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Behavioral neuroendocrinology in nontraditional species of mammals: Things the 'knockout' mouse CAN'T tell us

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Abstract

The exploration of many of the fundamental features of mammalian behavioral neuroendocrinology has benefited greatly throughout the short history of the discipline from the study of highly inbred, genetically characterized rodents and several other "traditional" exemplars. More recently, the impact of genomic variation in the determination of complex neuroendocrine and behavioral systems has advanced through the use of single and multiple gene knockouts or knockins. In our essay, we argue that the study of nontraditional mammals is an essential approach that complements these methodologies by taking advantage of allelic variation produced by natural selection. Current and future research will continue to exploit these systems to great advantage and will bring new techniques developed in more traditional laboratory animals to bear on problems that can only be addressed with nontraditional species. We highlight our points by discussing advances in our understanding of neuroendocrine and behavioral systems in phenomena of widely differing time scales. These examples include neuroendocrine variation in the regulation of reproduction across seasons in *Peromyscus*, variation in parental care by biparental male rodents and primates within a single infant rearing attempt, and circadian variation in the regulation of the substrates underlying mating in diurnal vs. nocturnal rodents. Our essay reveals both important divergences in neuroendocrine systems in our nontraditional model species, and important commonalities in these systems.

Keywords: Behavioral neuroendocrinology; Paternal care; Circadian rhythm; Seasonality

Introduction

The concentration of much laboratory research on a small number of mammals such as rats, hamsters, guinea pigs, and mice has both revealed fundamental broadly applicable principles (e.g., Phoenix et al., 1959), and provided the opportunity to attain a depth of understanding of relationships between neuroendocrine mechanisms and behavior that could never have been achieved with nontraditional models. For example, this work has led to the identification of co-factors that work with hormones to modulate the way in which they bind to and regulate the expression of genes that ultimately modulate specific features of lordosis behavior (Molenda et al., 2003). This level of analysis is really quite remarkable, and could never have been achieved without a history of many laboratories focused on behavioral neuroendocrinology in one or two well-studied laboratory rodents. So, why not just cheer the rat and mouse biologists on, and leave it at that? Why bother to look at other, less convenient, species whose genes can't be knocked in and out at will? There are a variety of reasons, having to do with the diversity of life forms that have evolved on the planet, and with the fact that natural selection, which has shaped that evolution, operates on outcomes, not mechanisms.

One of the issues is very simple: principles emerging from traditional mammalian models do not always apply to behaviors that we see in other species. We don't need to

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look far for examples. One is that the same sex differences in two species may develop in different ways. Male rats do not show lordosis behavior in adulthood both because estradiol and progesterone are not ordinarily present to activate it, and because perinatal hormones block development of the ability to show the behavior in response to the hormones. In ferrets, the former mechanism does not exist, only the latter (Baum et al., 1985). The behavior in question is the same in the two species (neither shows lordosis) but the mechanisms involved are not identical. Another principle derived from work with laboratory rats that is more limited in its application to other species is that the effects of testosterone on behavior depend on aromatization of testosterone to estradiol. This does not appear to be the case for sexual behavior in male ferrets, as inhibition of aromatase during an early critical period does not block the development of male-typical behavior in this species (Baum et al., 1983). In primates too, a role for aromatization in the mediation of the effects of testosterone has not been well-established (Becker et al., 2002). Clearly, examination of multiple species is the only way to determine which principles are most general, as well as to reveal new patterns of hormone–behavior relationships.

A second reason for looking beyond the traditional rodents is that a broader range of behaviors and patterns of hormone secretion can be explored. We cannot learn from a mouse how complex social behavior in group-living animals can moderate the effects that hormones might have on behavior (Wallen, 2001). Another example is dispersal. Among most mammals, males undergo a suite of behaviors that leads to their permanent departure from their natal home range, where females often remain throughout their lives (Greenwood, 1980). The pattern clearly suggests a role for hormones, but that role is difficult to evaluate in a laboratory setting with standard research models. A far better study animal is a Belding's ground squirrel, a diurnal species in which all males leave home, most within the first 3 months of life. Field work with these animals has led to identification of novel patterns of interaction between perinatal testosterone, energy stores, and season in the regulation of the behavior (Nunes et al., 1998, 1999).

