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Evolution of neuroendocrine mechanisms that regulate sexual behavior

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Whiptail lizards provide a unique system to study evolution of brain mechanisms because both ancestral (sexual) and descendant (parthenogenetic) species exist. Parthenogenetic whiptails enable us to avoid the two major confounds in sex differences research – males and females that differ both genetically and hormonally. Parthenogens are females that reproduce clonally, yet display alternately female-like and male-like pseudosexual behavior. Thus, the neural circuitry underlying male and female sexual behavior can be examined within the 'same' brain (same genome), enabling us to see how neuroendocrine mechanisms controlling mounting behavior change. In ancestral males, testicular androgens control sexual behavior, whereas male-like pseudocopulatory behavior is controlled by ovarian progesterone in parthenogens, revealing that progesterone is important in regulating sexual behavior in male vertebrates, including mammals.

The difficulty of studying evolution of brain mechanisms

The major challenge to studying the evolution of brain mechanisms is the absence of ancestors of present day animals. This problem has been addressed using two major approaches. One is the behavior–genetic approach, which traditionally utilizes selective breeding, now including more modern methods such as viral vectors, and the development of transgenic organisms. The problem inherent in this approach is that one is looking at the potential for change in the central nervous system, not how the mechanisms have actually changed in response to natural or sexual selection pressures. The other method used is the comparative phylogenetic approach, a method championed by Konrad Lorenz and today taking the form of molecular estimations of genetic distances between species, coupled with behavioral analyses. Here, the problem is that, except in rare instances, one does not know the history of extant species since their divergence from a common ancestor.

Natural experiments as model systems for the study of brain-behavior evolution

There is an alternative approach – one that utilizes species that exhibit unusual traits [1]. As Jared Diamond [2] has stated: 'Natural experiments permit one to examine conditions that cannot… be created experimentally… and reveal the end results of ecological and evolutionary processes'. The whiptail lizards (genus *Cnemidophorus*) are such a system. This genus consists of ~45 species, most which reproduce by sexual means. However, one-third of whiptail species are obligate parthenogens, consisting only of female individuals that reproduce by cloning. These parthenogenetic species are either diploid or triploid, and arose from the hybridization of closely related sexual species. In addition to *Cnemidophorus*, parthenogenesis has evolved independently in at least six lizard genera and one snake genus [3]. Molecular analyses have established that all of these parthenogenetic species are of hybrid origin, often are polyploid, and display high levels of nuclear gene diversity but lower levels of mitochondrial DNA diversity compared with their sexual ancestral species [3–7]. The power of these systems lies in the fact that representatives of both the ancestral and the descendant species still exist, thereby representing a 'snapshot of evolution'. Until relatively recently [8,9], most research on reproductive physiology or behavior has focused on the whiptail lizards. Molecular analyses indicate that the little striped whiptail (*C. inornatus*) is the maternal ancestor, contributing two-thirds of the genome of the desert-grasslands whiptail (*C. uniparens*) [5] – hereafter the ancestral or sexual species and the descendant or parthenogenetic species, respectively.

It was the observation that parthenogenetic whiptails display behaviors typical of both the male and the female of the ancestral species during the breeding season [10] that alerted me to the unique opportunity that these animals presented. Consistently and reliably, one individual will court and mount another individual, and assume the male-like copulatory posture characteristic of the genus (Figure 1, top panel). The mounted individual is receptive, remaining stationary to these courtship advances, allowing it to be mounted. The mounting lizard then assumes a contorted posture characteristic of a mating male of the ancestral sexual species, here called pseudocopulation (because the parthenogenetic species consists only of females). In the sexual ancestral species, females are receptive to male courtship when they are preovulatory, but will respond aggressively after ovulation; female–female mounting is never seen.

