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# 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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## 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**

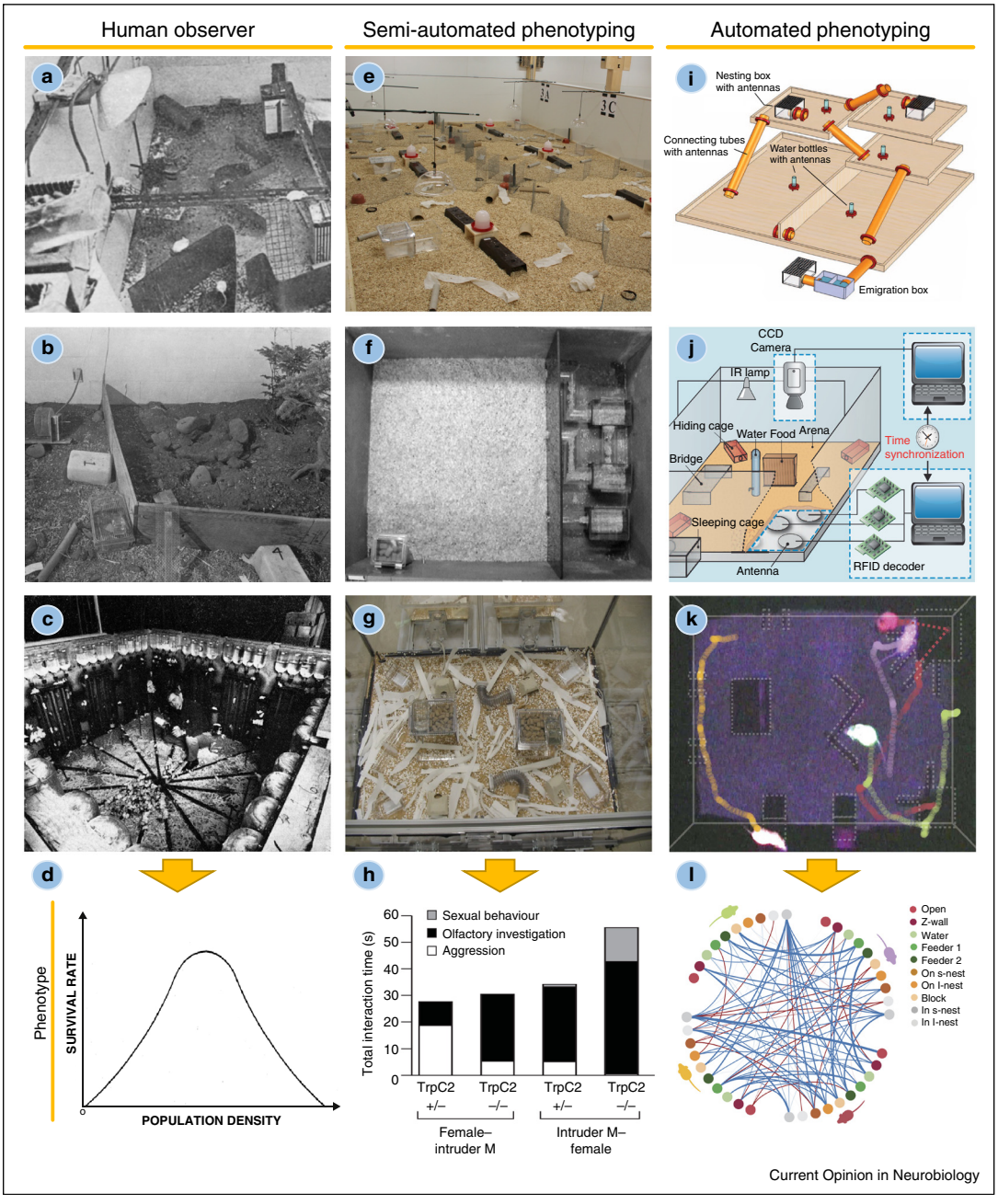
**Milestones in the research of reproductive behavior: from classic ethology to the modern lab**