A third important reason to go beyond traditional laboratory mammals is that an understanding of how the mechanisms associated with behavior can vary among species will help us understand how old patterns were transformed across evolutionary time into new ones. One example involves mating systems. Recent data suggest that an evolutionary transition between the polygynous pattern of a meadow vole and the monogamous one of a prairie vole may have involved selection favoring individuals with genes that regulate the expression of the V1a vasopressin receptor in a variety of neuronal populations (Young and Wang, 2004). These ideas come from work that applied viral vector gene transfer techniques, developed in laboratory mice, to species not traditionally used in behavioral neuroendocrinology. This line of work should lead to a better understanding of the evolutionary process that led the prairie vole to a monogamous way of life.

A fourth important point involves the value of field studies, which can be conducted more readily with non-traditional models. For example, some complex and subtle relationships between circulating hormones and social behavior have been revealed through field studies of spotted hyenas, studies that could not have been conducted in a captive setting (Holekamp and Smale, 1998). Specifically, while a relatively standard positive relationship between social rank and testosterone is apparent among males that have dispersed from their natal clans and settled in new ones, a very different relationship becomes apparent when comparing animals within their natal clan with same-age immigrants. In this case, although the males that have not yet left home are dominant to the immigrants, their levels of circulating testosterone are significantly lower. That is, the hormone–behavior relationship is the opposite in the two social contexts.

One additional value of nontraditional mammals is that they can help us understand neuroendocrine mechanisms that give rise to naturally occurring individual differences, the raw material on which natural selection acts. Individual differences in neuroendocrine mechanisms are seen in numerous species, and are the first of three issues we turn to now to illustrate the arguments we have made above in greater depth.

Regulation of the seasonal timing of reproductive behavior

To demonstrate the power of combining traditional and nontraditional models in the study of mammalian behavioral neuroendocrinology, we first turn to the use of nontraditional species to uncover mechanisms underlying intraspecific differences in adaptations to a seasonally changing world. The major elements of the neuroendocrine pathways that regulate reproductive behavior were identified in traditional model species, especially rabbits, laboratory rats and mice, sheep, and pigs (Everett, 1994; Silverman et al., 1994). In mammals, the major regulator of the reproductive axis is gonadotropin releasing hormone (GnRH), a master regulatory hormone of reproduction. GnRH is produced by a population of a few hundred to a few thousand neurons, depending upon the species. Secreted GnRH regulates secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) by the anterior pituitary, which stimulate sex steroid production and gametogenesis. Sex steroids, primarily estrogen (E) and testosterone (T), bind to receptors in brain areas that regulate sexual behavior. Reproductive status depends largely on the level and pattern of GnRH secretion; seasonal inputs, including changes in photoperiod, food, and other variable environmental cues, may modify GnRH secretion.

In most temperate zone mammals, specific changes in photoperiod suppress the reproductive axis and reproductive behavior. The neuroendocrine signal for light and photoperiod is processed by a complex neuroendocrine pathway that serves multiple functions (Ebling and Cronin, 2000; Goldman, 2001). Light input to the suprachiasmatic nuclei (SCN) entrains daily biological rhythms and the endogenous circadian clock, which is also necessary for assessment of daylength (Goldman, 2001). A multi-stage output of this pathway stimulates increased secretion of melatonin from the pineal gland at night, providing a general physiological signal for night, with the duration of that signal indicating the photoperiod (Bartness et al., 1993; Goldman, 2001; Prendergast et al., 2002). The melatonin signal acts indirectly (Ebling and Cronin, 2000; Morgan et al., 1994) through this system to modify the secretion or release of GnRH, which in turn regulates pituitary gonadotropin secretion and gonadal steroids. Variation in one output of this pathway, seasonal timing of reproduction, has been described extensively for wild mammals both among and within species (Bronson and Heideman, 1994). While some of this variation is related to latitude (and presumably to the length and severity of winter), much geographic variation (and all variation within populations) is not yet easily explained.