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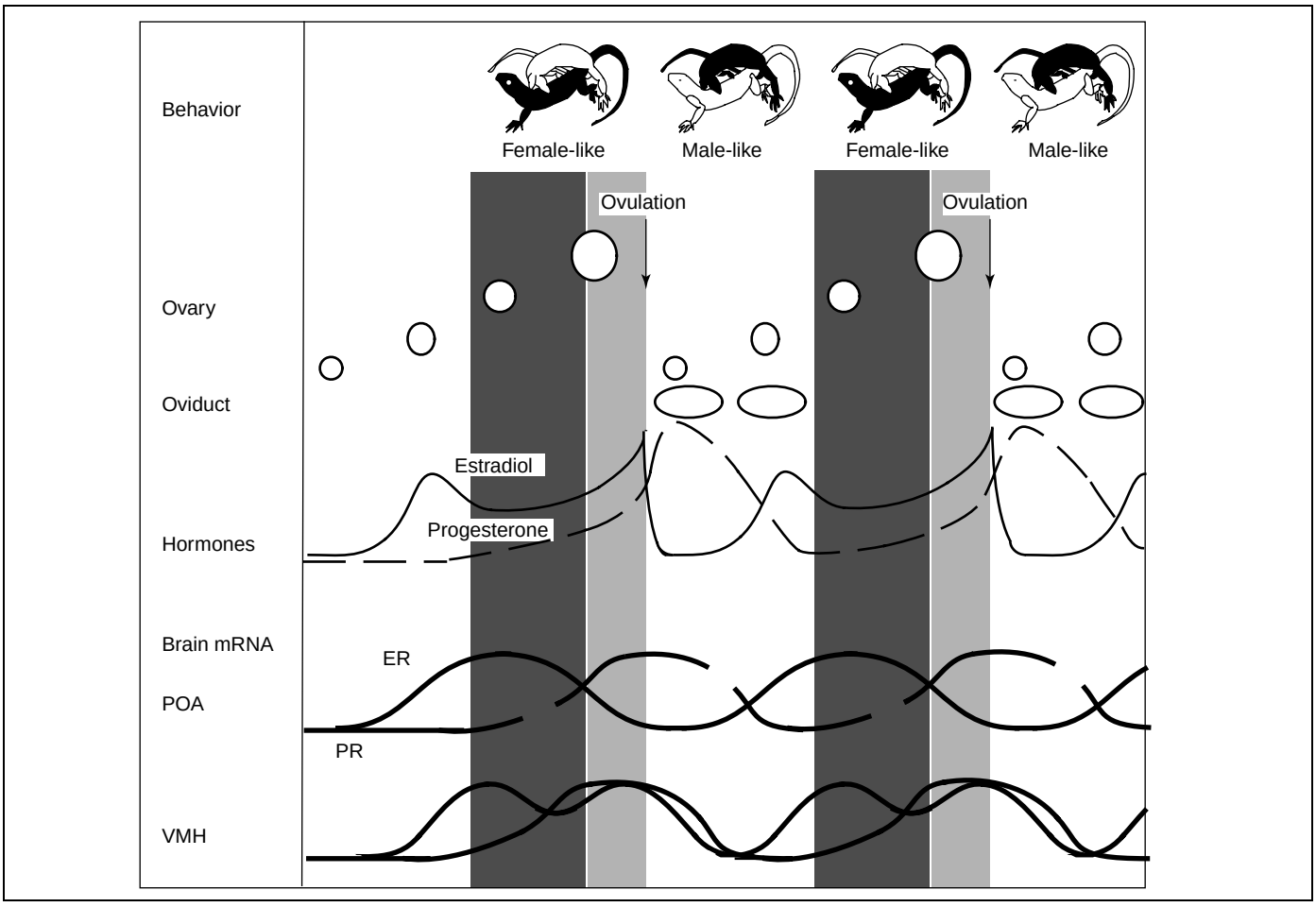

Figure 1. Relations between male-like and female-like pseudosexual behavior, ovarian state and circulating levels of estradiol and progesterone during different stages of the reproductive cycle of the parthenogenetic whiptail lizard. The transition from receptive to mounting behavior occurs at the time of ovulation (arrow). Also shown are the changes in abundance of the gene transcripts encoding the estrogen receptor (ER) and progesterone receptor (PR) in the preoptic area (POA) and the ventromedial hypothalamus (VMH), brain areas that are involved in the regulation of male- and female-like pseudosexual behaviors, respectively. Adapted from [39].

Does pseudosexual behavior occur in nature and what is its function? I and others have observed pseudosexual behavior in the field, and laboratory experiments demonstrate that it increases fecundity, much as male courtship facilitates female reproduction in its sexual ancestors; indeed, this behavioral facilitation of reproduction is conserved in all animals. Thus, despite the loss of male individuals (and their sperm), the need for male-like behavior remains in the parthenogenetic whiptail; the benefit to the mounted individual is to stimulate ovarian growth and ovulation, whereas the benefit to the mounting individual is to shorten the time to ovulation in the mounted animal, enabling a more rapid change in physiology and behavior.

Early studies established that during pseudocopulation the mounting lizard is postovulatory or has undeveloped ovaries, whereas the mounted animal has large, yolking follicles (Figure 1, bottom panel). After the mounted lizard ovulates, these behavioral roles are reversed. Thus, the role that each individual plays in pseudocopulation is reflected in complementary behavioral and ovarian states. In addition, the only time available during the reproductive cycle for this male-like behavior to occur is after ovulation or during the early stages of the next follicular cycle. This has significance for understanding how the male-like mounting behavior comes under the control of a new hormonal trigger.