| | | |
|---|---|---|
| 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

**Figure 1**




**Studying ethologically-relevant social behaviors under semi-natural conditions from classical observations to high-throughput automated phenotyping. (a)–(d)** Recording of behaviors by a human observer (in real time) to measure (a) social and territorial behaviors in mice [77], (b) dominance and sociability in gerbils [78], and (c,d) reproduction and survival of rodents [4,74]. **(e)–(h)** Recording of behaviors by audio or video devices followed by offline analysis, to measure (e) social behavior in groups of mice by combining USV recording and video-based recording in the research of social status, mating and reproductive success (unpublished data, courtesy of Prof. Dustin Penn), (f) pairwise and group social behaviors of rodents in the visible burrow system, containing many features of a natural habitat, including burrows, tunnels and an open surface area (courtesy of Prof. Caroline Blanchard), and (g,h) complex reproductive behaviors in a group of genetically modified female mice [42•]). **(i)–(l)** High-throughput automated behavioral phenotyping systems of multiple mice, used to measure (i) behavioral differences in correlation to neural development based on RFID tracking of multiple mice [92], (j) locomotion, pairwise social interactions and group social hierarchy based on fusion of video and RFID tracking [89•], and (k,l) complex interactions in a group of mice using fluorescence-based identification [15•].
*Source*: Images are reprinted with permissions from Elsevier (a), Koninklijke Brill NV (b), Oxford University Press (d), AAAS (i), and eLife Sciences (k,l).

**Box 1 The history of laboratory mice**

Wild mice were first described by the Swedish biologist Carl Linnaeus in 1758 [53]. However, they were first used in scientific research by Gregor Mendel who spent his initial heredity experiments trying to breed mice. Apparently, Mendel's bishop did not approve his work on animal reproduction, and thus forced him to switch to peas [54]. Mice were not considered again for scientific research until the beginning of the 20th century, although they were domesticated and raised as pet 'fancy mice' [55]. In Massachusetts, Miss Abbie Lathrop purchased mice from other fanciers and started breeding them as pets for sale. Some of her customers were scientists, including William Castle and Clarence Little, who worked on Mendelian heredity of mouse colors [55,56]. Lathorp herself, a retired school teacher, collaborated with Leo Loeb of the Washington University in St. Louis to study tumor development using her bred laboratory mice [57]. These scientists quickly saw the need for genetically-homogeneous mouse strains, and began inbreeding their mouse colonies. The first inbred strain, DBA, was created in 1909, and in 1929 Little established the Jackson laboratory, currently the largest collection of inbred (i.e., resulting from at least 20 generations of brother-sister mating) mouse strains [56]. Most of the commonly-used inbred strains of mice available today, including the popular C57BL/6, originate from the collection of fancy mice bred by Lathrop over a century ago [55].

females. For example, the majority of sexually naïve wild females presented inter-female aggression and pup-directed aggression. In contrast, sexually naïve laboratory females did not present inter-female aggression and were spontaneously parental to unfamiliar pups [14••]. Since these behaviors are absent in laboratory female mice, it is impossible to examine their underlying mechanism using common genetic techniques. One possible solution is to backcross laboratory transgenic mice with wild-derived mice. This method was successfully applied in our laboratory by generating mutant mice lacking a functional vomeronasal organ (VNO) [29] with a wild-derived genetic background. These wild-backcrossed mice displayed all the relevant behavioral traits that were lost during domestication and artificial inbreeding (and in similar levels to that of wild mice), allowing us for the first time to assess the role of VNO-mediated inputs on female aggressive behaviors [14••]. This study uncovered the crucial role of VNO-mediated signaling in the control of conspecific aggression in females, as was previously demonstrated in laboratory male mice [29,30]. Such unique methodology can enable researchers to integrate advanced molecular tools in studies on the neural basis of complex social and reproductive behaviors, without forsaking the genetic and behavioral diversity of wild mice.