The presence of differences among individuals in reproduction and reproductive behavior has been apparent from traditional models. Although this has been useful in identifying mechanisms, it has not provided insight into natural variation or led to new insights from that variation. Thus, the hypogonadal mouse (with a genetic defect disabling GnRH production; Mason et al., 1986), tau mutant hamster (with a genetic defect disabling normal circadian rhythms, thus disabling the ability to measure photoperiod normally; Loudon et al., 1998), and fosB knockout mouse (which fails to show normal nurturing behavior toward pups; Brown et al., 1996) have been useful in study of mechanisms, but all mutations of these forms would be highly maladaptive in the wild. In contrast, variation observed in neuroendocrine control of reproductive behavior within and among wild species of mammals is likely to have functional significance and provide insights into normal variation in reproduction and reproductive behavior in other species, including humans.

A major model for causes of within-species variation in seasonal timing has been the genus *Peromyscus*, especially *P. maniculatus* (the deer mouse) and *P. leucopus* (the white-footed mouse) (Blank, 1992; Heideman, 2004; Prendergast et al., 2001). Geographically distant natural populations differ in the response to season, and single populations contain selectable genetic variation for seasonal reproduction (Bronson and Heideman, 1994), indicating that at least some of the natural variation in seasonal reproduction is genetically based. Other factors, including food and age, can affect reproductive responses to season (Blank, 1992; Heideman, 2004; Prendergast et al., 2001), indicating that some variation in seasonal reproduction is environmental. Heideman et al. (Heideman, 2004) and others (Blank, 1992; Korytko et al., 1998) have used natural variation in these populations to study causes of intraspecific differences in seasonal reproduction. Lines of white-footed mice created by artificial selection from a single wild population (Heideman et al., 1999a) are being used to study natural genetic variation in this pathway. Lines selected for strong responses (strong reproductive inhibition) to short photoperiod (Responsive line, R) or for little or no response (no or weak reproductive inhibition) to short photoperiod (Non-Responsive line, NR) can be compared with each other and an unselected control line (Heideman, 2004).

Data from traditional laboratory models suggested that defects in the circadian system or melatonin production are major sources of variation in the pathway (reviewed by Majoy and Heideman, 2000). However, traditional models have been misleading when it comes to natural populations. In deer mice and white-footed mice, the evidence suggests that variation within natural populations is *not* due to an inability to secrete melatonin (Blank et al., 1988; Lynch et al., 1982; Ruf et al., 1997), to bind melatonin (Heideman et al., 1999b), to respond to melatonin (Blank, 1992; Heideman et al., 1999a), or an inability to produce normal circadian rhythms (Carlson et al., 1989; Majoy and Heideman, 2000).

Genetic variation in reproductive responses to season appears to arise from elements of the photoperiod pathway specific to reproductive regulation. First, selected lines of white-footed mice differed in amount, but not location of binding of radiolabeled iodomelatonin (IMEL). NR mice showed higher IMEL binding in the medial preoptic area and bed nucleus of the stria terminalis than R mice, but there were no differences in the dorsomedial nucleus of the hypothalamus or the SCN (Heideman et al., 1999b). The brain areas at which differences were observed are near those previously suggested to cause reproductive changes in response to melatonin in this species (Glass and Knotts, 1987). The fact that the two lines had similar binding in some but not all brain areas examined suggests that they both can detect melatonin signals, but that they differ in the mechanisms that respond to these signals. The latter interpretation is consistent with the observation that the selected lines respond to short photoperiod with similar decreases in body mass (Heideman et al., 1999a, in press).

Second, the number of neurons immunoreactive for mature GnRH was 50% higher in the NR line than in the R line (Avigdor et al., 2005). The differences between lines were present in both SD and LD, and were also present when mice from each line were castrated and given replacement testosterone (Avigdor et al., 2005). The higher number of immunoreactive neurons in both LD and SD in NR mice relative to R mice may indicate greater potential secretion of GnRH in response to inhibitory factors even in LD, including such inhibitory factors as stress or food shortage. If so, then the NR phenotype may be genetically

resistant to reproductive inhibition in general, rather than merely resistant to reproductive inhibition in SD. Regardless of the cause, this finding suggests that natural variation in the number (or location) of GnRH neurons contributes to variation in reproductive responses to photoperiod. This report from a nontraditional mammal is, to our knowledge, the first link between natural variation in neuron numbers and functional variation in phenotype.

