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Current Opinion in Neurobiology

From classic ethology to modern neuroethology: overcoming the three biases in social behavior research

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A typical current study investigating the neurobiology of animal behavior is likely restricted to male subjects, of standard inbred mouse strains, tested in simple behavioral assays under laboratory conditions. This approach enables the use of advanced molecular tools, alongside standardization and reproducibility, and has led to tremendous discoveries. However, the cost is a loss of genetic and phenotypic diversity and a divergence from ethologically-relevant behaviors. Here we review the pros and cons in behavioral neuroscience studies of the new era, focusing on reproductive behaviors in rodents. Recent advances in molecular technology and behavioral phenotyping in semi-natural conditions, together with an awareness of the critical need to study both sexes, may provide new insights into the neural mechanisms underlying social behaviors.

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Current Opinion in Neurobiology 2016, **38**:96–108

This review comes from a themed issue on **Neurobiology of sex**

Edited by **Barry J Dickson** and **Catherine Dulac**

For a complete overview see the Issue and the Editorial

Available online 12th May 2016

http://dx.doi.org/10.1016/j.conb.2016.04.014

Introduction

The study of animal behavior, termed 'ethology' [1], was founded by Nikolaas Tinbergen, Konrad Lorentz and Karl Von-Frisch in the middle of the 20th century. Initially, it consisted of the observation and characterization of behaving animals within their natural surroundings [2•]. Three central processes took place as this science transitioned into what is now referred to as behavioral neurobiology (Table 1).

The first was the domestication and inbreeding of animal models, alongside the focus on a few selected species, primarily laboratory mice [3•]. The second was the simplification of experimental settings, with a transition from field studies, through the seminal 'universes' founded by Calhoun in the 1960s (Figure 1c,d) [4], to the standard laboratory apparatuses commonly used today [5]. The third process was the narrowing of research focus to only one of the sexes, typically the male [6•].

The main advantages of these changes are genetic uniformity together with experimental standardization and reproducibility [7]. Notably, this reductionist approach has enabled remarkable discoveries, advancing the field of behavioral neurobiology to a state it likely would not attain otherwise [8–10]. However, these processes have also abolished much of the genetic diversity available in natural animal populations, substantially reducing the complexity of quantitative traits [11] and limiting the scope of the behavioral phenotypes observed in the laboratory [3•,12,13]. These boundaries are especially limiting when it comes to social and reproductive behaviors [14••,15•].

Thus, there is a cause for concern regarding the validity of using inbred laboratory mice and common experimental methodologies in studying ethologically-relevant social behaviors and identifying polygenic social traits. Such practices may have hampered progress in our understanding of how the brain controls the richness and complexity of a wide range of natural behaviors essential to the survival of all species, including humans.

Indeed, despite vast multidisciplinary advances in studying the mechanisms underlying social and reproductive behaviors, including sexual [16,17], parental [18], and aggressive [19] behaviors, the molecular and neural factors underpinning these complex behaviors in males and females are still poorly understood.

Here, we will discuss the caveats and advantages of modern research in laboratory animals, focusing on the neural basis of innate sexually dimorphic reproductive behaviors. We will provide examples of the overwhelming research biases in the chosen animal model, the conditions of the experimental environment, and the sex of the tested subjects. Finally, we will review recent studies that integrate new ethologically-relevant approaches with revolutionary molecular tools. These new paradigms might offer a deeper and more comprehensive understanding of how reproductive behaviors are governed by the brain.

The species bias: black mice as the model of choice

The early research of behavioral sciences used a large variety of model species ranging from insects to birds to

Table 1

Milestones in the research of reproductive behavior: from classic ethology to the modern lab

