

New Frontiers for the Integrative Study of Animal Behavior

A report of a workshop held at the
New York Genome Center, New York NY
August 6-9, 2014

This report was prepared in November 2014 by the participants of the workshop for the National Science Foundation. The workshop was sponsored by the National Science Foundation through Grant Number IOS-1439985 to Columbia University. Any opinions, findings, conclusions, or recommendations expressed in this report are those of the participants and do not necessarily represent the official views, opinions, or policy of the National Science Foundation.

Organizers

Dustin R. Rubenstein, *Columbia University*
Hans Hofmann, *The University of Texas Austin*

Participants

Erol Akcay, *University of Pennsylvania*
Suzanne Alonzo, *University of California Santa Cruz*
Elizabeth Archie, *University of Notre Dame*
Annaliese Beery, *Smith College*
Rebecca Calisi-Rodríguez, *Barnard College*
Karen Carleton, *University of Maryland*
Brian Chow, *University of Pennsylvania*
Josh Dubnau, *Cold Spring Harbor Laboratory*
Christina Grozinger, *Pennsylvania State University*
Ellen Ketterson, *Indiana University*
Andrew Leifer, *Princeton University*
Tim Linksvayer, *University of Pennsylvania*
Matthew MacManes, *University of New Hampshire*
Lynn Martin, *University of South Florida*
Kevin McGraw, *Arizona State University*
Lisa McGraw, *North Carolina State University*
Tamra Mendelson, *University of Maryland Baltimore County*
Lauren O'Connell, *Harvard University*
Alex Ophir, *Cornell University*
Luke Ramage-Healey, *University of Massachusetts Amherst*
Suzy Renn, *Reed College*
Timothy Roth, *Franklin and Marshall College*
Jenny Tung, *Duke University*
Sarah Woolley, *Columbia University*

Executive Summary

The organism is the central unit of integration for genes, development, and the environment. Behavior plays a key role in this organismal integration, and as such, it is a major determinant of biological form and function. Yet in recent decades, biological practice shifted considerably from studying organisms and their behavior to studying populations on the one hand and genes and molecules on the other. One reason for the shift was the Modern Synthesis of Mendelian genetics with Darwinian theory, which led to a productive focus on theoretical and modeling approaches and gave rise to population and quantitative genetics. The shift was also due to the rise of reductionist experimental approaches based on molecular biology, which focused on cellular functions common to all living organisms and resulted in many of the monumental discoveries and advances that define the current state of biology. However, as the discoveries have accumulated, the need for integration of neural, genetic, physiological, ecological, and evolutionary studies has become increasingly apparent. This is especially relevant to studies of animal behavior, since behavior is central to both neurobiology and population biology/genetics. Approximately 50 years after Tinbergen's seminal publication on levels of analysis in studies of animal behavior, the necessity of understanding behavior at the level of the organism has become pressing. If we are to provide a deeper understanding of what drives behavior and why/how it works; if we are to employ the study of behavior in new biomedical discoveries; and if we are to conserve biodiversity through knowledge of how animals adapt to climate change and other anthropogenic stressors, an integrative understanding of animal behavior—and organismal biology more generally—will be essential.

In response to this need, 26 animal behaviorists and scientists from related disciplines (most of them at early career stages) gathered in New York City in August 2014 to discuss new frontiers for the integrative study of animal behavior. The group's objectives were to (1) define the integrative study of animal behavior, (2) outline the benefits of employing integrative research in animal behavior, (3) discuss how best to conduct integrative animal behavior research, and (4) determine how the animal behavior community can acquire the skills necessary to make their work integrative. The group was unified in its stance that an integrative approach to the study of behavior will enable critical tests of the robustness of current knowledge, while also generating novel insights and new areas of inquiry. The group also agreed that the goal of promoting discovery of paradigm-shifting or paradigm-reinforcing findings in behavior will require a critical balance between developing novel ideas and testing existing ones. Integrative research in animal behavior must retain its tradition of beginning with a question, being hypothesis-driven, and/or being steeped in natural history and in theory, whether verbal or mathematical. Moving forward, however, the challenge will be to achieve an optimal balance between explicit hypothesis-testing, and the use of new technologies for exploratory work to promote hypothesis-generation. To achieve integration, researchers will not only need to collaborate with colleagues in other fields, but also be trained in a diversity of skills, particularly those that are quantitative in nature. This will require new training at all career stages, and we encourage the community to develop a summer course or workshop as has been done in other disciplines (e.g., neuroscience, evolution, genomics). The goal of this workshop should be to provide researchers competency and literacy in a range of core areas constituting the discipline of animal behavior and its application to the needs of society to understand the principles underlying behavior, to preserve biodiversity, and to provide breakthroughs in medicine.

Introduction

This document is the result of an NSF-funded workshop on *New Frontiers for the Integrative Study of Animal Behavior*, held at the New York Genome Center, New York NY, August 6-9 2014. The goals of this workshop were to (1) define the integrative study of animal behavior, (2) outline the benefits of doing such integrative research in animal behavior, (3) discuss how best to conduct integrative animal behavior research, and (4) determine how the animal behavior community can learn the skills necessary to make their work integrative. Here we describe in detail what was discussed at the workshop, arguing why integrative research will benefit the field of animal behavior and society at large, and outline a path forward, in terms of both training and infrastructure, needed to accelerate progress in the integrative study of animal behavior.

How animals behave and interact with each other and their environment has long fascinated scientists and non-scientists alike. Human and non-human animal behavior can be incredibly complex, as animals care for their young, use symbolic languages to convey information, build and use tools, cooperate, and migrate long distances. These sophisticated behaviors vary across space and time, among individuals, populations, and species, and are the outcome of extrinsic and intrinsic processes and evolutionary forces (Hofmann et al. 2014). However, due to both historical contingencies and methodological limitations, classical approaches to the study of animal behavior have been limited to relatively simple processes and questions, which often failed to holistically explain the complexity of animal behavior in natural ecological systems. Understanding physiological mechanisms and developmental constraints may not only provide a more holistic understanding of why and how behavior evolve, but such an approach may also help explain why animals sometimes behave in seemingly non-adaptive ways or if certain resources are indeed limiting, as is often assumed (Monaghan, 2014). Moreover, placing developmental and physiological results within their evolutionary and ecological context allows us to distinguish between how organisms *can* behave in a laboratory environment and how they *do* behave in their natural environment. With the development of new resources and tools in genomics, physiology, neurobiology, and behavioral tracking, for example, it is now possible to integrate across mechanisms, taxa, and scales of biological organization. Such '**integrative studies of animal behavior**' can revolutionize our understanding of the proximate and ultimate mechanisms underlying behavior and how evolution shapes these behaviors, including that of our own species. Furthermore, these studies can provide broad interdisciplinary training of the next generation, including scientists, educators, conservationists, and policymakers. Finally, this integrative approach to animal behavior can provide new perspectives and solutions to both biomedical research and discovery (e.g., the emergence of mental disorders, obesity and other metabolic-dependent disorders, the basis of family dynamics and health) and current societal issues (e.g., disease risk and spread, conservation of species, adaptation to climate change, habitat loss).

What Is the Integrative Study of Animal Behavior?

While integration across spatiotemporal scales and levels of analysis has become a goal of many modern scientific disciplines, it is particularly critical to the field of animal behavior (Hofmann et al. 2014). Behavior is the ultimate complex and integrated trait, shaped not only by gene, protein, neural, and endocrine interactions, but also by interactions among animals of the same and even different species, and the abiotic environment. Moreover, studying behavior is important because it is the currency of selection; selection does not work on genes, proteins, hormones, or ecosystems, but on the behavioral phenotype itself. At the heart of animal behavior is the organism. Integration means not only drilling down from the organismal level to the level of the gene or molecule, but also building up to the level of the population or ecosystem. Tinbergen's classic "Four Questions" have long been appreciated as a centerpiece

and motivator of advancing animal behavior research, but our framework of integration is not limited to Tinbergenian levels of analysis. Instead, we propose that the integrative study of animal behavior can be schematized along four major axes of integration, spanning conceptual, biological, and analytical frameworks and including (but certainly are not limited to): (1) Tinbergenian levels of analysis (i.e., development, mechanism, fitness benefit, phylogenetic) (Bateson & Laland, 2013); (2) levels of biological organization; (3) taxa and habitats, including across different temporal and spatial scales; and (4) research disciplines and their tools (Figure 1). This framework places equal emphasis on studies spanning cells to ecosystems, involving diverse mechanistic, theoretical, computational and comparative tools, and integrating diverse taxa.