Third, the two selected lines of mice and the unselected control line differ in voluntary food intake, but not in body mass, at 70 days of age (Heideman et al., in press). The NR line averaged 50% higher in nightly food intake than the R line; the unselected control line was intermediate in food intake. Preliminary data suggest that as mice in the two lines age, the difference in food intake eventually results in higher body mass in NR mice (Heideman, unpublished data). This genetic difference in feeding behavior exists independently of photoperiod, with similar differences in food intake among lines in both long photoperiod and short photoperiod (Heideman et al., in press). Thus, selection on a reproductive trait (degree of development of testes or ovaries in short photoperiod) has apparently produced a correlated response to selection that altered a very different behavior—food intake. This suggests a previously unsuspected link between variation in seasonal reproduction and variation in feeding behavior. An important point here is that this is a link that would be difficult to make in a traditional model, and even harder to extend to natural populations. For example, there are rat strains that differ in reproductive responses to photoperiod as well as in voluntary food intake, but these strains differ in so many other characteristics, including body mass (Heideman and Sylvester, 1997; Lorincz et al., 2001), that a connection between food intake and reproductive responses to photoperiod has never been proposed. It is not surprising that low voluntary food intake might be linked to lower reproductive performance, but it is intriguing that this might be an important source of natural variation in seasonal reproduction, and that selection on seasonal reproductive traits might cause changes in food intake.

In summary, laboratory study of this nontraditional model is providing insight into natural variation in reproduction that could not be gained from a traditional model. Even if similar findings were obtained in one of the more conventional species, it would not be possible to determine if the variation would occur in nature. In fact, studies of differences within more traditional species have found mechanisms that appear to be absent in natural populations (see above). Study of the natural populations has suggested a link between individual differences in food intake and in seasonal reproduction, a link whose significance would be difficult to assess if it had been noted first in a more conventional laboratory rodent. While traditional models will continue to be vital in studies of neuroendocrine mechanisms of behavior, nontraditional ones will be vital to study variation in context, and to apply an understanding of variation to humans and other species. In future, it should be possible to use modern molecular techniques developed for use in mice, such as microarrays and viral transfection, with nontraditional models such as *P. leucopus* or *P. maniculatus* to obtain a deeper of understanding of patterns on naturally occurring variability.

Daily rhythms

Another example of an issue that only a nontraditional lab mammal can help us address involves species differences in the temporal organization of female reproductive processes. For a female to reproduce successfully, there must be temporal coordination between reproductive behavior and physiology such that sperm will be available to meet her eggs around the time of ovulation. Endogenous circadian rhythms, originating within the SCN, play an important role in the regulation of virtually all aspects of behavior and physiology, including the events that occur at this time (Klein et al., 1991; Moore-Ede et al., 1983). In some species, such as rhesus macaques, the time of day at which estrus-related events typically occur is likely to be influenced by the circadian system only indirectly through its more general effects on arousal and activity. In others, however, the SCN plays a very direct and crucial role in the tight coordination of mating behavior and the neuroendocrine mechanisms associated with it. This is the case in rats, mice, and hamsters, nocturnal rodents that are all most likely to mate towards the beginning of the dark phase of a 24-h light–dark cycle (Schwartz, 2000).

The timing of female sexual behaviors in nocturnal rats is promoted both by changes in steroid hormone secretions and by rhythms in responsiveness to these hormones (Sodersten et al., 1981). Rhythms in behavioral responsiveness to hormones persist in nocturnal rodents maintained in constant darkness and are abolished by SCN lesions (Alleva et al., 1971; Sodersten et al., 1981). At least part of the circadian regulation of behavioral estrus may be mediated by direct projections that extend from the SCN to hypothalamic cells containing estrogen receptors (ER+ cells; De La Iglesia et al., 1995; Watson et al., 1995). Mating behavior is associated with an ovulatory surge in LH that occurs in these nocturnal rodents approximately 4 h before the lights go out on the day of proestrus (Schwartz, 2000). The timing of this event is dependent on high levels of ovarian hormones, but is gated by the circadian timekeeping system such that it can only occur within a relatively narrow window of time on the afternoon of proestrus. The circadian regulation of the LH surge appears to be mediated, at least in part, by projections extending directly from SCN cells containing vasopressin (VP) and vasoactive intestinal polypeptide (VIP) to GnRH-containing neurons in the hypothalamus (Palm et al., 1999; van der Beek et al., 1999). Because of this body of data on the pathways through which the SCN influences estrus-related

