Anesthesiology 2009; 111:5–7

Anesthetic Sensitivity

Learning to Fly

OUR ever-increasing knowledge of the human genome has revealed the genetic basis underlying many diseases while simultaneously providing us with a greater understanding of normal intraspecies genetic variability. This raises the possibility that, as 21st century physicians, we will be able to practice a personalized form of medicine specifically tailored to the genetic makeup of each patient. One key question for anesthesiology is how clinically relevant is genetic diversity in the day-to-day practice of anesthesia.

In the current issue of the journal, Alone *et al.* studied the effect of genomic copy number variation (CNV) on volatile anesthetic sensitivity.[1] Recent work has demonstrated that CNV is one of the most important determinants of genetic variability in humans.[2] CNV is a type of polymorphism defined as a segment of DNA, measuring several thousand to several million base pairs, for which the number of copies encoded in the genome differs among two or more individuals of the same species.[3] Chromosomal duplications, deletions, rearrangements, and insertions all give rise to CNV. It is thought that predisposition to a growing number of complex diseases might be explained by CNV, including some forms of neuropsychiatric illnesses, malaria, and human immunodeficiency virus susceptibility.[4]

Despite the overall small phenotypic variability in a population's volatile anesthetic responsiveness, reflected by the familiar steep slopes intrinsic to anesthetic dose-response curves, phenotypic variation to anesthetics does exist. This variation arises from both environmental and genetic factors.[5] It is conceivable that CNV might contribute to the diversity in anesthetic responsiveness, including rare phenotypes such as partial resistance or hypersensitivity. Among the many interesting ways such extreme phenotypes could manifest are awareness under anesthesia or delayed emergence. From a practical standpoint, however, genotyping CNV and determining the allelic state of any given individual in human studies remains technically challenging. Therefore, to study the impact of CNV on anesthetic sensitivity, Alone *et al.* have made use of the many advantages available for the *Drosophila* model system.[1]

The fruit fly, *Drosophila melanogaster*, might at first seem like an unusual choice of organism to study a human problem. Most are more familiar with modeling human conditions in mammals. Several tantalizing genetic sites are already known to affect sensitivity in rodents. Mice bred for differential sensitivity to alcohol also display differential sensitivity to a variety of general anesthetics that maps to a locus on the seventh chromosome[6], and a second distinct locus that confers differential sensitivity to isoflurane in inbred mouse strains has also been mapped to the seventh chromosome.[7] However, in the field of behavioral genetics, the fly must not be overlooked. *Drosophila* studies have enabled many advances in neurobiology and behavioral genetics. The fly genome has been completely mapped and sequenced. Moreover, a wide repertoire of genetic tools, including tissue-specific inducible expression, gene knockout, and selective gene rescue are available in flies to assist in unraveling a gene's function. Flies have a complex nervous system, with roughly 100,000 neurons that bestow the ability to sense and react to the world. They exhibit robust circadian rhythms and sleep-wake cycles, and they form spatial and contextual memories.[8–11] Not surprisingly, *Drosophila melanogaster* are also useful in studying the action of volatile anesthetics. At comparable concentrations to those used clinically, volatile anesthetics cause a loss of motor, postural, and escape reflex control in flies.[12–14] Genetic studies in flies have uncovered many genes in which mutations alter anesthetic sensitivity. Of these, the narrow abdomen gene (*na*) in flies deserves special mention. Using a nonbiased forward genetic screening strategy, Nash *et al.* previously discovered a novel nonspecific cation channel, narrow abdomen (*na*) that confers hypersensitivity to halothane in flies.[15] It would be only natural to temper one's enthusiasm about the applicability of isolated findings from a lowly insect to higher mammals. However, the discovery that *na* interacts with an ortholog of the worm *Caenorhabditis elegans*' *unc-79* gene and that mutations in either flies or worms affecting either gene partner significantly affect the organism's anesthetic sensitivity suggest an evolutionary conservation that reinforces the utility of findings from primitive model organisms.[16]

In the current study, Alone *et al.* studied 220 lines of flies with distinct and defined chromosomal deletions spanning up to 50% of *Drosophila melanogaster* genome. These lines contained a chromosomal deletion

This Editorial View accompanies the following article: Alone DP, Rodriguez JC, Noland CL, Nash HA: The impact of copy number variation on anesthesia in a model organism. ANESTHESIOLOGY 2009; 111:15–24.