'Integration' does not simply mean working at multiple points along an axis or even incorporating multiple axes into a single research project. Rather, the results should be mutually—but not necessarily equally—informative within and among these axes. For example, the identification of a set of genes from a transcriptomics study of a neuronal circuit can be used to examine the role of associated electrophysiological properties in shaping a behavior, while molecular evolution approaches can examine the selective pressures on these genes across populations. Using the advances generated along one axis can change the interpretation along other axes, leading to conceptual leaps that can inform the field of animal behavior as a whole.

Finally, while classical approaches to animal behavior have overwhelmingly emphasized hypothesis-testing (Platt 1964), an integrative approach embraces a **hypothesis-driven** framework that encompasses both an iterative process of '**hypothesis-generation**' (i.e., exploration using new tools to obtain, analyze, and synthesize large data sets) as well as more classical '**hypothesis-testing**'.

Why Be Integrative?

Although integration across multiple scales, levels, taxa, or disciplines is not always necessary to conceptually advance the field of animal behavior, we envision at least two central ways in which integrative research will critically advance the field of animal behavior, either by generating novel hypotheses or by rejecting or supporting long-standing ones (Monaghan, 2014):

(1) Paradigm-shifting: Integration can help generate or test novel hypotheses for behavioral phenomena. Data supporting such hypotheses, or methods newly devised to test them, would be an example of a '**paradigm-shifting**' outcome that can change how scientists view a problem. We expect these new hypotheses to arise primarily from approaches that cross disciplinary and conceptual boundaries. For example, the application of molecular tools more than two decades ago to the study of avian mating systems revealed surprisingly high levels of extra-pair paternity in most species and changed the way we think about avian mating systems (Cockburn, 2014). More recently, studies of epigenetic inheritance have been changing the way we think about adaptive evolution. For example, work in flies suggests that males can transmit features of their phenotype via non-genetic seminal-fluid-borne factors to their mate's subsequent offspring sired by other males (Crean et al. 2014). Moreover, work in mice has shown that a father's learned experiences can be transmitted to offspring epigenetically, presumably also through sperm (Dias and Ressler 2013).

(2) Paradigm-reinforcing: Integrative research in animal behavior also encompasses testing the robustness of current paradigms. These '**paradigm-reinforcing**' studies may support or modify well-accepted hypotheses. For example, findings with strong support on different biological

scales or within single taxa may fail to generalize well across scales or species. A robust paradigm is a cohesive, well-supported hypothesis that has predictive capacity across scales and contexts. Integrative approaches therefore are more likely to provide robust knowledge. On the other hand, longstanding hypotheses to explain behavioral phenomena may not necessarily be true, or may be poorly supported. A strong integrative study of animal behavior has enormous potential to generate results that challenge an accepted paradigm. For example, early work on the evolution of toxic seminal fluids (which increase male performance in sperm competition but decrease female survival; Chapman et al. 1995) in *Drosophila* was interpreted as evidence that females were caught in an evolutionary arms race with unavoidable costs of mating (Holland and Rice 1998, 1999). However, detailed work on the function and evolution of male seminal fluid proteins and the physiological pathways and gene products produced by females in response to male seminal fluid components revealed that females actively respond to these “toxic” proteins. Thus, the integration of evolutionary and mechanistic studies suggests that the dynamics of coevolution between the sexes should be seen more as an outcome of the correlated evolution of multiple traits within and between sexes, where females are active participants in the biological effects these “toxic” male proteins initiate (e.g., Sirot et al. 2009; Rubinstein and Wolfner 2013). A combination of arms races, shared interests, and mechanistic constraints simultaneously shape the observed patterns of behavior, which go well beyond—and are much more interesting than—the original “bad males hurting females with no escape” interpretation. Yet, without the integration of detailed mechanistic and evolutionary perspectives, these insights would not have been possible.

What Tools Are Needed to Be Integrative?

An often noted driver of our increased ability to perform integrative studies of animal behavior is the astonishing growth of technologies that permit unification of previously disparate fields of study. However, as new tools and techniques become available for an ever-increasing number of species, we emphasize that biological tools are a means to answer questions as appropriate, rather than a necessary requirement of integrative research. Integration is not simply the application of new tools to traditional studies of animal behavior. Instead, new tools can enable us to answer questions in new and innovative ways and often, lead to a merging of separate fields of study. For example the study of the evolution of developmental mechanisms (often referred to as “Evo-devo”) became an emerging discipline after developmental biologists adopted methods from molecular biology that allowed them to identify the so called “developmental-genetic toolkit” (e.g., homeotic genes such as the Hox gene cluster) to better understand the regulation of embryonic development. This area has since proven that phenotypic novelty can emerge quickly and plastically, which can provide the basis for dramatic evolutionary changes. Further, the application of modern techniques across disciplines may generate new and sometimes transformative insights into our own research questions, initiate conversations and collaborations amongst behavioral biologists who may not have previously interacted, and challenge us to think above and beyond our initial field of study. Although tools derived from disciplines like molecular biology, genetics, and neuroscience facilitate integration of behavioral research, it should not be overlooked that our study systems of choice are also important tools that can contribute to a truly integrative line of research. Importantly, the utilization of new tools and collaborations must balance an exploratory hypothesis-generating approach with the classic hypothesis-testing nature of the discipline.

Themes and trends of tools that are useful for integration

A variety of technological advances are rapidly expanding the toolkit available to the integrative animal behaviorists. In general, we can now track animal behaviors in richer spatiotemporal detail and in less restrictive and more naturalistic environments. Moreover, tools to probe

mechanisms that were previously relegated to laboratory confines (e.g., neurophysiological recordings) are increasingly deployed under field conditions. In Appendix 1, we briefly highlight some classes of methods and tools that are of special interest to the animal behavior community. However, since many of these tools require sophisticated equipment or laboratory settings, their use for many investigators, especially in the context of field-based studies, has been limited. For example, techniques requiring sophisticated *in vivo* manipulations in behaving animals, such as optogenetics, microdialysis, or electrophysiology, often require the animal to be tethered to light sources, recording devices, or collection tubes. This technical requirement has made the application of these tools to animals in their natural habitat difficult. There is a clear need for field-compatible technological advances that allow the measurement of various physiological (e.g., hormones, heart or respiratory rates, immunoglobulins) or neural changes (e.g., neuronal activity, neurotransmitter release) in real time in non-tethered animals. Such development will require the collaboration of animal behavior researchers with computer scientists, nanotechnologists, electrical engineers, and experts in other fields. Challenges moving forward include not only applying emerging technologies to non-traditional model systems in ecologically relevant contexts, but also incorporating training and infrastructure into the implementation of these tools. For example, animal behavior studies often ignore a rich toolbox of sophisticated quantitative measurements and process-based modeling, although an emphasis on training and collaboration would easily make these accessible. Moreover, the implementation of all these tools generates an enormous amount of data for which not only training is important, but also the infrastructure supporting such studies.

What Training Is Needed to Be Integrative?

Animal behaviorists already receive training in basic experimental design (e.g., statistical design or how to design a well-controlled behavioral test) and methods for observing and quantifying behaviors in the field or lab. However, the need for more specialized training often arises at different career stages because fields evolve rapidly and because researchers may move toward integration at different points in their research program. Conducting effective integrative studies of animal behavior will require training in a breadth of core competencies, many of which are quantitative in nature (Table 1). We recognize that mathematical theories, statistical methodologies, and computational methods have increasingly central roles in integrative animal behavior research. For example, evolutionary game theory and population genetics have long been central to understanding ultimate causes of behavior for a long time. Likewise, modeling developmental, behavioral, gene expression, and neural dynamics uses tools from stochastic processes theory and nonlinear dynamics. Moreover, statistical tools like Bayesian hierarchical modeling are becoming increasingly important for behavioral ecologists. Obtaining a level of literacy in these mathematical and statistical tools (i.e., understanding their basics, scopes, and limitations) is therefore crucial in the training of animal behaviorists. Greater mathematical literacy in the methods used in animal behavior will help a new generation of researchers to integrate theory and empirical work, something in which the field has an uneven track record in so far.

