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Emerging from the bottleneck: benefits of the comparative approach to modern neuroscience

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Neuroscience has historically exploited a wide diversity of animal taxa. Recently, however, research has focused increasingly on a few model species. This trend has accelerated with the genetic revolution, as genomic sequences and genetic tools became available for a few species, which formed a bottleneck. This coalescence on a small set of model species comes with several costs that are often not considered, especially in the current drive to use mice explicitly as models for human diseases. Comparative studies of strategically chosen non-model species can complement model species research and yield more rigorous studies. As genetic sequences and tools become available for many more species, we are poised to emerge from the bottleneck and once again exploit the rich biological diversity offered by comparative studies.

Biological diversity as a resource for neuroscience

Model species such as the fruit fly (*Drosophila melanogaster*), the nematode 'worm' (*Caenorhabditis. elegans*), zebrafish (*Danio rerio*), the rat (*Rattus rattus*), and, most predominantly, the mouse (*Mus musculus*) have played an important role in biology. A given species may offer particular advantages for the study of a biological process, such as rapid embryonic development, accessible nervous systems, or ease of maintenance in the laboratory. The advantages of model species have become more pronounced with the advent of the genomic revolution. Until recently, sequencing genomes was expensive and laborious, limiting the number of species for which genomic sequences were available. As the database of information for a given model species grows over time, there is an increasing incentive to use that species to investigate topics outside the narrow field of inquiry for which the species was initially chosen. 'Repurposing' of model species, however, can raise concerns – as seen in the ongoing debate about the value of inbred mouse (*M. musculus*) strains as models for understanding human mental disorders [1,2]. While the use of model species has clear practical benefits, adherence to a small number of model systems can limit or even distort the research that is conducted. Neuroscience has a rich history of exploiting a wide diversity of taxa, including mollusks, crustacea, fish, amphibians, birds, and 'exotic' (i.e., non-rodent) mammals, as has been commented on previously [3–5]. We contend that comparative studies of strategically chosen non-model species can complement model species research and address some of the limitations inherent in an over-reliance on a small number of model species. Combining the strengths of a comparative approach with the advantages of model systems will lead to more rigorous research in neuroscience.

Potential limitations of the model species approach

Over the past 20 years or so, neuroscience and much of biology in general has coalesced from the traditional embrace of diverse species down to a small number of model species. There are various practical reasons for this process of concentration. Model species tend to be readily available, easily maintained in captivity, and are feasible to breed in large numbers. As a species becomes a well-established model for a research community, there is an exponential growth in the amount of available information that serves as a platform for future research. With the advent of the genomic revolution, and the ensuing development of powerful molecular tools such as combinatorial systems for gene expression and optogenetics, the incentive to concentrate on a small number of species has become even more pronounced. Conservation of orthologous genes across diverse taxa shows that we can understand much about basic genomic structure and function by studying model species.

The current enthusiasm for a model species approach, however, brings with it several limitations that are too rarely acknowledged. The standard model species represent a vanishingly small percentage of the total biological diversity. As Manger *et al.* [6] wrote: '75% of our research efforts are directed to the rat, mouse and human brain, or 0.0001% of the nervous systems on the planet.' In principle, every species has something to offer to our understanding of and progress in biology. We recognize that it is inefficient and impractical in the current funding climate to devote limited resources to the study of all species that appeal to investigators. Nevertheless, it is important to periodically remind ourselves that this coalescence has brought with it a self-perpetuating myopia and amnesia about the past

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contributions of diverse species that jeopardize possible future contributions from what are currently non-model species. This myopia affects choice of research topic and funding decisions, and might cause biologists to miss out on novel discoveries.

The history of biology is replete with examples of novel discoveries emerging serendipitously through study of 'exotic' species. Some famous examples include the discovery of green fluorescent protein in jellyfish [7], conotoxins in cone snails [8], nerve growth factor in chicks [9], GABA in crabs [10], *Taq* polymerase from the bacterium *Thermopilus aquaticus* [11], and channel rhodopsins in algae [12,13]. Each of these discoveries led to profound changes in how we study and understand the brain, but it seems unlikely that the pioneering research behind these discoveries would be funded under the current model species approach. Do we believe that all of the far-reaching discoveries to be mined from biological diversity are already in hand, and that we can therefore afford to focus future efforts on a dwindling number of well-studied model species? Prudence would suggest that we continue to cast the net broadly, understanding that we can never predict where the next transformative discovery might emerge.