As would be predicted, in the ancestral sexual species male courtship and mating are abolished by castration and reinstated by androgen replacement treatment and female receptivity is controlled by estrogen secreted by the growing follicle. However, in the descendant parthenogen, androgen is not secreted at any stage of the follicular cycle, and the male-like pseudocopulatory behavior is under the control of the increasing levels of progesterone following ovulation (Figure 1, middle panel). The parthenogen will respond to androgens by displaying male-like pseudocopulatory behavior, as do females of the sexual ancestral species. In other words, the parthenogen has not lost its sensitivity to androgen, even though androgen is not secreted or involved in the induction of pseudocopulatory behavior. How then did this sensitivity to progesterone as a trigger for male-like pseudosexual behavior evolve?

Conservation of brain mechanisms underlying sexual behaviour

It is clear that there is conservation in the limbic areas involved in sex-typical behaviors among vertebrates. Parthenogens performing the male-like role have increased 2-deoxyglucose uptake in the preoptic area

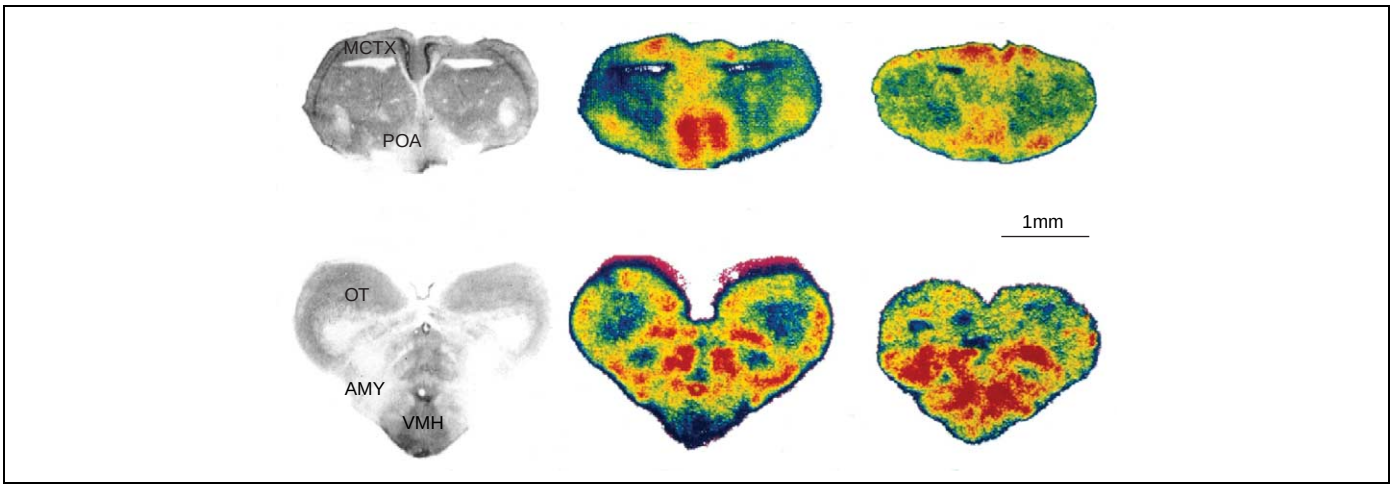

Figure 2. Metabolic activity in the brain of two parthenogens engaged in a pseudocopulation. The column on the left shows light-field photomicrographs of the brain of an individual exhibiting male-like pseudosexual behavior; the middle column shows the same sections, but they are pseudocolor images of 2-deoxyglucose accumulation, with red being most intense and blue the least intense. The right-hand column shows images of an individual behaving in the female-like receptive role. The top row is at the level of the preoptic area (POA), whereas the bottom row is at the level of the ventromedial hypothalamus (VMH). Note the complementary patterns of brain activity that reflect complementary behavioral roles, with the mounting individual showing greatest activity in the POA, and less activity in the VMH, relative to the individual in the receptive role, where pattern of activity is reversed, with less activity in the POA and greatest activity in the VMH. AMY, amygdala; MCTX, medial cortex; OT, optic tectum. (Scale bar = 1 mm.) Adapted from [40].