In addition to wild mice, other rodent strains can be used to explore new questions on the neurobiology of reproductive behaviors that cannot be examined in the classical laboratory mouse. For example, social attachment, pair bonding, and biparental care of offspring are displayed by prairie voles (*Microtus ochrogaster*) [31]. This rodent species displays sexual monogamy, a social trait presented by less than 5% of mammals [32], and thus allowed researchers to establish the involvement of the neuropeptides oxytocin (OT) and arginine-vasopressin (AVP) in social affiliations [33,34]. Specifically, early works showed that in female prairie voles, intracerebroventricular administration of OT promotes, while OT receptor antagonist inhibits, pair bonding [35]. In male prairie voles, the administration of AVP receptor antagonist into the ventral pallidum suppresses partner preference [36], while viral-mediated over-expression of the receptor advances this process [37].

Another rodent species that has been used in social behavior research is the deer mouse (genus *Peromyscus*) [38], which display several unique behavioral traits like the burrowing of complex architectures and season-specific feeding preferences [38,39]. The majority of *Peromyscus* strains are non-monogamous. However, at least two monogamous strains have evolved independently within this species with both displaying biparental behavior [40]. One of these strains, the California mouse (*Peromyscus californicus*), was used as an important model for the research of paternal behavior and its hypothalamic control [41]. The ability of monogamous and non-monogamous *Peromyscus* strains to mate and bare offspring provides a unique opportunity to unravel novel reproductive mechanisms [38].

It should be emphasized that many discoveries in life sciences in general and specifically in the neurobiology of social behavior, were realized only due to the extensive use of inbred laboratory mouse strains. These animal models allowed scientists to harness advanced molecular tools that were not available otherwise until very recently (e.g. transgenic and knockout mouse strains), and uncover substantial mechanisms underlying important social behaviors. In this framework, the crucial roles of specific ion channels within the VNO and the main olfactory epithelium (MOE) in mediating key reproductive behaviors were confirmed through the use of specific knockout transgenic mouse lines [29,30,42•,43,44]. The integration of transgenic mouse lines with advanced molecular tools like optogenetics or conditionally expressed genes enabled researchers to identify the distinct role of specific neuronal populations within various brain regions (like the olfactory bulbs [45], medial amygdala [46], and medial prefrontal cortex [47]) in mediating social behaviors. Nowadays, with the genome-wide sequencing of more and more organisms, behavioral scientists can utilize new molecular gene editing tools like CRISPR-Cas9 [48] and TALEN [49], which enable genetic modifications in non-traditional model systems. The CRISPR-Cas9 method has already been used successfully on non-murine rodents [50], and TALEN was employed in the field of reproduction to induce female-specific sterility in silkworms [51]. With these new and classical genetic tools,

applied on ancestor wild mice and rats as well as other species, we will hopefully face a revolution in the study of social behaviors [52].

## The environmental bias: simpler is not always better

Behavioral scientists have developed an abundance of experimental paradigms, modeling simple and complex behaviors alongside psychiatric illnesses, ranging from locomotion and anxiety, to depression and cognitive function, to social behavior deficiencies such as autism spectrum disorders (Table 1) [5,58,59]. The vast majority of these paradigms share one common feature — almost all are conducted in defined, small, and artificial apparatuses, and usually for a short period of time [5,7,58,59]. In addition, practically all the current behavioral paradigms, even those explicitly examining social behavior, use one or two animals at the most and usually with limited physical contact [5,60].