events in traditional laboratory rodents, this system provides a rare opportunity to evaluate the issue of how neural mechanisms may have been transformed during an evolutionary transition from a night-active to a day-active way of life. This issue has received little attention until now because all of the animal models traditionally used in circadian research are nocturnal. This situation is beginning to change as issues relating to diurnality are being explored with more diurnal mammals, including squirrels, degus, and grass rats (Smale et al., 2003).

The unstriped Nile grass rat (*Arvicanthis niloticus*), a diurnal murid rodent from sub-Saharan Africa, has opened the way for examination of questions related to whether and how circadian regulation of estrous behavior and neuroendocrine events associated with it differ in day- and night-active species. Recent field data have shown that these animals are most active in the middle of the day (Blanchong and Smale, 2000), and laboratory observations have revealed that mating behavior in these animals begins primarily around the time that lights come on in a 12:12 light:dark cycle (McElhinny et al., 1997) and the LH surge occurs approximately 4 h before that (McElhinny et al., 1999). These two components of estrus are therefore approximately 12 h out of phase in diurnal grass rats and the more traditional nocturnal, laboratory rodents. This provides the opportunity to begin to explore mechanisms that can produce temporal differences between diurnal and nocturnal species.

So, how are these differences produced? One mechanism involves a rhythm in behavioral sensitivity to steroid hormones (Mahoney and Smale, 2005). Ovariectomized female grass rats primed with estradiol and progesterone at different times of day and tested 4 h later exhibited lower rates of sexual behavior during the dark period than the light period. This pattern was apparent in the lordosis reflex and in rates of ejaculation, both reflexive components of the behavior. The rate of mounting behavior, which may represent a more motivational component, followed a rhythm that more closely parallels that of general activity. Species differences in behavioral responsiveness to steroid hormones may be promoted by SCN-driven rhythms within ER+ cells. Daily rhythms in estrogen binding in the hypothalamus (O'Conner et al., 1985), and SCN projections to ER+ cells, have been documented in lab rats (Watson et al., 1995), and in grass rats SCN efferent fibers, as indicated by staining for VIP, also appear to contact ER+ cells in regions of the grass rat hypothalamus involved in regulation of mating behavior (Mahoney, 2003). Differences in the timing of the LH surge of lab rats and grass rats are likely due to differences in GnRH neuron activation, as the rise in both LH and Fos expression in GnRH neurons occur 12 h out of phase with these events in lab rats (Mahoney et al., 2004). Appositions suggestive of synapses between VIP and VP fibers and GnRH neurons are seen in grass rats as is the case in lab rats (Mahoney, 2003). Taken together, these data suggest that rhythms in GnRH neuron function, as well as in behavioral responsiveness to estradiol, could be promoted by differences in direct signals from the SCN to GnRH and ER+ cells, or by responsiveness of these cells to such signals.

These patterns raise the question of whether the 12-h inversion seen in GnRH neurons is apparent in all SCN targets. The answer to that is "no". In two targets that do not serve such specialized functions as the GnRH neurons, very different patterns are seen. The SCN projects to cells just dorsal to it, a region referred to as the lower subparaventricular zone (LSPV), and in this region the rising phase of a rhythm in Fos expression occurs 8–9 h earlier in grass rats compared to lab rats (Schwartz et al., 2004). On the other hand, in the paraventricular thalamus (PVT), another major target of the SCN, Fos rhythms are almost identical in the two species (Novak and Nunez, 2000). Recent sequencing of the gene encoding PK2, a major signal sent from the SCN to the PVT, and of its receptor, has provided further evidence that some features of the SCN to PVT signaling pathways are the same in nocturnal and diurnal species (Lambert et al., 2005). One principle suggested by this comparative work is that there is not likely to be one simple "switch" that determines if the overall system is a diurnal or a nocturnal one. One question raised by these patterns is whether a complete 12-h reversal is a general characteristic of SCN targets that, like the GnRH neurons, serve highly specialized behavioral and neuroendocrine functions that are temporally inverted in day- and night-active animals. SCN targets with more widespread projections that modulate an array of functions may have a greater variety of differences and similarities in diurnal compared to nocturnal species. These issues can only be explored with further research on mammals that are not traditionally examined in the laboratory. Ultimately, the answers to the questions of diurnality will likely require further application of modern molecular techniques to such species.