(POA) and reduced activity in the ventromedial hypothalamus (VMH), as do males of the sexual species, whereas individuals performing the female-like role show decreased activity in the POA and increased activity in the VMH (Figure 2). Further study of these brain areas revealed a hormonal and behavioral specificity of function [11]. In both the ancestral species and in the all-female species, androgen implanted directly into the POA stimulates mounting behavior, but has no effect on mounting and receptive behavior when placed into the VMH. The opposite occurs when estrogen is implanted into the VMH: receptive behavior is elicited in the ancestral species and in the descendant parthenogen, but there is no effect on receptive or mounting behavior when estrogen is placed into the POA. In contrast, lesions of the POA of hormonally treated, gonadectomized lizards abolishes mounting behavior both in males of the sexual species and in the descendant parthenogen, whereas destruction of the VMH eliminates receptive behavior. Such findings conform to the literature on a wide variety of vertebrate species, indicating that these brain areas are ancient and conserved in their function [11].

Polymorphism in progesterone sensitivity in males of the ancestral species

To understand how a novel neuroendocrine mechanism controlling the display of male-like pseudocopulatory behavior during the postovulatory stage evolved in the parthenogen, I examined more closely the ancestral sexual species. That is, 'If we are to understand how a phenotype evolved, we have to understand how its regulation is organized and how it might have been pieced together during evolution from preexisting traits' [12]. Initial studies revealed that about a third of the males of the ancestral species also display the full repertoire of sexual behavior in response to physiological concentrations of progesterone after castration. This was a remarkable and novel finding that had never been reported in vertebrates. In these lizards, males typically secrete low levels of progesterone throughout the breeding season. Further studies confirmed that, although all males are androgen-sensitive, a subset of these males is also sensitive to progesterone – hereafter called progesterone-sensitive males.

An important aspect of these findings is that progesterone acts at the level of the brain only, because the androgen-dependent sex structures are not stimulated in males found to be progesterone-sensitive. Furthermore, it is evident that the progesterone acts as a native molecule and not as a precursor to androgen. Not only does co-administration of progesterone with a progesterone antagonist block the induction of sexual behavior in castrated males, but administration of a synthetic progestin that cannot be converted to androgen reinstates courtship and copulatory behavior in progesterone-sensitive males. It was also found that progesterone and androgen synergize in males to induce courtship and copulatory behavior in much the same way that estrogen and progesterone synergize in females in eliciting receptivity. In addition, neither is the androgen receptor (AR) of males of the ancestral species modified, because it has similar specificity and affinity to the AR of male mice. Taken together, these data indicate that progesterone acts via the progesterone receptor (PR).

Larry Young cloned and sequenced the PR, AR, and estrogen receptor (ER) and then used *in situ* hybridization to map their distribution in the brain [13]. One finding was that all three are localized in the POA of both the sexual ancestor and the parthenogenetic descendant species. This information made it possible to determine how progesterone might regulate sexual behavior and receptor expression in the area of the brain that controls mounting behavior of progesterone-sensitive males. In one experiment, sexually active males were castrated and then screened for progesterone sensitivity [14]. After removal of the subcutaneous implant of progesterone

and subsequent decline in behavior, both progesterone-sensitive and -insensitive males received an intracranial implant of progesterone into the POA. Those males that were progesterone sensitive responded by courting and mounting receptive females, whereas those that were progesterone insensitive ignored receptive females. In addition, *AR* and *PR* mRNA was differentially regulated by this treatment in the two types of males, with *AR* mRNA abundance increasing and *PR* mRNA decreasing in progesterone-sensitive males relative to progesterone-insensitive males. Thus, it is possible that in progesterone-sensitive males, progesterone acts via PR in the POA in a way that is functionally linked to the neural circuitry controlling mounting and copulatory behavior; these receptors could be either co-localized with AR in the same neuron or contained in separate neurons that synapse with AR-containing neurons. We presently are investigating whether a molecular mechanism underlies this polymorphism in progesterone sensitivity.

Systemic testosterone treatment significantly increases *PR* mRNA abundance in the POA of gonadectomized males and females of the sexual ancestral species and in the parthenogenetic descendant species, explaining the behavioral sensitivity to androgen in the parthenogen [11]. In contrast, estrogen treatment fails to upregulate *PR* mRNA in this brain region of the sexual ancestral species, but does so in the parthenogen (figure 3). Other experiments indicate that exogenous progesterone strongly inhibits receptivity in both females of the ancestral sexual species and the parthenogen, in addition to decreasing ER and PR abundance in the VMH, but has no effect on receptor abundance in the POA.