In addition to mathematical literacy, training in computational biology should be considered an emerging core competency for integrative animal behaviorists. To efficiently handle large datasets that result from genomics projects, mass-array recordings, automated tracking, or video recordings, researchers will need to develop computational skills. Specifically, researchers should receive training in a scripting language that will facilitate the handling of these data, as well as in how to test and validate the results of all custom software. In addition, given the recent push towards the automated handling of tracking, physiology, and image data via machine learning techniques, training in this mode of quantitative thinking may be beneficial

for researchers both implementing these methods as well as consuming them via the primary literature. Finally, many researchers might benefit from expertise in programming micro-controller circuits on inexpensive open-source platforms (e.g., <http://www.arduino.cc> or <http://www.raspberrypi.org>) in order to manufacture custom-designed data loggers, tracking devices, or transmitters.

Some of this education will necessarily come through standard graduate training, relying on a curriculum that draws upon existing courses within home institutions, or through stand-alone courses offered through various centers. Training in experimental techniques as well as computational and evolutionary analyses also exists in various summer courses and workshops (Table 2). Although many of these skills are currently offered at the identified courses, we can envision the creation of a new intensive, discovery-driven course, modeled after the iconic summer courses at the Marine Biological Laboratory and Cold Spring Harbor Laboratory, and specifically designed for animal behavior researchers at a range of career stages. Such a course would help build a vibrant integrated animal behavior community and focus on the core competencies including experimental design, molecular, cellular, and organismal tools, in addition to quantitative statistical, theoretical, and bioinformatics tools. Thus, although we have identified many courses and funding opportunities in other fields that currently exist to fill the training void in integrative animal behavior, we conclude that the community would benefit from developing its own course for the integrative study of animal behavior.

What Infrastructure Is Needed to Be Integrative?

Integrative animal behavior research requires access to a variety of tools and techniques that may necessitate additional infrastructure for the community. Specifically, it will require infrastructure in the form of physical, monetary, and personnel support to realize both training and research. While much of the integration and addition of new techniques and approaches might be accomplished through collaborations (either within or across institutions), additional physical infrastructure may be needed. For example, some institutions that have historic strengths in behavioral ecology may benefit from the addition of major research instrumentation in order to add integrative techniques. Meanwhile other universities with strengths in the neurobiology or physiology of animal behavior might benefit from additional resources for, and expertise in, field-based studies.

The animal behavior community recognizes the value of NSF-funded collaborative networks (e.g., Resource Coordination Networks [RCN]) and centers (e.g., National Evolutionary Synthesis Center [NESCent]) as sources of infrastructural support for both training and collaboration. For example, the EDEN Network in evolutionary developmental ecology (<http://edenrcn.com>), the Phenotyping RCN (<http://www.phenotypercn.org>), the Eukaryotic biodiversity research using High Throughput Sequencing (<http://eukhits.wordpress.com>), the BEACON Center for the Study of Evolution in Action (<http://beacon-center.org/>), the Epigenetics, Reproduction, and Behavior RCN (<http://nsfepigenetic.com/about>), and the Sociogenomics RCN (<http://www.sociogenomicsrcn.com>) currently already facilitate collaborations and trainee exchanges in their respective disciplines. In the future, the animal behavior community may benefit from a focused RCN, a Science and Technology Center, or a new Synthesis Center to provide beginning and established researchers opportunities either to gain new expertise with a critical tool or to establish a collaborations that enhance the integrative nature of their research. Similarly, other NSF funding mechanisms enable integrative research and training. For example, the midcareer supplement *Beyond the Genome* allows post-tenured researchers to get trained in “omics” techniques and bioinformatics. *Doctoral Dissertation Improvement Grants* allow graduate students to integrate additional approaches to

their thesis research, and the *Research Experiences for Undergraduates (REU)* continue to support undergraduate researcher training in integrative labs. Moreover, *Research Opportunity Awards (ROA)*, *Research Experience for Teachers (RET)*, and *Research Assistants for High School Students (RAHSS)* support training for researchers from undergraduate institutions, pre-service K-12 teachers, and high school students, respectively, that can be productively applied to skill development and community building in integrative studies of animal behavior.

What Can the Integrative Study of Animal Behavior Offer to Society?

People observe and intuitively analyze behavior every day. Why is a child crying? Will that driver shift lanes? In the classroom, fascination with animal behavior begins in elementary school and continues through the undergraduate curriculum. This accessibility provides an entry point for many people to science as a whole, and may well transform interest into the thoughtful study of many fields (e.g., neuroscience, evolutionary theory, endocrinology, genomics). The integrative study of animal behavior is one of the most important ways that we have of understanding ourselves as humans. It can be used to better explain large scale, complex human phenomena such as social relationships, politics, and economics. Behavioral biologists should be encouraged to participate in interdisciplinary discussions with general audiences such as in public forums, news, social media, and entertainment venues. In order to support these efforts, the scientific community should place greater value on, and create a reward system for participating in this type of outreach. By doing so, we take discoveries gained from the integrative study of animal behavior and share it with greater audiences, offering a deeper understanding of human nature.

Integrative approaches to behavior are also important for public health because they increase our understanding of the complex processes that lead to mental illness and social disorder (Insel 2010). Specifically, biological psychiatry can learn much from comparative approaches to neurobiology and behavior instead of solely focusing on the search for phenocopies of human behavior in standard laboratory animals (Insel 2007). For example, the study of endophenotypes—well-specified physiological or behavioral measures linking disease symptoms and risk genotypes—is inherently integrative and facilitates the dissection of complex, heterogeneous disease phenotypes such as schizophrenia (Gottesman and Gould 2003).

Finally, the study of animal behavior has important implications for the conservation of biodiversity and for understanding how animals adapt to anthropogenic stressors, including climate change (Buchholz 2007). Integration will allow for improved landscape-level measurements of animal movements, which could help reduce human-wildlife conflict. Moreover, studies of stress physiology in wildlife, particularly using non-invasive methods, will provide insights into how human behavior impacts animal behavior and physiology. Ultimately, understanding how animals respond to climate change will require an integrative approach that includes a mechanistic understanding of evolved and plastic responses to human-induced change.

Conclusions

Animal behavior is a rich and diverse discipline that encompasses researchers studying organisms at a range of scales of biological organization and levels of analysis. Classical approaches to the study of animal behavior were limited to relatively simple processes and questions, which often failed to holistically explain the complexity of animal behavior in natural ecological systems. However, integration via new resources and tools in genomics, physiology, neurobiology, and behavioral tracking is revolutionizing our understanding of the proximate and

ultimate mechanisms underlying behavior. Integrative research in animal behavior can be paradigm-shifting or paradigm-reinforcing, and it is important to strike a critical balance between developing novel ideas and testing existing ones. Greater integration allows us to test the robustness of current knowledge, while also generating novel insights and new areas of inquiry. However, we emphasize that integrative research in animal behavior must still begin with the question, and that studies that are both hypothesis-generating and hypothesis-driven are valuable, especially when steeped in theory, be it verbal or mathematical. Researchers must not only collaborate with colleagues in other fields, but to be truly integrative, they must be trained in a diversity of skills, particularly those that are quantitative in nature. This will require new training at all career stages, and we encourage the community to develop a summer course or workshop like has been done in other disciplines (e.g., neuroscience, evolution, genomics). The goal should be to give researchers competency and literacy in a range of core areas. Ultimately, the integrative study of animal behavior will improve our capacity to conserve biodiversity, understand how animals adapt to climate change and other anthropogenic stressors, and improve the way we treat diseases.

References

Bateson P and Laland KN. 2013. Tinbergen's four questions: an appreciation and an update. *Trends in Ecology and Evolution* 28:712-718.

Buchholz R. 2007. Behavioural biology: an effective and relevant conservation tool. *Trends in Ecology Evolution* 22:401-407.

Chapman T, Liddle LF, Kalb JM, Wolfner MF and Partridge L. 1995 Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature* 373:241-244.

Cockburn A. 2014. Behavioral ecology as big science: 25 years of asking the same questions. *Behavioral Ecology* 25:1283-1286.

Crean AJ, Kopps AM and Bonduriansky R. 2014. Revisiting telegony: offspring inherit an acquired characteristic of their mother's previous mate. *Ecology Letters* 17:1545-1552.