300BC	Aristotle and Erasistratus perform the first documented experiments on living animals	[149]
1849	Arnold Berthold demonstrates the role of gonads in reproductive behaviors in roosters	[150]
1859	Charles Darwin's theory of evolution, including the ideas of sexual selection and intrinsic behavior	[151]
1859	Isidore Saint Hilaire first uses the term 'ethology'	[152]
1900	Walter Heape discovers breeding seasons and the estrous cycle	[153]
1902	Ernest Starling and William Bayliss identify the first blood-driven hormone, secretin	[154]
1909	Jakob von Uexküll introduces the concept of Umwelt–the environment and inner world of animals	[155]
1921	Clarence Little breeds the mouse strain C57BL from female no. 57 of Abbie Lathrop's farm	[156]
1927	Karl von Frisch's book *The Dancing Bees* interprets the meaning of the waggle dance	[157]
1935	Konrad Lorenz describes the phenomenon of imprinting	[158]
1942	Hans Selye demonstrates the effect of a reproductive hormone on the neurobiology of rats	[159]
1951	Nikolaas Tinbergen's book *The Study of Instinct* describes innate behaviors and their adaptive value	[1]
1953	James Watson and Francis Crick uncover the double helix structure of the DNA	[160]
1956	John King uses semi-natural conditions to study the social behavior of domestic guinea pigs	[161]
1959	William Young demonstrates the role of testosterone in the sexual differentiation of guinea pigs	[162]
1960	Oliver Pearson designs an automatic photography system to monitor the activity of rodents	[163]
1962	John Calhoun establishes his first 'universe' to study how population density affects rodent behavior	[4]
1963	William Cochran develops an automatic radio-tracking system to monitor animal movements	[164]
1966	John Mackintosh examines the effect of intruders on resident mice in relation to olfactory stimuli	[63]
1971	Foundation of the Behavior Genetics Association and its journal *Behavior Genetics*	[165]
1975	Edward Wilson establishes the field of sociobiology	[166]
1977	FDA guidelines exclude women from participating in phase I and II clinical trials	[167]
1981	Production of the first transgenic mouse strain	[168]
1993	FDA and NIH guidelines mandate participation of women in clinical trials and data analysis by sex	[167]
1996	Development of Cre-recombinase-based conditional expression methods	[169]
1997	Discovery of vasopressin's role in pair bonding and parental behaviors of prairie voles	[170]
2002	A high-quality draft sequence and analysis of the C57BL mouse genome	[20]
2005	Optogenetics — the use of light to control modified neurons expressing light-sensitive ion channels	[171]
2006	Release of the Allen Mouse Brain Atlases — gene expression maps for the mouse brain	[22]
2012	CRISPR-Cas9 is first described as a genome engineering/editing tool in human cell cultures	[48]
2015	NIH issues mandate to consider sex as a biological variable in all NIH-funded research	[120]
2016	A transgenic primate model of autism is produced using CRISPR-Cas9	[143]

non-human primates (Table 1) [3•]. Various practical aspects, such as low maintenance, high reproductive rate, and short life cycle have gradually turned the laboratory mouse into the animal model of choice in biology and biomedical studies [3]. This process became even more profound in recent decades with the extensive increase in knowledge and available tools developed in the field of mouse genetics [20] and neuroscience [21, 22]. A process that occurred in parallel was the domestication and artificial selection of mice (Box 1), adapting them for breeding, maintenance, and study in the laboratory [23,24]. This deliberately selective process favored strains presenting traits that promote reproductive success, reduced aggression, and eased handling under laboratory conditions [14••,24,25••]. A striking example of a trait that has disappeared with artificial inbreeding and domestication is the adaptive avoidance of mating with close relatives [26,27]. The overall outcome of these human-driven processes was improved experimental consistency and reproducibility, which have led to significant discoveries in all areas of life sciences [24]. Yet, this genetic homogeneity produced phenotypes that present only a limited diversity of quantitative traits, especially those pertaining to animal behavior [23]. Behaviors like freezing, fleeing, and conspecific aggression evolved to maximize fitness in the natural environment, but possess no advantage (and even some disadvantages) under laboratory conditions and therefore became significantly reduced or even lost [28]. On the other hand, traits that carry disadvantages in nature but might be beneficial under laboratory conditions became common, like the production of large litters and early sexual maturation [24].

We have recently demonstrated robust differences between laboratory mice and mice derived from wild-caught individuals in several anatomical, physiological, and behavioral parameters [14••]. Wild mice were smaller, had extremely higher corticosterone levels, and displayed increased anxiety. However, the truly striking differences between the strains were seen in the social behaviors of