Repurposing model species from their initial use can distort research programs and funding priorities. An example is the current effort to develop the mouse as a model for visual neuroscience [14]. Vision in mice, in turn, is seen as an entry point for understanding higher processes including perception, consciousness, and decision-making [15]. There are, however, considerable limitations to the applicability of the mouse visual system [16]. Mice are nocturnal animals that rely far more on tactile and olfactory cues than on vision for orientation. They are estimated to effectively have vision on the order of 20/2000, which qualifies humans as legally blind (Niell in [16]). This poor visual acuity precludes mice from behavioral visual tasks such as facial recognition and object discrimination that are so fundamental to human vision. While the mouse visual cortex contains the same basic neural subtypes as the human visual cortex, the mouse cortex is not organized into different functional areas that are homologous to the human cortex. In addition, the mouse 'visual' cortex also serves other functions, unlike the human visual cortex that is dedicated to vision. Thus, while the mouse visual cortex may provide valuable insights into basic principles of cellular connectivity and computational processing in relation to vision, the mouse should not replace other animal models of vision such as cats and primates. Similar arguments apply in general to repurposing model species to the study of neural processes underlying sensory and behavioral processes for which they are not specialized.

Inbreeding of model species leads to extensive homozygosity and massive loss of genetic diversity. This approach ignores the important role of pleiotropy in gene function [17], and the polygenic regulation of most behaviors [18]. This loss of diversity and elimination of alleles will impact phenotypic molecular, physiological, and anatomical traits. Laboratory species are selectively bred to produce sedentary, obese, non-aggressive animals with reduced predator avoidance behavior, and are reared in conditions that lack normal social cues [18,19]. Chalfin *et al.* showed, for example, that laboratory mice are of limited use as models for studying the genetic basis of naturalistic behaviors and for identifying polygenic social traits that are relevant to mental disorders, compared with wild mice. For these reasons, the study of inbred model species can yield a picture of neural function that differs considerably from that seen in their wild ancestors.

The initial choice of a model species may be largely determined by practical considerations rather than for any particular biological reason. This fortuitous choice may then commit future generations of investigators to asking questions of this species that were never envisaged by the originator of the model. T.H. Morgan chose fruit flies as a model because they are easy to rear and maintain, have a short generation time, and reproduce in large numbers, and not for genetic considerations *per se* (http://www.nobelprize.org/nobel_prizes/medicine/laureates/1933/morgan-article.html).

The tremendous value of *Drosophila* for genetic studies established it as a model species, and this led generations of investigators to use it for research only indirectly or completely unrelated to genetics. Current investigators, for example, use fruit flies to study the neural basis of processes such as visually guided locomotion [20], olfaction [21], and courtship singing [22]. Given the small size of these flies, however, it is technically challenging to directly measure the electrical activity of single neurons from awake, behaving flies [23], but progress on this front has been made using larger non-model fly species such as blowflies [24–26].

Convergence on selected model species often carries an implicit assumption that mechanisms observed in one species are characteristic of all related species. A focus on any single species, however, fails to encompass the diversity of mechanistic adaptations present in even closely related species that differ behaviorally. An example can be seen in the coalescence of studies of the neural basis of song learning on the zebra finch (*Taenopygia guttata*) [27–29]. The zebra finch was initially chosen for practical considerations such as breeding readily in captivity, being widely available as a domesticated species, and having a single stereotyped song that is experimentally tractable (A.P. Arnold, personal communication). This species is now the dominant model used for avian studies of mechanisms of vocal learning, sensorimotor integration underlying song production, auditory encoding of biologically-relevant sounds, and mechanisms of sexual differentiation of brain and behavior ([30] for review). There are ~4000 species of songbirds, however, and there is extensive diversity in various aspects of song learning and production. No single species can capture all of this diversity, but the zebra finch in particular falls at one extreme on many dimensions of interest [31,32]. Coalescence on any single model species runs the risk of losing information on the diversity of neural and molecular mechanisms.