Dias BG and Ressler KJ. 2013. Parental olfactory experience influences behavior and neural structure in subsequent generations. *Nature Neuroscience* 17:89-96.

Gottesman II and Gould TD. 2003. The endophenotype concept in psychiatry: etymology and strategic intentions. *The American Journal of Psychiatry* 160:636-645.

Hofmann HA, Beery AK, Blumstein DT, Couzin ID, Earley RL, Hayes LD, Hurd PL, Lacey EA, Phelps SM, Solomon NG, Taborsky M, Young LJ and Rubenstein DR. 2014. An evolutionary framework for studying mechanisms of social behavior. *Trends in Ecology and Evolution* 29:581-589.

Holland B and Rice WR. 1998. Chase-away sexual selection: antagonistic seduction versus resistance. *Evolution* 52:1-7.

Holland B and Rice WR. 1999. Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. *Proceedings of the National Academy of Sciences USA* 96:5083-5088.

Insel TR. 2007. From animal models to model animals. *Biological Psychiatry* 62:1337-1339.

Insel TR. 2010. The challenge of translation in social neuroscience: a review of oxytocin, vasopressin, and affiliative behavior. *Neuron* 65:768-779.

Monaghan P. 2014. Behavioral ecology and the successful integration of function and mechanism. *Behavioral Ecology* 25:1019-1021.

Platt JR. 1964. Strong inference. *Science* 146:347-353.

Rubenstein CD and Wolfner M. 2013 *Drosophila* seminal protein ovulin mediates ovulation through female octopamine neuronal signaling. *Proceedings of the National Academy of Sciences USA* 110:17420-17425.

Sirot LK, LaFlamme BA, Sitnik JL, Rubinstein CD, Avila FW, Chow CY and Wolfner MF. 2009. Molecular social interactions: *Drosophila melanogaster* seminal fluid proteins as a case study. *Advances in Genetics* 68:23-56.

Table 1. Core competencies for the integrative study of animal behavior.

Core Competencies*	Example	Application
quantification of behavior	field methods, behavioral observations, natural history	knowing your animal, ethograms
ecology/evolution	ultimate causes, environmental determinants of behavior, measuring fitness	putting research questions in broader biological context
mathematical theory	game theory, evolutionary simulations	process based models of evolutionary and behavioral dynamics
statistics	Bayesian analysis	hierarchical models
computational methods	bioinformatics, machine learning	tracking, feature extraction, handling genomic data
genetics/genomics	functional genomics, gene expression	genotyping, gene function, gene manipulation
physiology/neurobiology	endocrinology, electrophysiology	characterizing neural/molecular mechanism

* *Competency refers to being literate in a given area, not necessarily being an expert*

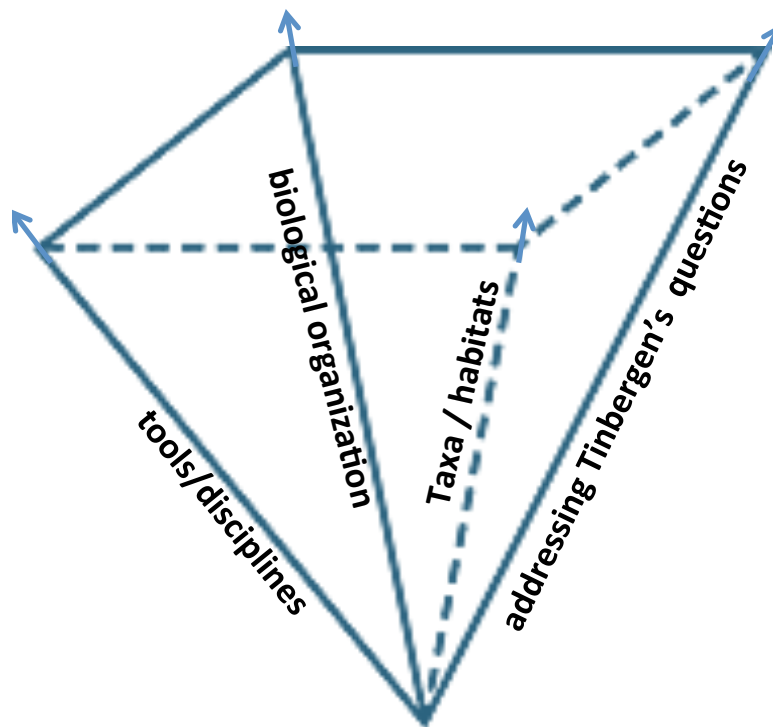
Table 2. Existing graduate courses and workshops. This is a non-exhaustive list of relevant courses that will inevitably change in time. We recommend that a list like this one be curated on a community website—perhaps that of the Animal Behavior Society—much like is done for the evolution community (e.g., http://evol.mcmaster.ca/cgi-bin/my_wrap/brian/evoldir/WorkshopsCourses).

Topics	Title	Location	
Neuroscience	Neural Systems and Behavior http://www.mbl.edu/nsb/	MBL	
	Neurobiology http://www.mbl.edu/education/summer-courses/neurobiology	MBL	
	Computational Neuroscience http://www.mbl.edu/education/special-topics-courses/methods-in-computational-neuroscience	MBL	
	Big Data Short Courses and Summer School http://ccbb.biosci.utexas.edu	University of Texas Austin	
Genetics and Genomics	The Genome Access Course http://meetings.cshl.edu/courses/2013/tgac13.shtml	CSHL	
	Programming in Biology http://meetings.cshl.edu/courses/2014/c-info14.shtml	CSHL	
	Computational and Comparative Genomics http://meetings.cshl.edu/courses/2014/c-ecg14.shtml	CSHL	
	Advanced Sequencing Technologies and Applications http://meetings.cshl.edu/courses/2014/c-seqtec14.shtml	CSHL	
	Next-Gen Sequence Analysis Workshop http://angus.readthedocs.org/en/2014/	Michigan State University	
	Molecular Evolution Workshop http://www.mbl.edu/education/special-topics-courses/workshop-on-molecular-evolution	MBL	
	Tutorial: Evolutionary Quantitative Genetics http://www.nimbios.org/tutorials/TT_eqg	NIMBioS	
	Workshop in Applied Phylogenetics http://treethinkers.org	Bodega Bay	
	Summer Institute in Statistical Genetics http://www.biostat.washington.edu/suminst/sisg/general	University of Washington	
	Computational Macroevolution and Phylogenetic Comparative Methods http://cba.anu.edu.au/news-events/computational-macroevolution-and-phylogenetic-comparative-methods	Australian National University	
	Other	Analysis of Organismal Form http://www.flywings.org.uk/MorphoCourse http://www.nimbios.org/tutorials/TT_eqg	Manchester University
		Modeling Dynamics in Biology http://www.transmittingscience.org/courses/syst-bio/intro-system-bio	Barcelona
Social Evolution http://biologie.cuso.ch/index.php?id=1128&clear_cache=1&tx_displaycontroller[table]=members&tx_displaycontroller[showUid]=1898		Switzerland	

MBL = Marine Biological Laboratory, Woods Hole

CSHL = Cold Springs Harbor Laboratory

Figure 1. Axes of behavioral integration. A study of animal behavior can vary in the degree of integration and conceptual advancement. We envision four primary axes of integration for the study of animal behavior. The degree of integration for any of the axes increases from bottom to top of the schematic. At the bottom of the axis is a single point that represents a research study that does not incorporate integration along any axis but may or may not provide a significant conceptual advance to the field. For example, this would be a study that is conducted on one Tinbergenian level of analysis, at one level of biological organization, using one study species or field/lab habitat, and using one major research tool or theory. Importantly, this framework can also span different spatial and temporal scales. Any study can integrate beyond this singular point and can be thought of as a point in the multidimensional vector space along one or more of the major axes of integration.



Appendix 1. Methods and tools for the integrative study of animal behavior.

