermates.

Conditional knockouts and knockins provide additional spatiotemporal control of genetic modifications, enabling experiments that probe neural systems levels between molecules and whole organisms. This is achieved by delivering a transgene in a way that its incorporation depends on specific “promoters,” genetic elements that control where and when genes are expressed during development. For example, the *Gfap* promoter couples transgene expression to that of glial fibrillary acid protein, a protein marker for glia, whereas the *Chat* promoter directs transgene expression to cells expressing cholineacetyl transferase, found in cholinergic neurons. The tissue and cell-type specificities of promoter elements vary. In this month’s report by Zhou *et al.*,¹ the promoter element that guided knockout of the HCN1 gene product was borrowed from calcium-calmodulin-dependent kinase II- α , which is expressed in forebrain but not hindbrain structures of adult mice. The resulting transgenic animals express HCN1 in cerebellum, but not in hippocampus, cortex, and other forebrain structures. The HCN1 conditional forebrain knockout reduces ketamine sensitivity (increases ED₅₀) by approximately 30%, an effect smaller than that in the global knockout. This new result suggests that forebrain HCN1 channels contribute to ketamine-induced hypnosis, but does not rule out significant contributions by hindbrain HCN1 channels or by other channels affected by ketamine. Forebrain-specific conditional knockouts of the GABA_A α 1 and β 3 subunits have also been created and studied for effects on anesthetic sensitivity.^{8,9} A role for forebrain structures in anesthetic loss of righting reflexes is also supported by etomidate studies in GABA_A β 3 conditional knockouts.⁹

The strength of inferences drawn from transgenic animal experiments also depends on other study design factors and results. Controls must be performed to demonstrate that the transgene is expressed with the correct spatial and/or temporal pattern. It is also important to demonstrate that the expected transgenic phenotype is observed at the cellular level, because other subunits may replace a knockout target, restoring function, whereas knockin mutations may alter cellular expression of the target. Ideally, motor strength, coordination, and pain transduction are unaltered by transgenic manipulations, as these can indirectly alter results of anesthetic sensitivity testing. Zhou *et al.*¹ present adequate control data demonstrating the expected distribution of HCN1 protein and the expected loss of neuronal sensitivity to ketamine. Furthermore, stronger inferences are drawn from loss-of-sensitivity than from gain-of-sensitivity results. Both the β 3N265M knockin and HCN1 knockout mice show reduced sensitivity to etomidate and ketamine, respectively. Increased sensitivity to various anesthetics, as seen in knockouts of dopamine hydroxylase¹⁰ and a critical mitochondrial complex I component,¹¹ is unlikely due to altered sensitivity in specific anesthetic targets.

Drug selectivity and quantitative considerations are also critically important. Threshold loss of righting reflexes dosing estimates by Liao *et al.*¹² indicate that β 3N265M knockin animals require approximately fivefold higher etomidate doses than wild-type, indicating a dominant role of GABA_A β 3 subunits in etomidate-induced hypnosis. In contrast, single-gene modifications have produced only modest loss of sensitivities (<50% increase in ED₅₀) to hypnosis and immobility by volatile anesthetics, perhaps because these effects are mediated through multiple targets. What about other potential targets for ketamine, such as *N*-methyl-D-aspartate receptors? Chen *et al.*⁵ argue that the case for HCN1 is stronger than the case for *N*-methyl-D-aspartate receptors, because the HCN1 knockout has no effect on etomidate sensitivity (but does decrease sensitivity to propofol, which inhibits HCN1), whereas global knockout of the *N*-methyl-D-aspartate receptor ϵ 1 subunit moderately reduces ketamine sensitivity, but also reduces sensitivity to pentobarbital, propofol, and benzodiazepines, all thought to act primarily *via* GABA_A receptors.¹³ Perhaps both channels contribute to anesthetic effects of ketamine.

Transgenic technologies continue to evolve. The relatively young field of optogenetics combines targeted neuronal expression of light-sensitive cation and anion channels with fiberoptically applied pulses of light, enabling unprecedented spatiotemporal control of neuronal activity.¹⁴ These approaches will undoubtedly help in revealing the inner workings of the “black box” that remains between our understanding of molecular targets of anesthetics and their profound behavioral effects.

Stuart A. Forman, M.D., Ph.D., Harvard Medical School, Boston, Massachusetts, and Department of Anesthesia Critical Care & Pain Medicine, Massachusetts General Hospital, Boston, Massachusetts. saforman@partners.org

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All Saints' Day of 1918 at Falzè di Piave: Lt. Paul Meyer Wood



As does the Po River to the south, Italy's alpine Piave River (to Venice's north) drains into the Adriatic Sea. A Columbia University medical student and future anesthesiologist, Lt. Paul Wood, served along the Piave as a commanding officer (C.O.) for his 557th Section, U.S. Army Ambulance Service, which was attached to the 22nd Army Corps of the 8th Italian Army. Pausing on November 1, 1918 in Falzè di Piave while besting the Austro-Hungarian forces during the Battle of Vittorio Veneto, the following "22nd C.d.A., 8^{vo} Armata" officers and support personnel assembled (*left-to-right above*): the 4th Field Hospital's C.O., Lt. Col. Gennatazio; the Sanitary Services Director, Col. Abelli; Lt. Amato (Abelli's adjutant); C.O. Maj. Gen. Vaccari; Lt. Paul M. Wood; Maj. Ghidoli (Vaccari's adjutant); and the Y.M.C.A.'s Mr. Edwin and Mr. Ray. Having persevered under shellfire, in treating and evacuating casualties, Lt. Wood's entire 557th Section received the Italian War Cross of Merit. (Copyright © the American Society of Anesthesiologists, Inc.)

George S. Bause, M.D., M.P.H., Honorary Curator, ASA's Wood Library-Museum of Anesthesiology, Park Ridge, Illinois, and Clinical Associate Professor, Case Western Reserve University, Cleveland, Ohio. UJYC@aol.com.