A particularly important limitation of a model system approach arises from the effort to use the lab mouse explicitly as a model for human disease, a concept we refer to as the '*homusculus*'. Given the biomedical orientation of much of neuroscience, coerced by the current translational

emphasis at the US National Institutes of Health (NIH), there is a strong incentive to develop animal models for disease (e.g., [33]). In this atmosphere, the results of model-species research may be pushed into clinical trials prematurely [34]. Efforts to develop disease models include attempts to 'humanize' model animal species by using genetic engineering methods to alter genes to express human coding sequences, or by grafting human cells into immune-compromised animals [17]. These methods are exciting and hold much potential for improving our understanding of disease processes.

There are, however, considerable limitations to the use of animal models of disease that must be acknowledged before making the transition to human clinical trials (see also [35]). As Beckers *et al.* point out, two important differences between mice and humans are in body size and lifespan. The small size of mice, with their large surface area to volume ratio, results in pronounced metabolic differences from humans. This difference raises serious doubts about the validity of the mouse as a model for brain disorders thought to be associated with metabolic dysfunction, including Alzheimer's, Parkinson's (PD), and Huntington's diseases, major depressive and bipolar disorders, and schizophrenia [36,37]. For example, no genetic model of PD fully duplicates the neural degeneration seen in humans with PD [38].

The short lifespans of mice and most other model species impose different selective pressures for mutation repair and stress responses than in long-lived humans, and this presents obvious limitations for the use of mice as a model for neurological disorders associated with aging, such as cognitive impairment, stroke, and amyotrophic lateral sclerosis (ALS). Using the mouse superoxide dismutase (*SOD1*) gene model of ALS, trials of 90 putative therapeutic compounds led to 11 clinical trials in humans as of 2009, all of which failed [39–41]. Riluzole remains the only approved medication for ALS.

Model species are typically housed under standardized laboratory conditions and are sedentary. Human disease etiology, by contrast, is influenced by exercise and external environmental, as well as endogenous, factors [17]. Studying model species in a controlled laboratory environment fails to replicate the complexity of environmental triggers encountered by humans. Using inbred animals with minimal genetic variability ignores the important contributions of single-nucleotide polymorphisms and copy-number variants to human disease susceptibility, and to resistance to diseases and therapies [17]. The attempt to humanize animal genomes may yield an inaccurate understanding of gene function by failing to replicate epistatic effects, polygenic regulation of complex phenotypic traits, and protein interactions that normally occur in intact human cells. Perrin [42] points out that mouse models typically have several copies of a disease-causing gene, and some or all copies may be lost during meiosis, with the consequence that some individuals in a colony may entirely lack the disease phenotype. A fundamental underlying assumption of animal models for human disease is that gene function and networks are highly conserved between model species and humans, but these traits commonly diverge during evolution [43].

These constraints help to explain why research on animal models has largely failed to translate to successful clinical treatments for disease [1,2,38]. Only 10–20% of interventions for a variety of diseases, including stroke, proposed from animal studies are actually approved for use in humans [42,44].

One final concern is that the high costs of maintaining large mouse colonies can sap the budgets of funding agencies. Because housing for flies, worms, fish, and many non-model species is much less expensive, these animals may offer a greater return on the dollar.

Benefits of comparative approaches

Having discussed the potential limitations of the model species approach, we will consider the positive benefits of the comparative approach in which studies are designed to exploit species diversity in neural mechanisms.