As with the species choice, a limitation of standard behavioral assays in studying complex social behaviors is that they are exceptionally restrictive [25], where even the mildest artifacts in environmental conditions can have a significant effect on the behavioral outcome [61,62]. For instance, aggression and mating are examined by the resident-intruder assay, typically conducted in the 'shoebox' home-cage of the subject mouse, which is housed individually and exposed to an unfamiliar conspecific (male or female) for 10–15 min [60,63]. Another laboratory method involves using the 'tube test' to analyze social hierarchy (dominance). In this assay, pairs of animals are inserted on two opposing sides of a narrow tube that only one of them can occupy. The dominant animal is the one that manages to cross the tube, forcing the submissive individual to retreat [64]. Such a test does not allow a direct examination of how social hierarchies are established and maintained as they typically are in nature [65,66]. Therefore, performance in the tube test might reflect differences in locomotion or anxiety, and not necessarily the social status of the animal [67,68]. This variety of behavioral assays has produced an abundance of knowledge, modeling bilateral social behaviors under laboratory settings. For instance, it allowed the identification of specific circuitry underlying aggressive behaviors [69–72] and of the relation between synaptic plasticity and social status [73]. However, the problem arises in the attempt to generalize their conclusions to complex social behaviors within groups.

The solution to this problem started to emerge with the employment of semi-natural enclosures where multiple animals, individually marked by ear tags or fur dye, were kept for many days, free to interact with each other and form complex social interactions [25••,74–76]. Meanwhile, their behavior was sampled by a monitoring human observer [27,77–79] (Figure 1a–d), or a video camera [42•,80,81] (Figure 1e–h). Technological advances in automated tracking and image processing [82–87], together with high-throughput data analysis [87,88], enabled the automatic analysis of such social behaviors in groups of animals over multiple days [15•,83,89•,90] (Figure 1i–l). For example, using a tracking system that can identify unique fur bleaching patterns [83], Neunuebel *et al.* recorded ultrasonic vocalizations (USV) in groups of male and female mice, and were able to isolate and assign specific USVs to individually videotaped mice, demonstrating that female mice also use USVs to communicate with males during courtship [91]. In another unique automated behavioral phenotyping setup, Shemesh *et al.* [15•] established a mice arena where individual detection was based on color-fluorescent fur labeling. The authors identified the positions of the mice and employed multiple mathematical models to analyze individual, pairwise, and higher-order correlations in their locations [15•] (Figure 1k,l).

Another technique to track multiple individual mice in large arenas utilized a radio frequency identification (RFID) system [92]. This tracking methodology allowed researchers to follow 40 mice for several weeks in an enriched environment, and to correlate their individual behaviors with hippocampal neurogenesis [90] (Figure 1i). Recently, a new tracking technology developed in our laboratory succeeded in fusing video footage with RFID data, thus identifying locations of individual mice within a group placed in semi-natural conditions for many days with high spatial and temporal resolution (Figure 1j) [89•]. The individual dynamic locations were then translated using designated algorithms into a complete behavioral phenotype that consists of individual, pairwise, and group behaviors. Specifically, the newly identified behaviors include locomotion and anxiety-related behaviors alongside social behaviors between pairs of mice, such as chasing, approaching, and avoidance. Moreover, the algorithms can also reveal complex group behaviors, particularly the formation of a social hierarchy [89•].

With the increasing use of such semi-natural automated methodologies for behavioral phenotyping, scientists should gain a more reliable representation of the mechanisms underlying complex ethologically-relevant social behaviors. The final goal should be to reveal the neural and molecular basis of complex social behaviors exclusive to groups of animals, such as social deficiency, group communication, and reproductive competition that can only be studied in such elaborate semi-natural systems.