Evolution of a novel neuroendocrine mechanism controlling sexual behavior

With this information it was possible to pose the question: what is different about the POA of the ancestral sexual species compared with the descendant parthenogenetic species? In other words, how is it that progesterone stimulates male-like pseudosexual behaviour in the parthenogen whereas it does not do this in females of its direct evolutionary ancestor? The answer appears to lie in the response to estrogen; that is, estrogen treatment upregulates PR in the POA of the parthenogen, but not in the sexual ancestral species (Figure 3). In other words, it appears that as estrogen levels increase in the circulation during follicular growth in the parthenogen, PR abundance increases in the POA. Thus, when the hormone ratio changes from estrogen dominance to progesterone dominance with ovulation, there is abundant PR in the brain area crucial to pseudocopulatory behavior at the time progesterone levels surge (Figure 1, bottom panel). This contrasts with what occurs in the sexual ancestral species. Here, under a similar scenario in the female, as estrogen increases in the circulation there is no change in *PR* mRNA in the POA and no mounting behavior occurs after ovulation. In both species, the rising level of estrogen from the growing follicles upregulates *PR* mRNA in the VMH, the brain area involved in sexual receptivity. In males of the ancestral species, androgen is normally the dominant hormone and testosterone strongly upregulates *PR* mRNA in the POA of the male (and in conspecific females and the parthenogen). Furthermore, neither estrogen nor progesterone is normally secreted in males, yet a response similar to that of the female is seen: estrogen treatment fails to upregulate *PR* mRNA in the POA. In progesterone-sensitive males, progesterone downregulates *PR* mRNA but upregulates *AR* mRNA in the POA.

Thus, the principal difference in the ancestral and the descendant species is how gene expression differs in the POA in response to estrogen. In addition, the sensitivity to progesterone in some males of the ancestral species may have been exploited, serving as the substrate on which selection acted, and resulting in progesterone activating male-like pseudocopulatory behavior in the parthenogenetic descendant whiptail. This suggests also that a progesterone-sensitive male would have been involved in the original hybridization event that gave rise to the unisexual species.

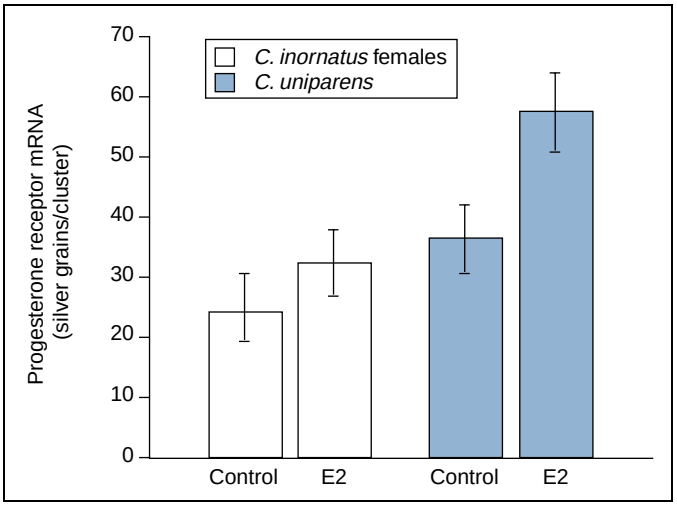

Figure 3. Levels of mRNA encoding the progesterone receptor in the periventricular preoptic area (PvPOA) of female *C. inornatus* (open bars) and *C. uniparens* (black bars) given either blank or estradiol-17β (E2) injections. An abundance of mRNA encoding the progesterone receptor is shown, measured as the average number of silver grains per cluster (mean±SE) in the PvPOA of the ancestral sexual (*C. inornatus*) and descendant parthenogenetic (*C. uniparens*) whiptail lizards. Adapted from [41].

New directions

How is progesterone capable of inducing this normally androgen-dependent behavior in some males of the ancestral sexual species and in the all-female descendant species? The model we are pursuing (Figure 4) focuses on neurotransmitters known to be involved in the control by androgen of male-typical sexual behavior of sexual species. In the present case, progesterone activates previously androgen-dependent mechanisms, resulting in the male-like phenotype of the unisexual species. Good candidates for an evolutionarily novel regulation of this kind are the neurotransmitters dopamine and nitric oxide (NO), both of which are regulated by androgens in some physiological contexts and by progesterone in others. Present research focuses on how in the parthenogen estrogen regulates dopamine and NO synthase (NOS).