Paternal behavior

Among mammals, provisioning of young offspring with food (i.e., milk) is, by taxonomic definition, restricted to females. Not surprisingly, most mammalian species, including the traditional models identified earlier in this essay, show exclusive or predominantly maternal care, and the endocrine substrates of maternal care has been a focus of behavioral endocrinology for decades. This cumulative literature shows clearly that the responsiveness of mothers to offspring is inextricably associated with the endocrine changes that occur during pregnancy, parturition, and lactation. Among the endocrine changes linked to maternal behavior and responsiveness to infants are high estrogen and falling progesterone concentrations in the late prepartum period, rising prolactin in the late prepartum phase, and high oxytocin associated with parturition and infant suckling (see review in Numan and Insel, 2003). In a small number of

mammalian species, distributed primarily among the canids, rodents, and primates, parental care is performed by adult males in addition to adult females. This section will focus on the contribution of studies of nontraditional rodents (hamsters, voles, and California mice) and primates (marmosets, tamarins, and human beings) to our understanding of the endocrine mediation of paternal care.

Early research on infant-directed behavior in male mammals focused on 'traditional' species that do not normally express high levels of these forms of caregiving, such as inbred laboratory rats and mice (see review by Brown, 1993). More recent evidence for specialized endocrine mediation of paternal care has accumulated from species in which male caregiving is the norm, and some important commonalities between the neuroendocrinology of maternal care and paternal care have emerged. In a variety of biparental mammals, levels of prolactin are related to paternal care in a manner similar to the patterns observed in postparturient females that are nursing and exhibiting maternal responsivity. Prolactin levels are higher in males that are interacting with offspring (Brown et al., 1995; Dixson and George, 1982; Gubernick and Nelson, 1989; Mota and Sousa, 2000; Reburn and Wynne-Edwards, 1999; Roberts et al., 2001b; Schradin and Anzenberger, 2004; Storey et al., 2000), prolactin levels are higher in males that are paternally experienced, relative to naïve males (Schradin and Pillay, 2004; Ziegler et al., 1996), and experimentally down-regulating prolactin concentrations is associated with reduced paternal responsivity (Roberts et al., 2001a).

The remainder of this section will focus on the involvement of the gonadal steroid testosterone (T) and its metabolic derivatives in the regulation of paternal care. There is considerable evidence from avian models that androgens inhibit paternal care, and much of the research in this area has centered around predictions derived from two heuristic hypotheses. The "challenge hypothesis" (Wingfield et al., 1990) predicts that seasonal changes in T will vary based on social system, level of male–male aggression, competition for reproduction, and presence of fertile females. The "trade-off hypothesis" (Ketterson and Nolan, 1999) proposes that circulating T concentrations in males of biparental species reflect a trade-off between male reproductive effort directed toward seeking, acquiring, and copulating with females (mating effort) versus those activities that are designed to promote offspring growth and survival (parental effort). Together, these hypotheses suggest that monogamous, biparental males should show increased T when male–male conflict is higher and when sexually receptive females are present, but decreased T when aggression and mate-seeking conflict with demands for male parental care.