## The sex bias: do we really believe females are just smaller males with less testosterone?

In a systematic review, Beery and Zucker examined sex bias in 10 different research fields in biology using animal

models, and found a male bias in 8 of them, most prominently in neuroscience [6•]. They also noted that even studies investigating both sexes usually do not analyze the results by sex. For the purpose of this review, we performed an independent analysis of the sex-bias in studies examining the molecular and circuit-level basis of aggressive, parental, and sexual behaviors in mice, indexed by PubMed in the past 5 years. Our analysis shows that out of a total 168 research articles, only 8 examined the same phenotypes in both sexes, while another 19 examined both sexes but did not analyze the same social behaviors between them. The other 141 (>80%) investigated the neural basis of these reproductive behaviors in only one of the sexes, mostly in males (see also Supplementary Figure 1). This bias probably stems from the desire to minimize the number of subject animals and to avoid the effects of cycling hormonal levels in female subjects [13]. These arguments are still being debated, since emerging studies suggest that data derived from female subjects is no more varying than male-derived ones [93–95]. However, considerations like higher variability of females cannot serve as an excuse in the study of reproductive behaviors, which are sexually dimorphic by definition [96•]. Moreover, we cannot simply assume that any underlying neurobiological mechanism identified in males can also be attributed to females and vice versa. Therefore, any research in the field must relate to both sexes and compare them appropriately. We will elaborate on some of the prominent works studying the neurobiology of reproductive behaviors in recent years, emphasizing the need to examine both sexes in the same study.

Sexually dimorphic behaviors in rodents are usually triggered by pheromones that are detected by the VNO and the MOE [97]. Isogai *et al.* used in situ hybridizations to identify which ligands and stimuli activate the VNO pheromone receptors, demonstrating clear differences between males and females even at the levels of sensory perception [98]. By exposing mice to various odor stimuli, the authors identified 28 receptors detecting conspecific cues, out of which 26 detected either male or female cues exclusively, and in each sex at least 2 receptors detected opposite sex cues exclusively [98]. From the VNO, pheromone processing is relayed to the accessory olfactory bulb (AOB), and then to the medial amygdala (MeA) [97]. Bergan *et al.* [99•] recorded the activity of neurons in the mouse accessory olfactory system in response to urine stimuli from predators and conspecific males and females. In the MeA they showed that in both sexes, neurons responding most strongly to predator stimuli resided separately from neurons responding to conspecific stimuli [99•]. Within the conspecific-responding neurons, the authors identified a striking sexual dimorphism, namely that most of the male units show greater responses to female stimuli and vice versa for female units [99•].

An example of sex bias in the research of pheromone-mediated sexual behaviors comes from studies that identified exocrine-gland secreting peptides (ESPs) as pheromonal signals. ESP22, which is detected through the VNO, was found in tears of juvenile mice and was shown to repress sexual behaviors in adult male mice [100]. The effect of this pheromone on female reproductive behavior, however, was not examined. In contrast, VNO-mediated ESP1 signaling was shown to enhance sexual receptivity in females a few years ago [101], but its effects on males were only examined very recently, demonstrating an aggression-inducing effect [102]. An additional category of pheromones is the major urinary proteins (MUP). These proteins, highly secreted in the urine, were shown to possess multiple roles in the social communication between individuals [103,104]. For the most part, the effects of MUPs have been investigated in males, where they were designated as facilitators of inter-male aggression [103,105]. In contrast, MUPs appear to play a different role in females, for example Darcin, which was identified as a male-emitted MUP, promoting attraction of females [106]. Moreover, it was recently shown that cycling progesterone levels control the specific perception of male MUPs in the female mouse VNO [107•]. During diestrus, neurons which exclusively detect male MUPs are silenced by progesterone, while other neurons like predator-specific neurons are not affected [107•]. The authors in this study point to a parallel effect in which juvenile or subordinate male mice are typically indifferent to aggression-inducing MUPs emitted by other males [107•]. This further supports the notion that studies exploring the neural basis of reproductive behaviors should examine both sexes, even for phenomena which seem completely unique to one sex.

