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is appreciated!

Computer Models as an Alternative to Animal Models

Animals are used extensively in biomedical research laboratories across the globe to model disease, study biochemical and physiologic processes, and to test the safety, toxicity, and effectiveness of drugs and surgical procedures[2,3,4]. However, in accordance with the three R's (reduce, replace, and refine) as first proposed by Russell and Burch (1959)[7], Federal laws and regulations as well as Public Health Service policy, require that non-animal alternatives be considered, evaluated and if suitable, implemented as a way of reducing the use of research animals. Furthermore, animal research is expensive, whereas non-animal alternatives are often cheaper and thus used whenever possible. Computer simulations are one alternative to animal models that have been evaluated and, to some degree, shown to be useful. Simula-
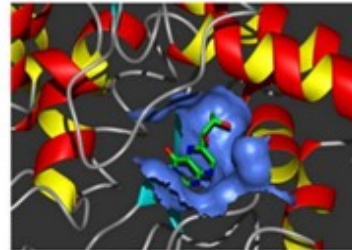
Computer Aided Drug Design CADD is one alternative used to predict receptor binding sites
Image credit: http://www.slideshare.net/FlorentBarbault/molecular-docking-andvirtualscreening?related=7

tion refers to the use of computer models that predict particular outcomes[2]. For example, there are computer-based models that attempt to predict the various possible biological and toxic effects of a potential drug

candidate without using an animal model. Moreover, there are simulations that use anatomic and physiologic patient data that aid in surgery planning and predicts treatment outcome or performance of a device[8]. A powerful digital simulation of the human brain (Human Brain Project) is being developed and once completed would allow scientists to repeat experiments under many different conditions[6]. Another example of a successful simulation is provided by *Computer Aided Drug Design* (CADD) used, for example, to predict the receptor binding site of a potential drug[2], thus avoiding the need to test chemicals having no biological activity; only the most promising candidates are evaluated *in vivo*. *Structure Activity Relationship* (SARs) and *Quantitative Structure Activity Relationship* (QSAR) computer programs are used to predict biologi-

Highly Immunocompromised Mouse Models: A Resource for Investigators

The highly immunocompromised NSG mouse, developed by Dr. Leonard D. Shultz at the Jackson Laboratory (JAX), is one of the most immunodeficient mouse strains available for use in biomedical research[2,10]. The strain was created in '04 by crossing a female NOD.CB17-Prkdcscid/J with a male bearing the X-linked B6.129S4-Il2rgtm1Wjl/J allele. It was originally developed to permit engraftment of human hematopoietic stem cells (HSC)[11]. The resulting male offspring were then back-crossed to the female NOD.CB17-Prkdcscid/J mice for eight generations. Inbreeding of the heterozygous offspring resulted in the double knockout strain that we know today by its acronym NSG. The
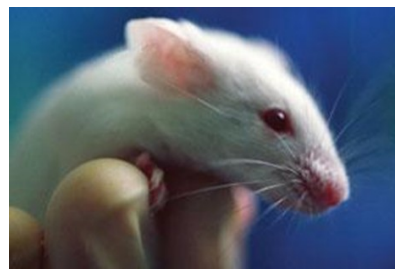
NOD SCID GAMMA (NSG)
Image credit: http://www.nature.com/news/2009/090603/

strain is homozygous for both the Prkdcscid and homozygous (female) or hemizygous (male) for the Il2rgtm1Wjl alleles[10]. The official strain name for the NSG strain is NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ, but other common names include "NOD scid gamma," "NOD-scid IL2Rgammanull," and "NOD

-scid IL2Rgnull." The NOG (NOD.Cg-Prkdcscid Il2rgtm1Sug/JicTac) mouse is another severely immunocompromised strain that was developed in '00 by Mamoru Ito *et al* at the Central Institute of Experimental Animals (CIEA) in Japan[4,6]. NSG and NOG mice are nearly identical except for the modification of the γc chain receptor. In both strains, triggering through the γc chain receptor is disabled; however, in NSG mice the receptor is completely knocked down, while in NOG mice the intracytoplasmic tail is truncated[5].