Administration of the D1 dopamine agonist SKF 81297 to castrated, progesterone-sensitive male *C. inornatus*

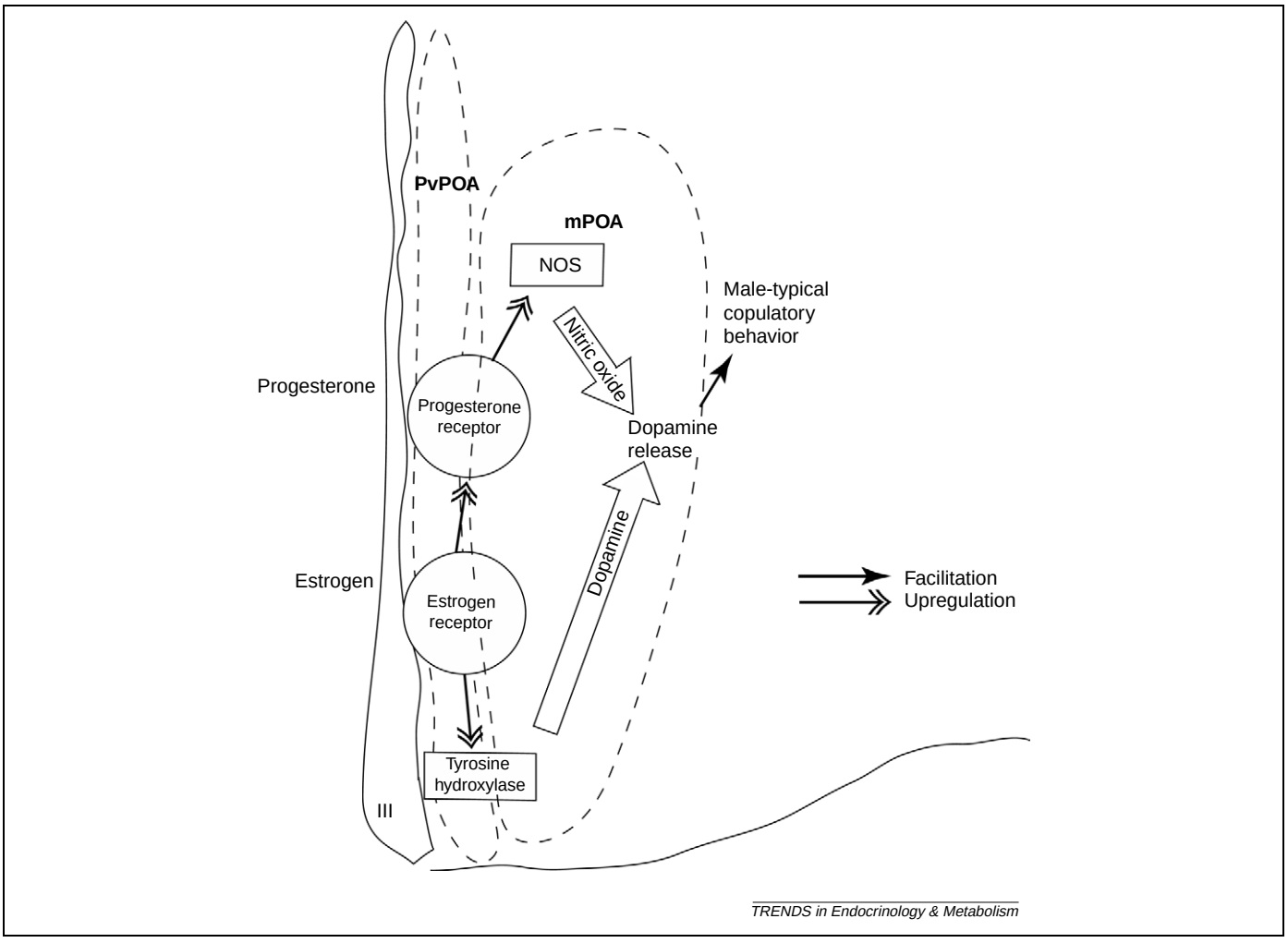

Figure 4. Model of the neuroendocrine mechanisms by which sex steroid hormones act on the preoptic area of the parthenogenetic whiptail lizard (*C. uniparens*) to enable male-like pseudocopulatory behavior. Estrogen, acting through its nuclear receptor (ER), upregulates the progesterone receptor (PR). Estrogen also facilitates development of the tyrosine hydroxylase (TH)-containing dopaminergic neurons of the periventricular preoptic area (PvPOA). Subsequent exposure to progesterone activates the PR, and the ligand-bound receptor upregulates the production of nitric oxide synthase (NOS) in cells of the medial preoptic area (mPOA). The nitric oxide produced by these neurons facilitates the release of dopamine from the varicosities and termini of dopaminergic axons from the PvPOA, and this dopamine release gates the expression of male-like copulatory behavior in response to stimuli from a receptive female.

and ovariectomized *C. uniparens* increases the display of mounting behavior in the ancestral species and in the descendant parthenogen (Figure 5) [15]. In addition, the number of tyrosine hydroxylase immunoreactive cells (TH-ir) changes across the reproductive cycle [16,17]; in the parthenogen there are fewer cells before ovulation, when the individual is exhibiting receptive behavior, compared with the postovulatory phase, when the individual is displaying pseudocopulatory behavior; there is no change in TH-ir cell number in the ancestral species. Consistent with other neuroendocrine traits [13], the somal size of TH-ir cells is larger in the parthenogen than in the sexual species because of their triploid nature.