In another line of research, a major role has been described for the MeA and ventromedial hypothalamus (VMH) in regulating both aggressive and mating behaviors in male mice (reviewed in [19,96•]), with very little exploration of these regions' parallel function in females. In males, optogenetic stimulation of MeA GABAergic neurons was shown to induce mounting behaviors towards male and female intruders [46]. In the VMH, researchers recorded increased activity in males during investigation of females, but not during later consummatory sexual behaviors [70]. In one of the few rare studies examining both males and females, the authors discovered that high-intensity optogenetic activation of estrogen receptors expressing neurons in the VMH promotes aggression in males and social investigation (but not attack) in females [108••]. In contrast, low-intensity activation of these neurons induced mounting behaviors in both sexes [108••].

In most mammals, parental care relies almost entirely on the female. This dimorphism is well-manifested in laboratory mice, where sexually-naïve females present spontaneous maternal behaviors towards unfamiliar pups,

while sexually-naïve males ignore or attack them [18]. Thus, researchers focus almost exclusively on maternal behaviors and disregard paternal behaviors. In females, oxytocin inputs from the paraventricular nucleus (PVN) to the auditory cortex were shown to enhance neural responses to pup calls [109], after inhibitory inputs from the PVN to the primary auditory cortex are modulated during the transition to motherhood [110]. Also, oxytocin signaling in the female medial prefrontal cortex was found crucial for expression of sexual receptivity in estrus females, with no apparent effect on social behaviors of diestrus females or males [111]. In male mice, a distinct sub-region in the medial preoptic area (MPOA) was identified as a gradual modulator between infanticide and paternal behaviors [112], in a VNO-dependent manner [113].

Two recent papers exploring the neural basis of parental care demonstrate the necessity of testing both sexes. In the first, the authors discovered a subset of galanin-expressing neurons within the MPOA that control parental behavior in both male and female mice. In male mice these neurons are also involved in directing mating behaviors and reducing aggressive responses towards both male conspecifics and unfamiliar pups [114••]. One notable conclusion of this study was that the same molecularly-defined circuit regulates parental care in both sexes [18]. In the second study, researchers focused on tyrosine-hydroxylase expressing neurons ($TH^+$) in the anteroventral periventricular nucleus (AVPV) that display a robust female-biased dimorphism. In female mice, these neurons regulate maternal behavior, without affecting other aspects of reproduction. In male mice, $TH^+$ AVPV neurons do not influence paternal behavior, but inhibit aggressive responses in general [115••]. This study's underlying conclusion was that there might be two distinct circuits that regulate maternal and paternal behavior [115••]. The question of whether maternal and paternal behaviors are coordinated by a single or two separate neural circuits remains open, warranting further research that simultaneously compares the molecular and neural mechanisms underlying pup-directed behaviors in males and females, whether in mice or in other animal species.

Unlike most studies that investigate one specific sexually-dimorphic reproductive behavior, a few recent papers comprehensively described an array of sexually dimorphic behaviors in both sexes [116•,117,118]. Xu and colleagues identified sexually-dimorphic expression of several genes in the mouse hypothalamus and amygdala. Two of these genes regulate mating and aggression in males, while two others control sexual receptivity, maternal care, and maternal aggression in females [116•]. Later work from the same laboratory showed that progesterone receptor-expressing neurons in the mouse VMH control mating and aggressive responses in males and sexual behavior in females [117]. Finally, another study revealed that aromatase-expressing neurons in the mouse MeA regulate inter-male aggression in males and maternal aggression in females [118]. It was suggested that the inability to evoke non-maternal aggression in females might rely on the specific strain of laboratory mice or rats used [119]. In the case of aggression, it is

**Box 2 implications of sex bias in the research of autism spectrum disorder (ASD)**

ASD is a neurodevelopmental disorder with a strong male bias (a ratio of 2–3 males per female diagnosed [121]) that has been studied extensively in male mouse models (e.g. [122–124]). However, the few studies that have employed and compared both male and female rodents have already uncovered interesting findings regarding the neuronal basis of ASD. Using the common BTBR mouse model for autism, researchers have shown that chronic intranasal administration of oxytocin improves social behavior, but only for male mice [125]. In contrast, rearing alongside neurotypical C57BL/6 mice alleviates the social deficits of BTBR males and females in a similar manner [126].