NSG mice combine the features of the NOD/ShiLtJ strain, the severe combined immune deficiency mutation (scid) and an IL2 receptor gamma chain deficiency. NOD mice lack hemolytic complement because of a

Image credit: http://www.cns.nf.ca/
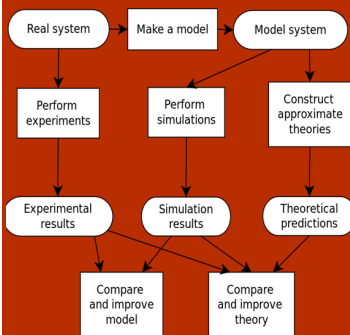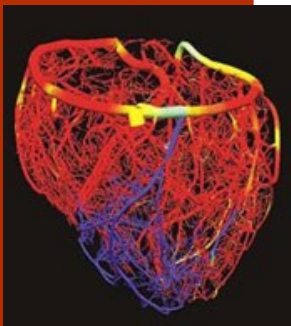

Process of building a computer model and the interplay between experiment, simulation, and theory

Image credit: https://en.wikipedia.org/wiki/

Dr. Dennis Noble's virtual heart

CCMP's eLearning Platform Brings Learning To Your Doorstep

One of the key elements to building an effective instructional program is knowing and understanding the needs of the students. This may sound like a simple task, but providing effective learning to the diverse population of learners at MSKCC and WCMC, for many of whom English is not their native language, is extremely challenging. Scientists with varied years of experience, from novice to expert levels, join our institutions yearly. Providing a quality educational experience that meets all the needs of this audience is challenging.

Until recently, most Institution's animal care and use programs followed the standard training model that most of us experienced in school, instructor led training. This type of learning is moderated by an instructor providing lectures accompanied by visual aids. This rarely meets the needs of the entire audience. This is where *eLearning* fills the gap.

Simply stated, *eLearning* is an educational experience accessed via electronic devices. Although the instruction is most often provided from online modules,

properly designed *eLearning* allows student interaction and participation. This interaction can be provided through a link for emailing questions, complex branching scenarios that dynamically react to student responses and feedback presented after a knowledge assessment is completed. Taking advantage of the self-paced nature of *eLearning* instruction, a well-developed *eLearning* program has the ability to meet the needs of a much broader student population. Each student proceeds through the course at a pace that best en-

Computer Models as an Alternative to Animal Models

cal activity of drug candidates with respect to their carcinogenicity and mutagenicity[2]. Finally computer models have been successfully used in demonstrations of experimental procedures to teach research scientists and medical students. In a study conducted in India evaluating the use of computer simulations as a teaching tool for pharmacology students, test scores and student feedback showed that the students gained a better understanding of the mechanisms of action of drugs in a shorter time[1].

However, a key point to consider is how computer models are developed and improved. It involves the interplay between mathematical algorithms and *in vivo* experiments that allows scientists to capture biological patterns, predict how a system behaves, and subsequently refine the model[6]. Given this developmental process, the accuracy of a model must be validated with actual data (most often acquired using animal models). Moreover, before a computer simulation that models a particular physiological process can be developed, an understanding of the process is fundamental. This understanding, most often, also comes from animal models. Another limitation of computer models is the processing power required. For example, a simulation of half a mouse's brain required the

use of the world's fastest supercomputer (Blue Gene/L) and the simulation still did not provide an accurate representation[6]. Computer models have also been used to simulate the function of whole organs, but currently the focus of most models is on interactions at the tissue level ignoring minor individual cell level variations. Dr. Dennis Noble (2006) who is part of a team designing a virtual heart explained: "I would say the real benefit of the [computer] model is that it can do a preliminary filter of your compounds, and that can replace some of the very early stages in animal experimentation"[5].

In sum, computer models provide many benefits to biomedical research and, at this point, may, in some cases, serve as a first line of analysis before transitioning to animals. They can serve as a filter reducing the number of animals used to address a research target as our understanding of biochemical, physiologic and disease processes advance and when increased computer processing power becomes available, better and more accurate models will be developed. However in the interim, the fact that animal models are still widely used shows that, for some types of research, computer models (and other alternatives) cannot supplant the use of animals.

~Christopher Cheleuitte-Nieves, DVM, PhD

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NSG: A Resource for Investigators

eLearning Platform

two base pair deletion in the C5 structural gene and carry a unique MHC haplotype which leads to defective NK cell function and defects in the immunoregulation of antigen presenting cells, such as macrophages and dendritic cells[10]. The lack of the Prkdc gene expression in scid mice results in a double stranded DNA repair defect and an inability to rearrange genes that code for antigen-specific receptors on lymphocytes[10]. As a result, scid mice lack detectable IgM, IgG1, IgG2a, IgG2b, IgG3, or IgA. The IL2 receptor γ chain also known as the common cytokine receptor gamma chain, is shared with receptors for IL-4, IL-7, IL-9, IL-15 and IL-2110. The null mutation in the IL2γ receptor leads to deficiencies in cytokine signaling and failure of clonal lymphocyte expansion[10]. All of these mutations result in a strain that lacks mature T and B as well as NK cells and hemolytic complement, and are deficient in cytokine signaling[10].