A clear benefit is the potential for discovering novel adaptations that may have broad transformative impact. An example is the study of ongoing neurogenesis in adult brains. The addition of new neurons to the brain of adults of higher vertebrates was first suggested in the pioneering studies of Altman and Kaplan on rats [45,46]. Their claims, however, met with skepticism [47]. The study of adult neurogenesis was dropped for nearly 20 years in the face of the dogma that neurogenesis was completed by birth [48]. This prevailing view only began to be overturned when Nottebohm and colleagues demonstrated neurogenesis in the forebrain of adult songbirds [49–52]. This work in songbirds stimulated investigators to re-examine this topic in mammals. It soon became clear that new neurons are added throughout life to the dentate gyrus and olfactory bulb of mammals including humans [53–57]. Since these initial confirmatory reports, there has been explosive growth in study of the mechanisms and functions of adult neurogenesis in the mammalian brain. The songbird brain provided a more convincing proof of concept for adult neurogenesis than did rats, because they have more widespread neuronal addition in the telencephalon and higher levels of neurogenesis [58–61]. This illustrates how non-model species may be better suited to the identification of novel but fundamental processes than more commonly studied model species. There are numerous other examples, including the discovery of GFP in jellyfish, GABA in crustacea, and neurotrophins in chicks, as discussed above. Who knows what other important phenomena remain to be discovered that might be missed by focusing future research on a small number of model species?

Studying the neural substrate of fundamental processes in 'specialist' species that have evolved an elaborated form of that process has been extremely productive. This approach characterizes neuroethological investigation. Classic vertebrate examples include: the study of sound localization in barn owls (*Tyto alba*) by Konishi, Knudsen, and colleagues [62,63], which provided the first empirical evidence for neuronal delay lines; computational processing of sensory stimuli in weakly electric fish by Bullock, Heiligenberg, and colleagues [64], the first delineation of a complete sensorimotor circuit in a vertebrate brain and unambiguous evidence for 'neuronal democracies' or parallel processing; and prey capture in the common toad

(*Bufo bufo*) by Ewert and colleagues [65], which provided the first computational model of pattern recognition in the visual system. Studies of these elaborated systems have yielded basic insights that then inform investigation of these same processes manifested in a less-elaborated form in non-human primates and humans (e.g., [66]).

Invertebrate species with relatively simple, accessible nervous systems have been crucially important in understanding fundamental processes such as action potential propagation studied in squid giant axon by Hodgkin and Huxley [67], synaptic mechanisms of learning studied in *Aplysia* by Kandel and colleagues [68], central pattern generators first studied in locusts by Wilson [69], and neuromodulation studied in the crustacean stomatogastric nervous system by Selverston, Marder, and colleagues [70]. These studies of invertebrates have been so productive to a large extent because they provide tractable nervous systems that can be functionally dissected. It is difficult to exaggerate the impact that this work has had on our understanding of the core topics of membrane excitability, neural and molecular mechanisms of learning, pattern-generating neural networks, and neuromodulation. Invertebrate models continue to offer the advantage of having accessible nervous systems that are more complex and functionally linked to more interesting behaviors than can be found in a simple model such as *C. elegans*, but which present a more accessible model than found in vertebrates [71].

Comparative study of species from different phyletic lineages can be useful for critical tests of hypotheses. Closely related species, such as rats and mice, may share neural mechanisms because of recent common ancestry. Selective study of a small number of related model species may consequently lead to the conclusion that shared mechanisms are essential for the regulation of a given phenomenon. A good example of this bias comes from the study of grid cells in the entorhinal cortex (EC). These cells fire when an animal moves through the vertices of a periodic hexagonal grid that spans the environment, and they are therefore thought to encode a neural representation of space [72]. In rodents grid cells co-exist with ongoing theta band (4–10 Hz) oscillations, and it has consequently been hypothesized that interference between theta oscillations in the soma and dendrites of single neurons is necessary for transformation of a temporal oscillation into the spatial response grid [73]. Yartsev *et al.* [74] tested this hypothesis in the Egyptian fruit bat (*Rousettus aegyptiacus*). They found grid cells in the EC that were similar to those in rodents, but no evidence of continuous theta-band oscillations and essentially no theta-band modulation of grid cell activity. This clever comparative study refuted the dominant model of grid cell spatial selectivity arising from theta oscillation interferences, a hypothesis that came from a selective focus on rodents. This example nicely demonstrates the value of exploiting species diversity to test mechanistic hypotheses, as well as the risk of limiting analysis to one or a few closely related model species.

Any one model species has limitations in what it can tell us about neural mechanisms. Expanding the palette to include analysis of diverse species can mitigate these limitations. An example is the growing emphasis on the use of the mouse as a model for the visual system, as discussed above. The wide availability of mutant strains provides a powerful tool for manipulation of the visual system, but there are severe challenges in generalizing the results of mouse studies, given the many limitations already presented [16]. We see the value of a simplified model such as the mouse visual system is in developing tools and framing questions that can then be applied to other species that more closely approximate humans.