Among transgenic mouse models for autism, some notable studies explored mutations in the SHANK gene family that were discovered in ASD patients with a sexually dimorphic phenotypic display and a gradient severity of ASD symptoms [127]. Knockout mouse models of SHANK genes present a similar gradient of ASD-like severity, but are inconsistent with regards to the sex bias. In $Shank1^{-/-}$ mice, the only autism-related deficits were in the social communication of adult males and female pups [128]. In $Shank2^{-/-}$ mice, both sexes displayed marked autism-related social deficits compared to wild-type littermates, although some behavioral displays were sex specific [129]. In $Shank3^{-/-}$ mice, the autism-related behavioral deficits were more severe [130], with no significant differences between males and females [131].

An autism-like state was also induced in animal models by prenatal exposure to valproic acid (VPA). Male VPA rats display deficits in social and cognitive behaviors with alterations in several immunological parameters. In contrast, female VPA rats exhibit only partial behavior deficits and minor immunological alterations [132]. Moreover, only VPA males display aberrations in the cerebellum [133] and in post-synaptic markers [134]. Finally, these differences between VPA males and females in expression of post-synaptic markers were shown to be modulated by methyl-CpG-binding protein 2 (MeCP2) [135], an X-linked gene implicated in several neurodevelopmental disorders including Rett syndrome (RTT), an ASD presented exclusively in females [136]. A notable animal model for RTT are $Mecp2^{+/-}$ mice, which present several RTT-related behavioral deficits in females, including social, cognitive, and anxiety related behavioral deficits. On the contrary, male $Mecp2^{+/-}$ mice display only some motor deficits [137•]. The important contribution of this animal model, distinguishing between the sexes similarly to the human condition, is in demonstrating the genotype-sex interaction, such that despite the fact that male mice were completely MeCP2 null their symptoms were far less severe [137•]. This animal model has been used in several other studies since, among them preclinical studies such as a recent study investigating the therapeutic effect of PTP1B in RTT [138].

These and other studies highlight the need to investigate the mechanism of ASD, and of other sexually-dimorphic neuropsychiatric disorders, by using and comparing both sexes, as these differences might be crucial for understanding the neurobiological basis of these disorders [139,140•]. Additionally, the current sex bias in preclinical studies that investigate neuropsychiatric disorders [140•,141] has created the dangerous situation of developing treatments designed for men so women may not respond to them or may endure adverse side-effects [141].

possible that in wild (undomesticated) rodent species the neural circuit underlying this behavior might not be so sexually dimorphic, as we have recently suggested [14••].

In the future, we anticipate that scientists will become increasingly aware of the need to study both sexes (Box 2), in part because of the recent timely decision by the National Institutes of Health to balance sex in all its funded cell and animal studies (Table 1) [120].

## Conclusions: the best is yet to come!

Until very recently, most studies on reproductive behaviors have manifested profound biases and therefore did not encompass the full extent of these behaviors as they are in nature. Focusing on selected inbred mouse strains in standard behavioral assays has allowed scientists to utilize the genomic revolution and reveal many underlying neurobiological mechanisms. However, a growing understanding of the limitations in this reductionist approach [3•,24,25••,120], along with technological advances [2•,142,143], will allow scientists to combine advanced molecular tools with high-throughput behavioral phenotyping in various animal models. Such animal models can arise from wild rodents like prairie voles [144] or naked mole rats [145,146], or from non-murine undomesticated mammals such as bats [147] or primates [143,148], all of which have already been used to study the neurobiology of social behaviors under laboratory or field conditions. Eventually, integrative research of this sort will promote our understanding regarding the molecular basis of social behavior and related pathological states in a manner that cannot be achieved using the common laboratory approaches. Thus it is time for scientists to go ahead and think outside the Skinner box.

## Conflict of interest statement

Nothing declared.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.conb.2016.04.014.

## References and recommended reading

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