Because NSG mice are severely immuno-compromised, husbandry practices that are normally sufficient to maintain nude and other immunodeficient strains, such as SCIDs, are often inadequate for this strain. JAX maintains their NSG colonies in a maximum barrier, in which the mice are provided sterilize individually ventilated cages, feed, and drinking water; staff use an air shower entry and extensive protective personal equipment (i.e., scrubs, smock and shoes, gloves, bonnet, mask, and face shield[7]. In order to maintain a healthy colony, JAX acidifies the drinking water to pH 2.5 – 3.0 before autoclaving to help prevent infection by *Pseudomonas* spp.[7] In addition, they conduct cage changes under a laminar flow hood to prevent the accumulation of commensal organisms within the cage environment and handle their mice with disinfected forceps or gloved hands to help prevent the spread of opportunistic organisms. These special husbandry practices are implemented to minimize the incidence of spontaneous disease within NSG colonies; however, despite these extra precautions, it is still possible for NSG mice to develop opportunistic infections, with ascending bacterial infections of the urinary tract being the most common[3]. Despite their immunocompromised condition, NSG mice are good breeders producing up to 8 pups per litter and 7-8 litters over a 6 month period[7].

Since its development thirteen years ago, the NSG mouse has made significant contributions to biomedical research. It is a highly versatile model having and continuing to be used in various scientific disciplines, including immunology, infectious diseases, oncology, diabetes, and stem cell/regenerative medicine[8]. Importantly, a transgenic NSG mouse, expressing human membrane-bound Kit ligand proteins, has been developed to improve myeloid engraftment without preconditioning irradiation that allows for the engraftment of human hematopoietic stem cells[1]. Other NSG related mouse strains have been developed, including the NRG mouse, which are NOD-congenic mice harboring the Rag1null mutation (Rag1KO or Rag1tm1Mom) on chromosome 2 and the IL2rγnull mutation (IL2RγcKO or Il2rgtm1Wjl) on the X chromosome. This strain was produced by breeding NOD-Rag1null mice with NSG mice[8]. Offspring were intercrossed and bred to be homozygous for the Rag1null mutation, homozygous (females) or hemizygous (males) for the X-linked IL2rγnull mutation, and wild-type for the scid mutation[8]. NRG have been used extensively in transplantation studies since they tolerate much higher levels of irradiation conditioning.

In a relatively short period of time, the NSG mouse has been demonstrated to be an extremely valuable animal model for use in a variety of disciplines. The Research Animal Resource Center (RARC) offers special husbandry practices to investigators wishing to house and maintain severely immunocompromised mouse models. Because of these animals' immune status, dedicated animal rooms as well as sterile caging and supplies are used for housing. As these models are typically engrafted with human cells, a biological safety cabinet, providing both animal and personnel protection, is routinely employed for cage changing and manipulation. Contact RARC for additional details if you determine that the NSG or NOG mouse models will be utilized.

~ Samantha M. Peneyra, DVM

For detailed information on
NOD SCID GAMMA (NSG™): THE MOST VERSATILE IMMUNODEFICIENT MOUSE
go to the following link:
https://www.jax.org/jax-mice-and-services/find-and-order-jax-mice/nsg-portfolio

ables them to absorb the content. A learner can skim through sections they are familiar with or move slowly to focus on information that is new. *eLearning* modules also provide benefit to English as a Second Language (ESL) learners. The self-paced nature of the modules enables these learners to review the information presented in a manner that allows better comprehension. The inclusion of written documents and guidelines embedded in the modules and available at the touch of a button, enable ESL students to read the information presented, rather than relying on their understanding of a didactic lecture presentation.

Another key benefit to *eLearning*, is the ability for learners to complete their training on their own schedule. Research staff need to juggle mandatory training around their research and clinical responsibilities. Making time for mandated training can be challenging. An *eLearning* module can be completed at any time, day or night, onsite or from a remote location and doesn't have to be completed in one sitting. eLearning also allows for *just in time learning* that can easily fit into even the busiest schedule. This allows learners to synchronize their training with the onset of their work to optimize the learning experience and maximize content retention.

In summary, CCMP has chosen to use eLearning as a way to provide our research community high-quality, self-paced, learning available around the clock.