The POA plays a central role in this testosterone-dependent gating [18] by a mechanism involving the facilitation of dopaminergic transmission by NO [19]. NO is produced in neurons from arginine by NOS and is thought to play crucial roles in both peripheral and central control of reproductive behavior [20]. Whereas in male rats NO is involved in the control of androgen-dependent copulatory behavior [21], in female rats it has been shown to influence progesterone-mediated lordosis behavior [22]. This responsiveness to the two different steroids suggests a possible involvement in the neural control of pseudocopulation in the parthenogen, which normally displays male-like pseudosexual behavior under the influence of progesterone, but can be made to display the same behavior with androgen treatment. Indeed, administration of the arginine analog *N*-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NOS, suppresses pseudocopulatory behavior in androgen-treated parthenogens [23] (Figure 6). NO synthesis is also crucially involved in the local control of erection [24]. However, unisexual whiptail lizards have entirely female-typical morphology and no erectile or intromittent structures, so this model system avoids the confounding peripheral effects of NO. Our findings support the idea that NO is involved in central neural control in a way that is independent of penile erection.

Thus, it is possible that estrogen, acting through ER, upregulates PR and facilitates development of the tyrosine hydroxylase-containing dopaminergic neurons of the POA. Subsequent exposure to progesterone activates the PR, and the ligand-bound receptor upregulates

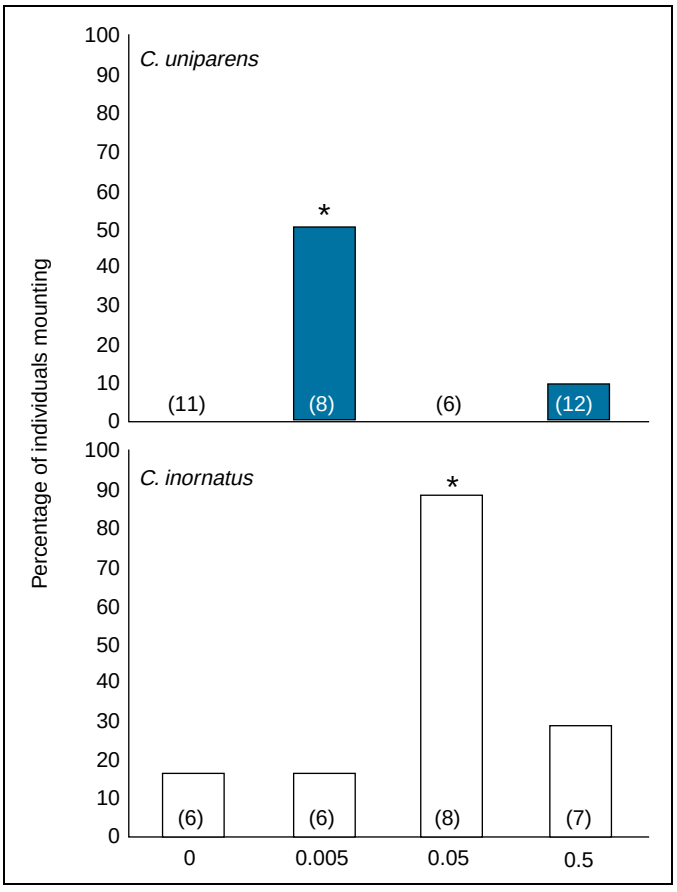

Figure 5. Percentage of castrated, progesterone-sensitive male *C. inornatus* (ancestral species, bottom panel) and ovariectomized *C. uniparens* (descendant species, top panel) mounting after administration of the full D1 receptor agonist SKF 81297. Within each species, the asterisk indicates a dose that is significantly different to the vehicle treatment ($P<0.05$); samples sizes shown in parentheses. Adapted from [15].

the production of NOS in cells of the POA. The NO produced by these neurons facilitates the release of dopamine from the dopaminergic axons from the POA, and this dopamine release gates the expression of male-like pseudosexual behavior in response to stimuli from a receptive parthenogen.