Accepted for publication February 20, 2009. Supported by grant NIH GM077357 from the National Institutes of Health (Bethesda, Maryland) to the University of Pennsylvania Institute of Translational Medicine and Therapeutics, and by the Harold Amos Medical Faculty Development Program, the Parker Francis Fellowship, and the Department of Anesthesiology and Critical Care, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania. The authors are not supported by, nor maintain any financial interest in, any commercial activity that may be associated with the topic of this article.

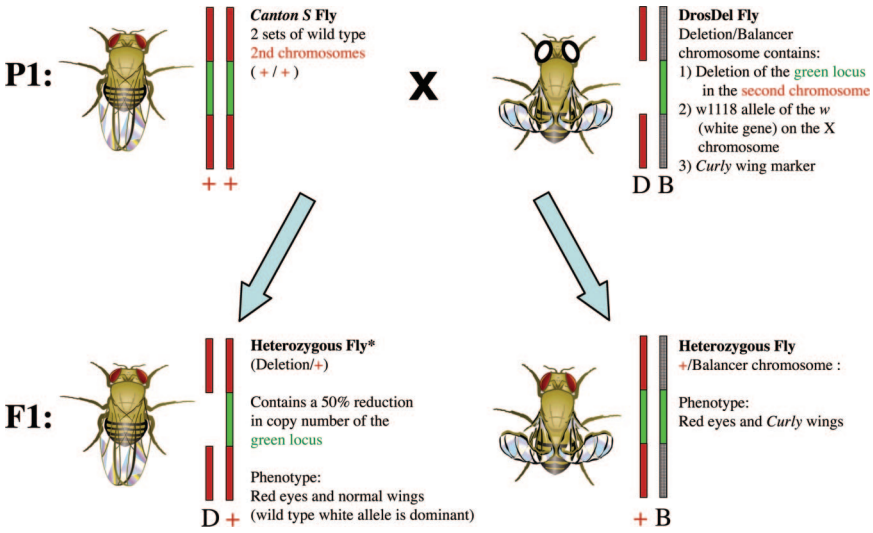

Fig. 1. Schematic representation of *Drosophila* genetic cross depicting inheritance of hypothetical deleted locus on the second chromosome. The parental flies (*P1 generation*) are a *Canton-S* wild type fly (+/+) mated with a fly from DrosDel Collection carrying the desired deletion (missing green element). The complementary chromosomal strand for this fly is a "balancer chromosome" (*gray*) that carries multiple inversions and deletions that prevent recombination events. Its presence is easily inferred by the inheritance of the *Curly* wing marker. The F1 progeny (first filial generation) can only have two possible combinations. The first (*left*) carries a chromosomal arm with the deletion opposite a wild-type chromosome. These flies make up the experimental group. The second group (right) does not carry the deletion and is recognized visually by its curly wings. Note that eye color is determined by the wild-type dominant allele at the white (*w*) gene locus. White is present on the X chromosome, which is not depicted in this figure.

(also termed deficiency) with a mean size in the range of a typical CNV. By crossing these flies with another line of flies in whom the deficiency was not present, resulting heterozygous progeny that carry only one copy of the deleted locus serve as a model of natural CNV (fig. 1).

Flies with reduced copy number were then tested by using a well-validated locomotion-dependent assay.[12,17] For the vast majority of tested mutants, no significant changes in EC_{50} were found as compared to control counterparts. However, in eight different deletion lines, reduced copy number caused a significant change in halothane's EC_{50}. The existence of only eight loci (with 50% coverage of the genome) is perhaps not surprising in light of the steep Hill slopes that characterize volatile anesthetics. It would be a mistake, however, to interpret this small number of CNV sites as evidence of a minor effect of genetic variability upon anesthetic sensitivity. After all, as the *na* gene itself illustrates, a significant shift in sensitivity only occurs when two dysfunctional copies of the gene are present in the genome. Moreover, while the associated effect size of reduced the copy number (+16% to −25% change in EC_{50}) appears at first to be small, resulting from the steepness of the Hill slope, these shifts in EC_{50} represent a change in the population's sensitivity of roughly one SD.[5] Several other points bear mention. Reducing copy number was associated with an equal number of partially hypersensitive and resistant strains. This is important because it refutes the notion that CNV progeny were unwell, in which a bias toward anesthetic hypersensitivity might be expected. Also, the discovery of both concordant changes in anesthetic sensitivity across volatile agents (as in the case of +/ED4065) as well as discordant changes across different volatile anesthetics, highlighted by the case of +/ED2751, refute a simple change in baseline CNS function as an underlying causal explanation.