The technologies that allow us to probe mechanisms of animal behavior have advanced rapidly in the past few decades. What are the features that make these tools particularly well suited for the integrative study of animal behavior? One feature is the ability to bridge multiple descriptions (e.g., anatomy, physiology, genetics). For example, optogenetic approaches (Fenno et al. 2011, Miesenböck 2009) provide insight into the neural basis of behavior in an integrative way, by enabling the perturbation or monitoring of activity with high spatial resolution (i.e., anatomy) and temporal and biochemical precision (i.e., physiology) in a targeted cell type in awake behaving animals. Importantly, these cellular level physiological perturbations have been shown to alter behavior in animals ranging from invertebrates to non-human primates (Gerits et al. 2012, Cavanaugh et al. 2012) to establish a causal link between neural activity and animal behavior. Another feature is the emerging interoperability between different species, such as the novel development of CRISPR/Cas9 technology for genome editing that can be applied to multiple study systems (Mali et al. 2013, Sander and Juong 2014). Likewise, next-generation sequencing is malleable in that it can not only provide genomes, but also reveal new insights in proteomics and epigenetics, importantly, by only varying sample preparation and analysis. These two examples are certainly not the only features of powerful tools, nor are they necessary features to be impactful. However, they illustrate how technology can reduce the multi-dimensional space that is the integrative study of animal behavior by spanning multiple axes of integration.

Here we briefly summarize some of the available tools that might be useful for the animal behavior community. We place these tools into three categories: (1) “omics” technologies; (2) methods for quantifying animal behavior; and (3) techniques for measuring and manipulating neurons and biological signals. This list is not meant to be exhaustive. Instead, it is meant to illustrate the types of tools available to behavioral biologists. We note that some of these tools are not yet commercially available or usable off-the-shelf, and there is recognition that even when off-the-shelf components can be used for multidisciplinary technologies, they often require outside expertise from computer science, electrical engineering, physics, genomics, or other disciplines for integration into animal behavior experiments. Consequently, collaborations should be considered invaluable components of the integrative behaviorist’s toolkit, and an important goal will be to reach out to colleagues across disciplines to collaborate on animal behavior questions.

“Omics” Technologies

Genomic approaches are probably the most relevant of the “omics” technologies for animal behaviorists because they can provide novel phenotypic measures of behavior and gene function. The typical application of genomic technologies can be considered a “forward behavioral genomic approach”, in which individuals are first classified into their distinct behavioral states, and subsequently the functional genomic profiles are examined to identify the underlying mechanisms. It is also possible to use a “reverse behavioral genomic approach”, in which differences in expression profiles can be used to predict behavioral differences (Harris and Hofmann 2014, Manfredi et al. 2014). This reverse behavioral genomic approach can be incredibly powerful, since behavioral differences can be subtle and difficult to measure, and there are undoubtedly behaviors that have not been previously quantified and studied. Similarly, transcriptomic approaches can be used to functionally annotate suites of genes, rather than relying on annotation systems that have been developed in a small set of traditional model species (Landry and Aubin-North 2007). For example, studies of host-parasite interactions in honey bees have demonstrated that expression of a suite of “immune response genes”

identified using a transcriptomic approach (Richard et al. 2012) that are significantly regulated by parasite infection (Holt et al. 2013), while a suite of immune genes identified using classical annotation based on *Drosophila* (Evans et al. 2006) are not significantly regulated.

The development of genomic tools and resources for a broad variety of species has provided us with exciting new insights and approaches to study animal behavior in a highly integrative manner, spanning the molecular mechanisms underpinning the expression of complex behavior at the level of the individual (including individuals interacting in groups), mechanisms underlying behavioral variation and evolution at the level of the population, and evolution, and co-evolution across populations and lineages (Aubin-Horth and Renn 2009, Toth and Robinson 2009, O'Connell and Hofmann 2011). Genomic approaches involve (1) identifying global patterns of gene expression associated with different behaviors and behavioral states using quantitative real-time PCR, microarrays, and transcriptome sequencing (RNA-seq) (Clayton 2013, Harris and Hofmann 2014), (2) characterizing genomic variation underpinning behavioral variation between lineages and within natural populations using approaches such as quantitative trait locus mapping and genome-wide association studies (Robinson et al. 2013, Santure et al. 2013), (3) quantifying behavioral variation resulting from epigenetic mechanisms such as DNA methylation and chromatin remodeling using bisulfite-sequencing or Chromatin Immunoprecipitation followed by deep sequencing (ChIP-seq) (Stolzenberg et al. 2011), and finally, (4) by experimentally verifying inferred mechanisms by manipulating expression levels of candidate genes using techniques like RNA interference (RNAi) or genome editing (e.g., TALEN or CRISPR/Cas9) technologies (Shalem et al. 2014, Wang et al. 2014).

Genome assembly and annotation

For researchers interested in leveraging the full power of genomics to understand the behavioral phenotypes—leveraging techniques such as CRISPR/Cas9 (Shalem et al. 2014, Wang et al. 2014) and ChIP-Seq (Furey 2012)—generating an annotated genome sequence is necessary. In addition to these things, research questions where cis- regulatory elements (e.g., Ament et al. 2012) are important will benefit from the generation of these data, as these genomic elements may lie many thousands of nucleotides away from coding regions, making them inaccessible to transcriptome based efforts. Though beyond the scope of this review (see Bradnam et al. 2013, Narzisi and Mishra 2011), genome assembly and annotation typically requires a moderate financial investment (\$15-30k), in addition to investment in a collaboration with bioinformaticians trained in genome assembly. These projects may take a year or more to complete—far less time than earlier genome projects.

Genome manipulation and editing

The relative affordability of completing sequenced genomes and transcriptomes has made testing hypotheses about novel candidate genes and gene pathways an attainable goal in a wide range of species. For example, virus-mediated gene delivery or gene knockdown methods in particular brain regions have been used to alter social behavior in *Microtus voles* (Lim et al. 2004) and to alter vocal learning in zebra finches (Haesler et al. 2007). One recently developed technology that has caused great excitement in the community is based on the CRISPR/Cas9 pathway adopted from bacteria that, in principle, allows direct editing of any genome (Mali et al. 2013, Sander and Juong 2014). Remarkably, unlike gene targeting techniques that are specific to a few laboratory species (i.e., mice and *Drosophila*), CRISPR/Cas9 has already been demonstrated to work in wide variety of species including monkeys (Niu et al. 2014), silkworms (Daimon et al. 2014), Syrian hamsters (Fan et al. 2014), and pigs (Whitworth et al. 2014). Refining the CRISPR/Cas9 system to create gene knock-outs or knock-downs, conditionally expressed genes, and tagged RNAs and proteins in a wide range of non-model organisms

shows great promise for gaining a mechanistic understanding of the roles of genes in orchestrating complex behaviors.

Epigenetics

The term epigenetics can refer to DNA-sequence independent inheritance of information either across cell division or generations, and is often used to refer to specific modifications to DNA or chromatin structure that do not alter the sequence of DNA. Our understanding of the dynamics of specific epigenetic modifications (e.g., DNA methylation and histone acetylation) is growing rapidly, particularly in the fields of functional genomics, development, and cancer biology. Epigenetic changes occur during development and across the life course. Intriguing recent work suggests that behavioral factors may contribute to and respond to these changes, offering a tantalizing view of how animal behavior may relate to the mechanistic regulation of the genome. In the coming years, epigenetic studies are likely to emerge as another facet of research in integrative animal behavior. Such work has the potential to make substantial contributions to understanding the biological embedding of social adversity, the mechanistic basis of behavioral plasticity, and the persistent effects of early life challenges on fitness-related traits. However, both methods for measuring and analyzing epigenetic data and a general understanding of the impact of epigenetic marks are rapidly evolving. Major considerations for animal behavior researchers include: (1) effect sizes, which tend to be small and hence challenging to identify with certainty with modest sample sizes; (2) cell and tissue specificity, which make it difficult to extrapolate findings in readily accessible tissues to other tissues, present statistical challenges at the analysis stage, and make non-invasive methods challenging; and (3) taxonomic breadth, as most of what we currently know about the role of epigenetic modifications in behavior comes from a small set of taxonomic groups, particularly eusocial insects, humans, and other mammals. Many sequencing and microarray based methods are being developed for assessing methylation (reviewed in Stolzenberg et al. 2011), and epigenetic marks can be manipulated by pharmacological means to study effects on behavior (e.g., Wang et al. 2013).

Quantifying Animal Behavior

New techniques are changing the way in which we observe and record animal behavior across a wide range of length- or time-scales. Here we present emerging trends for quantifying behaviors including an animal's location over time, its instantaneous posture, and the position and appearance of all of its appendages.