Among some biparental rodents, T titers in males across phases of the breeding cycle are consistent with these predictions. For instance, in hamsters, males of the biparental *P. campbelli* showed reduced T immediately after birth, at a time when males interact extensively with newborn pups (Reburn and Wynne-Edwards, 1999; but see Schum and Wynne-Edwards, 2005). In male gerbils, T levels dropped immediately after their mates gave birth to pups (Brown et al., 1995), and adult castration increased males' preference for, and attraction to, young pups and the stimuli emanating from them (Clark et al., 2004). There may also be some important organizational effects of T. Male gerbils that developed in a 2F position (that is, between two female fetuses), and hence were exposed to lower levels of T in utero, spent less time interacting with potential sexual partners and substantially more time caring for young pups, than males that developed in a 2M position (developing between two male fetuses; Clark and Galef, 2000). The organizational origin of this effect requires further evaluation, since 2F males also have lower circulating T as adults, when compared to 2M males (Clark and Galef, 2000).

Several studies have suggested a connection between T concentrations and paternal responsiveness in human males. Men who showed reduced T concentrations immediately after the birth of their child, and men who were highly reactive to a simulated encounter with an infant, had the largest drop in T levels after the encounter (Storey et al., 2000). Fleming et al. (2002) exposed fathers and non-fathers to infant cries and monitored endocrine, autonomic, and emotional responses to these stimuli. Overall, fathers were more sympathetic and alert to the cries than non-fathers, but in both groups T levels were negatively correlated with sympathy. That is to say, men with higher T reported less sympathy or need to respond to the infant cries.

In the callitrichid primates, there is some evidence that paternal care is related to T profiles in a manner consistent with the predictions derived from the challenge and trade-off hypotheses. In common marmosets, paternal males were more likely than non-fathers to have low T titers (below 10 ng/ml: 4/5 paternal males, 2/10 nonpaternal males; Dixson and George, 1982). Research on Wied's black tufted-ear marmoset (*Callithrix kuhlii*) provides important converging evidence that paternal care is facilitated by low androgen levels. In this species, males are primary caregivers (in terms of energetically–costly offspring transport and sharing of solid food) during the weeks 3 and 4 of post-natal life. Urinary T concentrations vary according to the male's carrying effort, such that levels of T were reduced to 60% of prepartum baseline concentrations during the period of maximal male care (Nunes et al., 2000). Similar postpartum patterns of T excretion were noted whether or not males were continuously exposed to infant stimuli, suggesting that proximal cues for this transition derive from short-term exposure to infants or cues from other sources (e.g., female mates). Males with more paternal experience showed greater reductions in T during infant caregiving (Nunes et al., 2000), showing that the dynamics of T regulation may be influenced by parental experience. Individual differences in paternal care are also correlated with differences in underlying androgen activity. Male marmosets that engaged in

extensive paternal care (carrying infants on average 35 min each hour) had significantly lower levels of T, relative to males that were less involved in paternal care (carrying only 16 min each hour; Nunes et al., 2001). The differences in T excretion were substantial, with high effort fathers excreting 1.2 μg/mg Cr in weeks 3 + 4 and low effort males excreting 2.9 μg/mg Cr T. In both classes of males, T levels across the first 6 weeks of infant life were negatively correlated with infant carrying effort (Nunes et al., 2001). Together, these studies suggest that variation in the timing of infant care, and in the overall level of infant care expressed by males, are influenced by T. The recent demonstration that female marmosets also show androgen-correlated changes in maternal responsiveness (Fite et al., 2005) highlights the possibility that T-sensitive neural circuits may be involved in both paternal and maternal care.

The relationship between T and parental care has been assessed in two other species of callitrichid primates. In the cotton-top tamarin (*Saguinus oedipus*), Ziegler et al. (2000) noted no significant changes in T as fathers became active in infant care. A recent data set on the same species, however, suggests that T levels may drop in the presence of newborn infants. If the baseline in Fig. 1 of Ziegler et al. (2004) is recalibrated for the month immediately prior to parturition, then males had substantially reduced T in the first month postpartum, on the order of a 2-fold reduction. In free-living golden lion tamarins (*Leontopithecus rosalia*), males also showed reduced levels of fecal androgens in the period immediately after the birth of infants, relative to concentrations measured during the mating and pregnancy phases of reproduction (Bales et al., under review). Thus, evidence is accumulating that the regulation of androgen activity may have an important determining role in the timing, quality, and quantity of paternal care provided to offspring by male rodents and primates.