The comparative approach is of value even when focusing on rodents. While researchers intensively focus on THE standard lab mouse, there are 2000 species of rodents (500 in the family including rats and mice). Many other rodents show promise for tackling translational questions. As an example, grasshopper mice (*Onychomys torridus*) from the Sonoran desert prey on scorpions and are resistant to their stings. A sodium channel specific to nociceptors (Nav1.8) has evolved in grasshopper mice to be blocked rather than activated by scorpion venom [75]. Understanding the interaction between the venom peptides and sodium channels could lead to new non-addictive analgesics. Another example is the naked mole rat (*Heterocephalus glaber*) from Africa. Wild mole rats live as long as 40 years underground in hypoxic, hypercapnic conditions, whereas most wild rats and mice live less than 1 year. Mole rats do not develop cancer and thus have much potential to help us understand mechanisms of cancer resistance and anoxia tolerance [76].

Concluding comments: looking backward, looking forward

During the years when neuroscience was emerging as a distinct field of study, pioneering investigators worked on an eclectic variety of wild species, choosing the species for the question [5], rather than the question for the (model) species – as is too often the case now. Research on marine invertebrates, insects, fish, salamanders, frogs, turtles, chicks, and bats played a large role in developing this field. Pioneering neuroscientists and physiologists such as Ted Bullock, Steven Kuffler, Per Scholander, and George Bartholomew felt free to work on an extraordinary diversity of species, following their curiosity where it led. The work done by this generation yielded astonishing insights that laid the foundations for the explosive growth of neuroscience and physiology. We look back with longing on these free-ranging early days of neuroscience and ponder upon what has been lost in the narrowing of the field to study of so few species.

The success of the early pioneers of neuroscience contributed to the coalescence of research on a smaller number of selected species. Students and postdocs working in their laboratories built their careers around species that their mentors showed to be productive for investigation of particular questions. As the amount of background information for these systems increased exponentially, the impetus for other scientists to focus their efforts on these selected species became ever greater. In this way what started out as novel species for study morphed into established model species. In the past, zebra fish, fruit flies, *C. elegans*, and even rodents must all have seemed like

exotic animals to use in research, though this is difficult to comprehend now.

The narrowing of the research enterprise to a very few model species moved into overdrive with the onset of the genomic revolution. Initially only a few model species were selected for laborious and expensive genome sequencing. The availability of genetic sequences allowed the development of powerful molecular tools for manipulating gene expression such as knockouts, knock-ins, manipulation of transcriptional switches through combinatorial methods, and optogenetics. The availability and successful application of these tools for only a limited number of species further reinforced the coalescence of research on a few model species. The limited availability of genome sequences and the tools they allowed essentially formed a bottleneck that has only reinforced the concentration of research around a small number of species.

The good news is that we are now poised to emerge from that bottleneck and once again broaden the range of species used for research. As the costs and labor required for genome sequencing decrease, many more species are being sequenced. Efforts such as the Genome 10K project (https://genome10k.soe.ucsc.edu), which aims to sequence 10 000 vertebrate species, hold tremendous promise of making genetic information and tools available for vertebrates in essentially every genus. An important step toward this goal is the recent series of reports on the whole-genome sequencing of 48 bird species spanning 32 of the 35 recognized orders [77]. The Global Invertebrate Genomics Alliance (http://nova.edu/ocean/giga/) has a similar goal. These genomic sequences, combined with new methods such as TILLING [78], TALENS [79], and CRISPR/Cas9 [80] that enable precise DNA editing, hold the potential to generate transgenic lines of a wide range of species. The first generation of studies to use these genomic editing tools in non-model species is already appearing [81–85]. The availability of sequence data should facilitate the adaptation of optogenetic and RNA-interference methods to manipulate gene expression in non-model species. Given the benefits of studying diverse species discussed above, we believe that we are on the threshold of an exciting renaissance of comparative approaches to neuroscience. Grad students, dust off your field boots!

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