Integrating behavior, physiology, and movement of free-living animals

One of the major logistical limitations of studying animal behavior is the process of collecting unbiased behavioral data, especially in free-living animals. Remote devices to collect behavior are often limited in scope and can collect only basic information (e.g., spatial location in the case of a GPS). Moreover, collecting physiological data, especially on an untethered and freely moving animal, can be extremely difficult or impossible in many cases. Animal tracking in the field has benefited from a proliferation of inexpensive accelerometers, affordable GPS receivers, and more powerful light-weight batteries that enable animals to be tracked over greater distances for longer times. Recent advances in the miniaturization of GPS devices and the sophistication of remote telemetry devices now allows the collection of information integrating behavior, physiological variables, and spatial location simultaneously. Wireless (i.e., untethered) EEG loggers allow behaviors such as sleep and sleep stage to be collected physiologically in real time in free-living animals (e.g., Lesku et al. 2012). Similarly, advances in GPS technology allow researchers to simultaneously collect spatial movement data along with accelerometer and magnetometer data to infer behavior and energetics (e.g., Wall et al. 2014, Williams et al. 2014). Proximity loggers can also be incorporated into this devices to understand social

dynamics, predator-prey interactions, and disease transmission (e.g., Hamede et al. 2009). The miniaturization of such technology is allowing researchers to track the large-scale movements and migration patterns of ever-smaller animals with incredible precision, such as geolocators (Fudickar et al. 2012), satellite telemetry (Willemoes et al. 2014), and GPS (Peron and Gremillet 2013). Finally, GIS provides a powerful tool to understand animal behavior and movement on a landscape scale, through both space and time. A GIS allows multiple layers of information to be superimposed and manipulated to describe and understand interactions between animals and their environment. One of the primary strengths of the tool is that behavior can be modeled on existing or virtual landscapes, producing testable predictions of activities (e.g., movement, biotic interactions, habitat use) based on spatially- and temporally-explicit environmental factors.

Posture and short-distance motion

New methods for analyzing digital video recordings of behaving animals have revolutionized the quantification of animal posture and animal behavior over short distances. Collectively, these methods constitute an emerging field called “computational ethology” that combines techniques from computer science and classical ethology to automate the scoring of behaviors and to capture behaviors that may be less-well suited for human description (Anderson and Perona 2014). Some of these techniques require high spatiotemporal resolution recordings, and they use a variety of “big data” analytical techniques such as dimensionality reduction to create unbiased quantitative descriptions of behavior (see Vogelstein et al. 2014, Brown et al. 2013, Berman et al. 2014). For example, machine learning algorithms enable mining digital video recordings to automatically classify behavioral motifs (Kain et al. 2012) or perform automatic phenotyping (Brown et al. 2013). As computer vision algorithms become more sophisticated they are increasingly being used in the laboratory to track social interactions between two or more animals, or even behavior dynamics of entire population such as schools of fish (Pérez-Escudero et al. 2014, Swierczek et al. 2011).

Image analysis and morphometrics

Automated software tools are allowing researchers to batch-process digital images of animals for quantification of morphometrics (e.g. size, shape), posture/position, color, and pattern (Stevens et al. 2007, Bergman and Beehner 2008, McKay 2013). Light environments, habitats, and scenes can also be captured and combined with animal visual systems to permit digital analysis of animal colors as they see them. Digital photograph-based quantification of animal appearance is ideal for upscaling, and using citizen science and smartphone app technologies to transmit and acquire animal images across the globe.

Techniques for Measuring and Manipulating Neurons and Biological Signals

Areas of biology that have recently focused on genes, neurons, and molecules are increasingly recognizing the importance of studying biological function in the context of behaving animals. Neurobiology, in particular, acknowledges the study of animals in naturalistic environments as a high-priority research area (NIH BRAIN 2014) and is working to bring methods for probing biological mechanisms to behaving animals. Much of this work, such as the development of virtual reality environment for mice or flies, is still laboratory-bound and restricted to traditional model organisms, but other advances such as portable implantable wireless electrode arrays in bats and rats (Ulanovsky and Moss 2007, Szuts et al. 2011) are beginning to blur the line between laboratory and field. Integrative studies of animal behavior can benefit from these advances, as they develop, to probe neural and biological mechanisms that drive behavior.

Quantification and perturbation of hormone and neurochemical pathways

The measurement of circulating hormones has long provided a window into the inner workings of the behaving animal (Nelson 2011). While traditionally limited to low-throughput radioimmunoassays (RIAs) and enzyme-linked immunosorbent assays (EIAs), many hormones and other (neuro-)chemicals can now also be measured in bead-based multiplex assays (Lynch et al. 2014) or with liquid chromatography combined with mass-spectrometry (Keevil 2013). Moreover, in aquatic animals such as fishes and amphibians, circulating levels of steroid hormones can often be reliably inferred from measuring waterborne hormones (Kidd et al. 2010).

Traditional pharmacological perturbations of endocrine and neurochemical systems will continue to be important, especially as implant technologies become ever more sophisticated. The further development of specific receptor agonists and antagonists will thus remain a crucial component of research. Importantly, however, new, more specific modes of controlled release and delivery of small molecules, peptides, proteins, and viruses can be achieved *in vivo* or *in vitro* over long periods (i.e., days, weeks, months) in highly precise and predictable patterns (e.g., steady release, pustule release, or interleaved release of multiple formulations) using bioengineered means (e.g., poly(lactic-co-glycolic) acid [PLGA] microparticles and other biodegradable polymer matrices) (<http://qrono.com>). Also, recent advances in nanotechnology have driven the development of multifunctional molecular platforms that can transport drugs across the blood-brain barrier, target specific cell types or functional states within the brain, release drugs in a spatially and/or temporally controlled manner, and enable visualization of the physiological processes of interest *in vivo* using conventional imaging systems (Ramos-Cabrer and Campos, 2013). Similarly, recent advances in antibody technology have resulted in the development of nanobodies, which are derived from single-chain antibodies from camelids and share some unique properties. These antibodies exhibit high stability, are easily produced in large quantities (also as recombinant molecules), readily bind active site of target proteins, and can cross the blood-brain barrier (Rissiek et al. 2014).

Use of these delivery platforms will increasingly enable researchers to design creative ways to manipulate behavior traditionally reserved for work involving cannulation or repeated administration using cumbersome delivery methods (Choleris et al. 2007). Long term release of a desired deliverable from a single injection or surgery can now function in an analogous way to the way hormone implants have been used, with the added advantage of specific targeting and/or genetic levels specificity. These techniques may soon provide a powerful approach to lab and field neurobiology, endocrinology, and animal behavior.

Engineered devices with form factors useful for field work

The recent development of several device technologies aimed at implantable bioelectronics and improving healthcare in developing nations may benefit field work if properly adapted. Injectable biosensors and wireless electronics are now available that degrade *in vivo*, including conformal electrodes (Kim et al. 2013, Kim et al. 2010). Paper microfluidic devices can be made for pennies for blood and sputum analysis, which can be incinerated after use (Whitesides 2006). Moreover, functional paper-based fluorescence microscopes are now available that can be manufactured for less than one dollar (Cybulski et al. 2014).

Neurophysiology and Optogenetics

Neurobiology is currently undergoing a technological revolution. Large electrode arrays are replacing single cell recordings, and optogenetic methods are making it easier to record or manipulate specific populations of neurons. Many of these methods are being adapted for use

in behaving animals. For example, it is increasingly common to record neural activity from animals such as mice or *Drosophila* that, although head-fixed, remain free to run on a ball or fly in a virtual reality environment (Harvey et al. 2009, Dombeck and Reiser 2012). Tethered (Fee and Leonardo 2001) and wireless telemetry systems (Ulanovsky and Moss 2007, Szuts et al. 2011) allow animals to roam freely in contained arenas. Finally, optical calcium indicators allow for recording large populations of neurons during closed loop fictive locomotion (Ahrens et al. 2012).