~Desiree Ehleiter, BA, MAED, LATG

The self-paced nature of eLearning places knowledge at your fingertips as close as your keyboard!
Image credit: http://gettingsmart.com/2013/10/students-outsmart-school-laptops-thats-good-thing/

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Olfactory Communication as a Mediator of Aggression in Lab Mice

Laboratory mice play a vital role in bio-medical research. While they may model human disease, it is crucial that we remember the unique natural history and sensory biology of the mouse. For instance, the mouse's sense of smell is far more developed and intricate than our own. It is due to this complexity that mice are able to communicate much of their social hierarchy and stability through odors[1]. This article will discuss the murine olfactory system and its implications on conspecific aggression in the laboratory setting.

The mouse employs two distinct olfactory systems and a high percentage of neural tissue volume is devoted to olfactory processing. The first system corresponds to what many mammals utilize exclusively: the perception of airborne odorants by the main olfactory epithelium of the nose. These signals are then transmitted to higher brain regions such as the olfactory cortex resulting in learning and behavior. The second system consists of the perception of fluid phase odorants and pheromones by the vomeronasal organ (VNO). As a more primitive structure, the VNO projects to the limbic and autonomic nervous systems, bypassing cortical structures entirely[1, 2, 3, 4]. The resulting changes in behavior and physiology mediated by this system are more instinctive and less cognitive. Together, these systems form a robust and multifaceted mechanism for mice to interact with their olfactory environment.

The natural habitat of mice is rich with olfactory cues from their own social group, distantly related mice, predators, and the environment itself. Urine, as well as the products of several scent glands, provides the primary source of murine odor cues[5, 6]. Due to the complex nature of olfactory processing, these scent marks are able to convey information regarding the individual's species, sex, immune status, social or reproductive status, diet, and microbiome[1, 5,6,7,8]. This information is then used to establish territories, social hierarchies, and mating pairs. It thus follows that alteration in the ability to appropriately scent mark could interfere with olfactory mechanisms of social communication and stability.

The laboratory environment provides few similarities to the mouse's wild habitat. Captive mice have a small home range limiting their ability to escape aggressors or perceived predators (us). Odor communication via scent marking is constantly altered by husbandry and cage changes. While individually ventilated cages prevent airborne odor contamination, fluid phase odors on the gloves, lab coats, or equipment of the technical and investigative staff provide a source of potentially stressful olfactory stimuli[9,10]. Mice are also exposed to novel odorants any time they are removed from their caging for experimental purposes. Additionally, handling and manipulation lead to the release of alarm pheromones that in turn affect mice in the home cage[7, 9,10]. As such, care must always be taken to change personal protective equipment when working with different species or sometimes, even different cages. Equipment and experimental materials must similarly be devoid of organic material that can serve as an odor source. Current biosecurity practices incidentally limit many of these concerns.

When mice establish their territories in the wild they are claiming dominance over resources and potential mates. A dominant male is the one that most successfully lays down scent marks and can defend that territory through overt aggression. In fact, research shows that urine marking predicts dominance status such that the male mouse that scent marks more will also initiate the most aggressive encounters with cage mates. In a natural setting a submissive mouse would avoid escalation through decreasing pheromone concentrations and overall scent marking[6,11,12]. In the lab setting, mice live in a closed housing environment that combines a high odor concentration and an inability to escape. This leads to an issue of both experimental and welfare significance: fighting[12]. Laboratory animal scientists and medical professions are always seeking to better understand and develop methods of minimizing injury-inducing aggression.

Investigations into the effects of odors on mouse behavior and physiology have been illuminating. Mice exposed to the odors of sexually mature male mice, and recently human males, display increased stress and a sympathetic response including elevations in blood pressure and heart rate[13,14]. This is also born out in a husbandry refinement study where either dirty bedding or nesting material was transferred at cage change to identify effects of odor transfer on aggression. Interestingly, the transfer of bedding exacerbated fighting, while nesting material transfer resulted in an abatement of aggression compared to controls. The authors hypothesize that since mice avoid urinating in their nest there is likely a non-urine source mediating this pro-social effect of transferring established odor cues in nesting material[15].

Mouse olfaction and its implications in the laboratory setting remains an understudied area of research. Species' differences in sensory biology must be acknowledged and accounted for in both study design and animal husbandry. By better understanding the sensory experience of the mouse, we can better adapt our methods to improve both animal welfare and scientific advancement.

~Mimi Gallo, DVM, MS
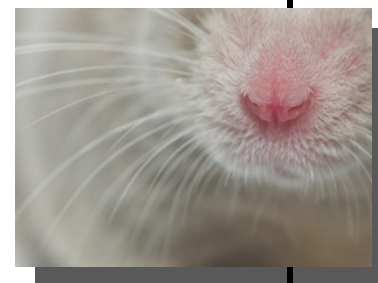
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