As might be expected, the story is not as simple as "elevated androgens inhibit paternal care" in biparental male mammals. In some species, elevated androgens in males may, in fact, be critical for the stimulation or facilitation of male parental care. In the prairie vole (*Microtus ochrogaster*), castration in adulthood reduced parental behavior, and T implants reinstated paternal responsiveness (Wang and De Vries, 1993), although this finding was not fully replicated (Lonstein and De Vries, 1999). On the other hand, neonatal castration of male voles led to a reduction in later paternal responsiveness, suggesting an organizational effect of neonatal T on paternal responsiveness (Lonstein et al., 2002). A conspicuous exception to the general rule is the California mouse (*Peromyscus californicus*). Initial studies revealed no link between androgen concentrations and the likelihood that males would behave paternally (Gubernick and Nelson, 1989), but more recent work suggests that high T titers may be necessary for the expression of caregiving. Castration reduced pup grooming and huddling in males, relative to intact males, while castration + T replacement reinstated these behavioral patterns to levels similar to or higher than those in intact males (Trainor and Marler, 2001). The mode of action of T on paternal behavior in this species appears to be mediated via the actions of estrogens, a conclusion supported by both pharmacological and neuroanatomical studies. In castrated male California mice, paternal behavior was reinstated by treatments of T alone or E plus the aromatase inhibitor fadrozole, but treatment with T + fadrozole (a condition that blocks the conversion of T to E) or vehicle did not restore paternal responsiveness, suggesting that the behaviorally-relevant steroid is estradiol (Trainor and Marler, 2002). Aromatase activity in the medial preoptic area of the hypothalamus, a nucleus associated with both paternal and maternal care, was higher in male California mice interacting with pups than in males paired with females but not exposed to pups (Trainor et al., 2003). Together, these data provide compelling evidence in this species that gonadally-derived T provides an important substrate for facilitating both paternal responsiveness and aggressive behavior. The divergent responsiveness to T in this species, relative to other biparental mammals, reveals that there are multiple actions of T on paternal responsiveness. It is surely the case that T exerts its modulating effects on paternal behavior through interactions with other important neurotransmitter systems. For example, we know that vasopressin synthesized in androgen-sensitive neurons project to the lateral septum, a system known to play a role in modulating paternal care (Wang and De Vries, 1993). Exploring the distinction among species in which T either inhibits or facilitates paternal responsiveness, both from the perspective of understanding the different socioecological factors that may favor one action over the other, and the critical differences in the organization of the CNS and its response to T and its metabolites, remains an important challenge for the future.

Overall summary

The examples highlighted in our essay collectively provide a compelling scientific rationale for the use of nontraditional mammalian models in the study of behavioral neuroendocrinology. It is clear that important lessons are being learned from these species in a variety of phenomena involving differing time scales (across seasons, across weeks, and across days). Studies of organisms like wild-derived *Peromyscus* allow scientists to identify mechanisms underlying the development of alternative phenotypes, reflecting the action of natural selection on underlying neurobiology (e.g., differences in the GnRH system in photoperiod responsive vs. nonresponsive mice). The sensitivity of these effects of selection is highlighted by comparative studies of diurnal grass rats and traditional nocturnal laboratory rats, showing that some components of the circadian timing system are altered in diurnal vs. nocturnal rodents (e.g., GnRH and ER+ cells) while not influencing others (e.g., Fos rhythms in the thalamus).

Finally, our examination of biparental care in male rodents and primates reveals both commonalities in the neurobiology of a behavioral trait that is highly dimorphic in traditional mammals (e.g., the responsiveness of the prolactin system to stimuli associated with offspring in both males and females) and important differences (the observation that T can either inhibit or facilitate paternal care, depending on the species). To return to our original question: "Why not just cheer the 'knockout mouse' models as representative of a generalized mammalian system in behavioral neuroendocrinology?" The examples we provide, and many others not covered, amply demonstrate the utility of nontraditional mammals for exploring both divergent and convergent evolution, and the impact of this evolution on variation in the genes and mechanisms that regulate complex physiological and behavioral phenotypes.

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