Optogenetics (Fenno et al. 2011, Miesenböck 2009) allows for the perturbation of genetically targeted cell types in intact tissue, using light via the heterologous expression of natural or engineered ionotropic photoreceptors. Upon illuminating the photosensitized cells, their activities are manipulated with tremendous temporal resolution (millisecond-to-seconds), spatial resolution (microns), and biochemical precision (e.g., activities include depolarization, hyperpolarization, GPCR signaling, transcription factor activation). For example, by expressing a light-sensitive cation channel in a neuron or excitable cell, one can cause specific populations of cells in a neural circuit to fire, thus making a causal link between the activity of cell types and complex behaviors, cognition, and perception. Importantly, optogenetic protein-based sensors can also report myriad activity in genetically targeted cells, including calcium signaling, transmembrane potential, pH, ATP/ADP ratio, among others. There is a vastly expanding set of community resources that aids the deployment of optogenetic tools. These include genetic targeting technologies (e.g., floxed AAV viruses and transgenic mice to cross with Cre-driver lines) and hardware for light-delivery in freely behaving mammals and invertebrates (currently, their use is limited to the laboratory, and has not been implemented in the field).

Pharmacogenetics

Pharmacogenetics (Shapiro et al. 2012) is an approach similar to optogenetics, where a heterologously expressed membrane receptor or ion channel is agonized by a chemical ligand that is orthogonal to the mammalian receptor repertoire. These include naturally occurring proteins, such as ivermectin-sensitive glutamate-gated chloride channels (McCavera et al. 2009) and engineered GPCRs (Rogan and Roth 2011) and ligand-gated ion channels (Magnus et al. 2011). Although the functional diversity of the toolbox significantly lacks behind that of optogenetics, an appealing feature of pharmacogenetic approaches is that the *in vivo* perturbation is responsive to traditional pharmacological methods of dosing. The temporal resolution is set by the pharmacokinetics of the ligand.

References

- Ahrens MB, Li JM, Orger MB, Robson DN, Schier AF, Engert F and Portugues R. 2012. Brain-wide neuronal dynamics during motor adaptation in zebrafish. *Nature* 485:471–477.
- Ament SA, Wang Y, Chen C-C, Blatti CA, Hong F, Liang ZS, Negre N, White KP, Rodriguez-Zas SL, Mizzen CA, Sinha S, Zhong S and Robinson GE. 2012. The transcription factor ultraspiracle influences honey bee social behavior and behavior-related gene expression. *PLOS One* 8:e1002596.
- Anderson DJ and Perona P. 2014. Toward a science of computational ethology. *Neuron* 84:18-31.
- Aubin-Horth N and Renn SC. 2009. Genomic reaction norms: using integrative biology to understand molecular mechanisms of phenotypic plasticity. *Molecular Ecology* 18:3763-3780.
- Bergman TJ and Beehner JC. 2008. A simple method for measuring colour in wild animals: validation and use on chest patch colour in geladas (*Theropithecus gelada*). *Biological Journal of the Linnean Society* 94:231-240.
- Berman GJ, Choi DM, Bialek W and Shaevitz JW. 2014. Mapping the stereotyped behaviour of freely moving fruit flies. *Journal of the Royal Society Interface* 11:20140672.
- Bradnam, KR, et al. 2013. Assemblathon 2: evaluating de novo methods of genome assembly in three vertebrate species. *GigaScience* 2:10.
- Brown AEX, Yemini EI, Grundy LJ, Jucikas T and Schafer WR. 2013. A dictionary of behavioral motifs reveals clusters of genes affecting *Caenorhabditis elegans* locomotion. *Proceedings of the National Academy of Sciences USA* 110:791–796.
- Cavanaugh J, Monosov IE, McAlonan K, Berman R, Smith MK, Cao V, Wang KH, Boyden ES and Wurtz RH. 2012. Optogenetic inactivation modifies monkey visuomotor behavior. *Neuron* 76:901-907.
- Choleris, E, Little SR, Mong JA, Puram SV, Langer R and Pfaff DW. 2007. Microparticle based delivery of oxytocin receptor antisense DNA in the medial amygdala blocks social recognition in female mice. *Proceedings of the National Academy of Sciences USA* 104:4670e4675.
- Clayton DF. 2013. The genomics of memory and learning in songbirds. *Annual Review of Genomics and Human Genetics* 14:45-65.
- Cybulski JS, Clements J and Prakash M. 2014. Foldscope: origami-based paper microscope. *PLOS One* 9:e98781.
- Daimon T, Kiuchi T and Takasu Y. 2014. Recent progress in genome engineering techniques in the silkworm, *Bombyx mori*. *Developmental Growth Differences* 56:14–25.
- Dombeck, DA and Reiser MB. 2012. Real neuroscience in virtual worlds. *Current Opinion in Neurobiology* 22:3–10.
- Evans JD, Aronstein K, Chen YP, Hetru C, Imler JL, Jiang H, Kanost M, Thompson GJ, Zou Z

and Hultmark D. 2006. Immune pathways and defence mechanisms in honey bees *Apis mellifera*. *Insect Molecular Biology* 15:645-56.

Fan Z, Li W, Lee SR, Meng Q, Shi B, Bunch TD, White KL, Kong IK and Wang Z. 2014. Efficient gene targeting in golden syrian hamsters by the CRISPR/Cas9 system. *PLOS One*. 9:e109755.

Fee MS and Leonardo A. 2001. Miniature motorized microdrive and commutator system for chronic neural recording in small animals. *Journal of Neuroscience Methods* 112:83-94.

Fenko L, Yizhar O and Deisseroth K. 2011. The development and application of optogenetics. *Annual Review of Neuroscience* 34:389-412.

Fudickar AM, Wikelski M and Partecke J. 2012. Tracking migratory songbirds: accuracy of light-level loggers (geolocators) in forest habitats. *Methods in Ecology and Evolution* 3:47-52.

Furey, TS. 2012. ChIP-seq and beyond: new and improved methodologies to detect and characterize protein-DNA interactions. *Nature Reviews Genetics*, 13:840-852.

Gerits A, Farivar R, Rosen BR, Wald, LL, Boyden ES and Vanduffel W. 2012. Optogenetically induced behavioral and functional network changes in primates. *Current Biology* 22:1722-1726.

Haesler S, Rochefort C, Georgi B, Licznanski P, Osten P and Scharff C. 2007. Incomplete and inaccurate vocal imitation after knockdown of FoxP2 in songbird basal ganglia nucleus Area X. *PLOS Biology* 5:e321.

Hamede RK, Bashford J, McCallum H and Jones M. 2009. Contact networks in a wild Tasmanian devil (*Sarcophilus harrisii*) population: using social network analysis to reveal seasonal variability in social behaviour and its implications for transmission of devil facial tumour disease. *Ecology Letters* 12:1147-1157.

Harris RM and Hofmann H. 2014. Neurogenomics of behavioral plasticity. *Advances in Experimental Medicine and Biology* 781:149-168.

Harvey CD, Collman F, Dombeck DA and Tank DW. 2009. Intracellular dynamics of hippocampal place cells during virtual navigation. *Nature* 461:941-946.

Holt HL, Aronstein KA and Grozinger CM. 2013. Chronic parasitization by *Nosema microsporidia* causes global expression changes in core nutritional, metabolic and behavioral pathways in honey bee workers (*Apis mellifera*). *BMC Genomics* 14:799.

Kain J, Stokes C, Gaudry Q, Song X, Foley J, Wilson R and de Bivort B. 2013. Leg-tracking and automated behavioural classification in *Drosophila*. *Nature Communications* 4:1910.

Keevil BG. 2013. Novel liquid chromatography tandem mass spectrometry (LC-MS/MS) methods for measuring steroids. *Best Practices & Research Clinical Endocrinology & Metabolism* 27:663-674.

Kim T-I, McCall JG, Jung YW, Huang X, Siuda ER, Li Y, Song J, Song YM, Pao HA, Kim R-H, Lu C, Lee SD, Song I-S, Shin G, Al-Hasani R, Kim S, Tan MP, Huang Y, Omenetto FG, Rogers JA and Bruchas MR. 2013. Injectable, cellular-scale optoelectronics with applications for wireless optogenetics. *Science* 340:211-216.

Kim, D-H, Viventi J, Amsden JJ, Xiao J, Vigeland L, Kim Y-S, Blanco JA, Panilaitis B, Frechette ES, Contreras D, Kaplan DL, Omenetto FG, Huang Y, Hwang K-C, Zakin MR, Litt B and Rogers JA. 2010. Dissolvable films of silk fibroin for ultrathin conformal bio-integrated electronics. *Nature Materials* 9:511-517.