Finally, another promise of the fly as a model organism is illustrated in studies at the ED1 locus, where initial complementation rescue studies have narrowed the list of candidate genes down to three. Additional complementation experiments should precisely define the essential missing DNA elements in each of the eight outlier lines. With this knowledge, human researchers will be able to focus their efforts on studying smaller orthologous regions of the vast human genome and can then determine whether endogenous polymorphisms or mutations exist in human chromosomal DNA and whether such genetic variability similarly affects anesthetic sensitivity in actual patients.

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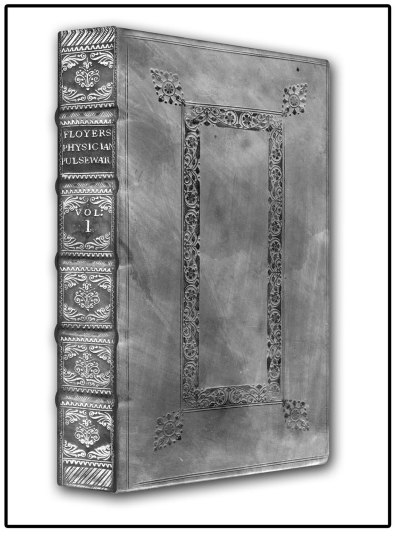
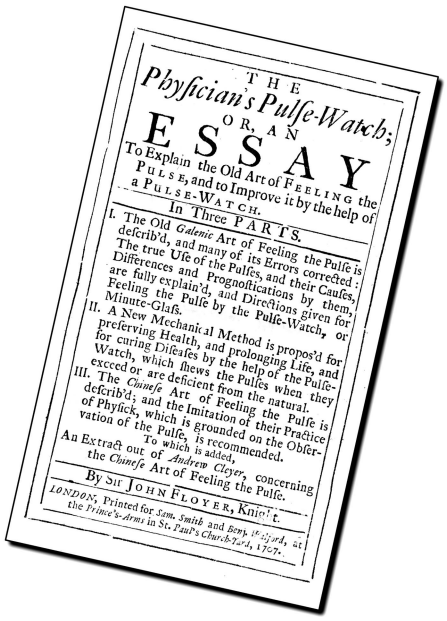
ANESTHESIOLOGY REFLECTIONS

The Physician's Pulse Watch




THE
Physician's Pulse-Watch;
OR, AN
ESSAY
To Explain the Old Art of FEELING the PULSE, and to Improve it by the help of a PULSE-WATCH.
In Three PARTS.
I. The Old *Galenic* Art of Feeling the Pulse is describ'd, and many of its Errors corrected: The true Use of the Pulses, and their Causes, Differences and Prognostications by them, are fully explain'd, and Directions given for Feeling the Pulse by the Pulse-Watch, or Minute-Glass.
II. A New Mechanical Method is propos'd for preserving Health, and prolonging Life, and for curing Diseases by the help of the Pulse-Watch, which shews the Pulses when they exceed or are deficient from the natural.
III. The *Chinese* Art of Feeling the Pulse is describ'd; and the Imitation of their Practice of Physick, which is grounded on the Observation of the Pulse, is recommended.
To which is added,
An Extract out of *Andrew Cleyer*, concerning the *Chinese* Art of Feeling the Pulse.
By Sir JOHN FLOYER, Knight.
LONDON, Printed for *Sam. Smith* and *Benj. Walford*, at the Prince's-Arms in St. Paul's Church-Yard, 1707.

An eccentric experimentalist and lifelong asthmatic, Sir John Floyer (1649–1734) had earned his M.D. from Oxford and a lifetime appointment as Justice of the Peace from Lichfield before receiving his politically-linked knighthood. Ridiculed for advocating cold hydrotherapy, then hailed for penning a classic treatise on asthma, Floyer dedicated the first of his two "pulse" volumes to a British monarch as self-obsessed as himself about personal health, Queen Anne. When his fellow politician from nearby Coventry, sheriff Samuel Watson, moved to London as a clockmaker and then Royal Mathematician-in-Ordinary, Floyer popularized that horologist's use of a seconds hand and a stopping lever in watches designed for taking the pulse. As pictured above from the Huston Rare Book Room of the Wood Library-Museum, Floyer's 1707 volume of *The Physician's Pulse Watch* revolutionized clinical monitoring and diagnosis by quantifying "the natural pulse and the excesses and defects from this . . .." (Copyright © the American Society of Anesthesiologists, Inc. This image appears in color in the *Anesthesiology Reflections* online collection available at www.anesthesiology.org.)

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