Landry, CR and Aubin-Horth, N. 2007. Ecological annotation of genes and genomes through ecological genomics. *Molecular Ecology* 16:4419-4421.

Lesku JA, Rattenborg NC, Valcu M, Vyssotski AL, Kuhn S, Kuemmeth F, Heidrich W and Kempenaers B. 2012. Adaptive sleep loss in polygynous pectoral sandpipers. *Science* 337:1654-1658.

Lim MM, Wang Z, Olazabal DE, Ren X, Terwilliger EF and Young LJ. 2004. Enhanced partner preference in a promiscuous species by manipulating the expression of a single gene. *Nature* 429:754 –757.

Lynch HE, Sanchez AM, D'Souza MP, Rountree W, Denny TN, Kalos M and Sempowski GD. 2014. Development and implementation of a proficiency testing program for Luminex bead-based cytokine assays. *Journal of Immunological Methods* 409:62-71.

Mali P, Esvelt KM and Church GM. 2013. Cas9 as a versatile tool for engineering biology. *Nature Methods* 10:957-963.

Magnus CJ, Lee PH, Atasoy D, Su HH, Looger LL and Sternson SM. 2011. Chemical and genetic engineering of selective ion channel–ligand interactions. *Science* 333:1292-1296.

Manfredini F, Lucas C, Nicolas M, Keller L, Shoemaker D and Grozinger CM. 2014. Molecular and social regulation of worker division of labour in fire ants. *Molecular Ecology* 23:660-72.

McCavera S, Rogers AT, Yates DM, Woods DJ and Wolstenholme AJ. 2009. An ivermectin-sensitive glutamate-gated chloride channel from the parasitic nematode *Haemonchus contortus*. *Molecular Pharmacology* 75:1347-1355.

McKay BD. 2013. The use of digital photography in systematic. *Biological Journal of the Linnaean Society* 110:1-13.

Miesenböck G. 2009. The optogenetic catechism. *Science* 326:395-399.

Narzisi G and Mishra B. 2011. Comparing de novo genome assembly: the long and short of it. *PLOS One* 6:e19175.

Nelson RJ. 2011. *An Introduction to Behavioral Endocrinology*, 4th Edition, Sinauer Associates.

Niu Y, Bin Shen B, Cui Y, Chen Y, Wang J, Wang L, Kang Y, Zhao X, Si W, Li W, Xiang AP, Zhou J, Guo X, Bi Y, Si C, Hu B, Dong G, Wang H, Zhou Z, Li T, Tan T, Pu X, Wang F, Ji S, Zhou Q, Huang X, Ji W and Sha J. 2014. Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos. *Cell* 156:836-843.

- NIH. 2014. BRAIN 2025: a scientific vision, brain research through advancing innovative neurotechnologies (BRAIN. Working Group Report to the Advisory Committee to the Director, <http://www.nih.gov/science/brain/2025>).
- O'Connell LA and Hofmann HA. 2011. Genes, hormones, and circuits: an integrative approach to study the evolution of social behavior. *Frontiers in Neuroendocrinology* 32:320-335.
- Pérez-Escudero A, Vicente-Page J, Hinz RC, Arganda S and de Polavieja GG. 2014. idTracker: tracking individuals in a group by automatic identification of unmarked signals. *Nature Methods* 11:743-748.
- Peron C and Gremillet D. 2013. Tracking through life stages: adult, immature and juvenile autumn migration in a long-lived seabird. *PLOS One* 8:e72713.
- Ramos-Cabrer P and Campos F. 2013. Liposomes and nanotechnology in drug development: focus on neurological targets. *International Journal of Nanomedicine* 8:951-960.
- Richard FJ, Holt HL and Grozinger CM. 2012. Effects of immunostimulation on social behavior, chemical communication and genome-wide gene expression in honey bee workers (*Apis mellifera*). *BMC Genomics* 13:558.
- Rissiek B, Koch-Nolte F and Magnus T. 2014. Nanobodies as modulators of inflammation: potential applications for acute brain injury. *Frontiers in Cellular Neuroscience* 8:344.
- Robinson MR, Santure AW, DeCauwer I, Sheldon BC and Slate J. 2013. Partitioning of genetic variation across the genome using multimarker methods in a wild bird population. *Molecular Ecology* 22:3963-3980.
- Rogan SC and Roth BL. 2011. Remote control of neuronal signaling. *Pharmacological Reviews* 63:291-315.
- Sander JD and Joung JK. 2014. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nature Biotechnology* 32:347-355.
- Santure AW, De Cauwer I, Robinson MR, Poissant P, Sheldon BC and Slate J. 2013. Genomic dissection of variation in clutch size and egg mass in a wild great tit (*Parus major*) population. *Molecular Ecology* 22:3949-3962.
- Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, Mikkelsen TS, Heckl D, Ebert BL, Root DE, Doench JG and Zhang F. 2014. Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science* 343:84-87.
- Shapiro MG, Frazier SJ and Lester HA. 2012. Unparalleled control of neural activity using orthogonal pharmacogenetics. *ACS Chemical Neuroscience* 3:619-629.
- Stevens M, Parraga CA, Cuthill IC, Partridge JC and Troscianko TS. 2007. Using digital photography to study animal coloration. *Biological Journal of the Linnean Society* 90:211-237.
- Stolzenberg, DS, Grant PA and Bekiranov S. 2011. Epigenetic methodologies for behavioral scientists. *Hormones and Behavior* 59:407-416

Swierczek NA, Giles AC, Rankin CH and Kerr RA. 2011. High-throughput behavioral analysis in *C. elegans*. *Nature Methods* 8:592-598.

Szuts TA, Fadeyev V, Kachiguine S, Sher A, Grivich MV, Agrochão M, Hottowy P, Dabrowski W, Lubenov EV, Siapas AG, Uchida N, Litke AM and Mesiter M. 2011. A wireless multi-channel neural amplifier for freely moving animals. *Nature Neuroscience* 14:263–269.

Toth AL and Robinson GE. 2009. Evo-devo and the evolution of social behavior: brain gene expression analyses in social insects. *Cold Springs Harbor Symposium on Quantitative Biology* 74:419-426.

Ulanovsky N and Moss CF. 2007. Hippocampal cellular and network activity in freely moving echolocating bats. *Nature Neuroscience* 10:224–233.

Vogelstein JT, Park Y, Ohyama T, Kerr RA, Truman JW, Priebe CE and Zlatic M. 2014. Discovery of brainwide neural-behavioral maps via multiscale unsupervised structure learning. *Science* 344:386-392.

Wall J, Wittemyer G, Klinkenberg B and Douglas-Hamilton I. 2014. Novel opportunities for wildlife conservation and research with real-time monitoring. *Ecological Applications* 24:593-601.

Wang H, Duclot F, Liu FY, Wang Z and Kabbaj M. 2013. Histone deacetylase inhibitors facilitate partner preference formation in female prairie voles. *Nature Communications* 16:919-924.

Wang T, Wei JJ, Sabatini DM and Lander ES. 2014. Genetic screens in human cells using the CRISPR-Cas9 system. *Science* 343:80–84.

Whitworth KM, Lee K, Benne JA, Beaton BP, Spate LD, Murphy SL, Samuel MS, Mao J, O’Gorman C, Walters EM, Murphy CN, Driver J, Mileham A, McLaren D, Wells KD and Prather RS. 2014. Use of the CRISPR/Cas9 system to produce genetically engineered pigs from in vitro-derived oocytes and embryos. *Biological Reproduction* 91:78.

Williams TM, Wolfe L, Davis T, Kendall T, Richter B, Wang Y, Bryce C, Elkaim GH and Wilmers CC. 2014. Instantaneous energetics of puma kills reveal advantage of felid sneak attacks. *Science* 346:81-85.

Willemoes M, Strandberg R, Klaassen RHG, Tottrup AP, Vardanis Y, Howey PW, Thorup K, Wikelski M and Alerstam T. 2014. Narrow-front loop migration in a population of the common cuckoo, *Cuculus canorus*, as revealed by satellite telemetry. *PLOS One* 9:e83515

Whitesides GM. 2006. The origins and future of microfluidics. *Nature* 442:368-373.