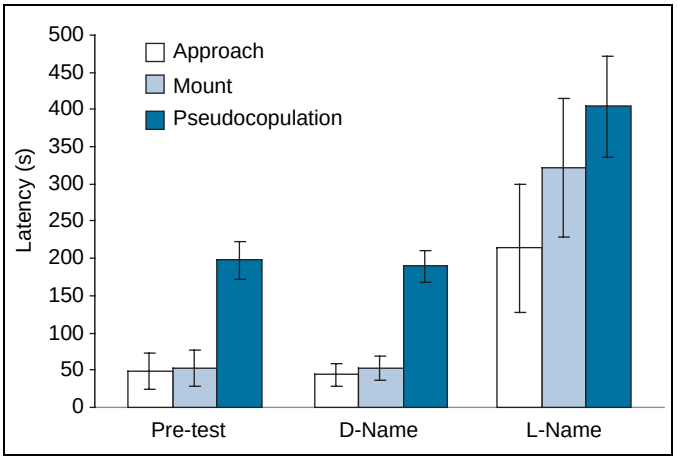

Figure 6. Inhibition of nitric oxide synthase suppresses pseudocopulatory behavior in unisexual whiptails. Using a repeated measures design, ovariectomized, androgen-treated lizards that were displaying robust pseudocopulatory behavior were treated with 600 μg per individual of L-NAME in physiological saline 1 h before testing with a receptive stimulus animal, and compared with the same dose of the inactive isomer D-NAME (an inactive isomer of L-NAME). The average (mean ± SE) latencies (seconds) to approach (open bars), mount (gray bar) and pseudocopulation (black bar) are shown. Adapted from [23].

Implications for other vertebrates

Before our work the common wisdom was that progesterone inhibited sexual behavior in male birds and mammals. However, this conclusion was based on relatively few studies and in all of them progesterone had been administered in pharmacological rather than physiological concentrations. Thus, the inhibition of behavior observed was a consequence of the negative feedback of progesterone at the level of the hypothalamus, culminating in testicular regression and a decline in circulating concentrations of androgens. However, in rats there is a substantial diurnal rhythm in progesterone secretion, with the greatest frequency of sexual behavior coinciding with peak progesterone levels [25]; furthermore, exogenous progesterone increases circulating androgen levels in male rats [26]. Human males secrete 1–5 mg of progesterone daily, with the peak occurring during the early morning hours when male libido is highest; this level is only slightly higher than that seen in females during the luteal phase [27–30].

Our results with lizards led us to study rodent model systems to determine whether progesterone and its receptor might play a role in sexual behavior in males. Administration of progesterone at physiological levels will reinstate sexual behavior in castrated male rats to the level of that seen with testosterone replacement treatment [31]. In addition, co-administration of progesterone and testosterone will completely restore sexual behavior in castrated males, which testosterone alone will not do. Finally, infusion of progesterone stimulates sexual behavior in castrated males, whereas RU486 inhibits the action of progesterone [32]. Studies with knockout (KO) mice indicate that naïve progesterone PRKO males are not as efficient in mating as wild-type males, show a more rapid decline in sexual behavior after castration and are less responsive behaviorally to exogenous testosterone than their wild-type litter mates [33].

Since our studies were published, other workers have investigated the role of progesterone in other vertebrates. In tree lizards, Moore and colleagues found that neonatal treatment with progesterone to simulate the increase in progesterone in circulation perinatally induces the development of male phenotype in tree lizards [34,35]. In both the rat and the mouse, males but not females have abundant PR-immunoreactive cells in the POA around the time of birth, when sexual differentiation of the brain is occurring [36,37]. Prenatal treatment with testosterone will increase PR synthesis in females to similar levels seen in normal males, whereas administration of a progesterone antagonist at this time prevents the male pattern abundance of PR-ir cells in the POA, resulting in males that exhibit female-typical behavior as adults. In the mouse and the guinea pig, estrogen upregulates PR in the medial POA, both in males and females [18,38]. Genetic dissection of this response using the ERKO mouse model indicates that ERβ interacts with PR to maximize this response [38].

Conclusions

Many studies have established that the POA, its functional output and the neurochemical mediators of its activity in regulating male sexual behavior are conserved across evolution. However, the steroid hormones and associated receptors that are involved in this process are much more diverse. For example, it has long been known that in some vertebrates estrogen is the active steroid in eliciting male sexual behavior, whereas androgen serves this role in other species. Our studies, which began with lizards and then extended to mammals, reveal a role for progesterone in modulating male sexual behavior. This, along with studies showing pronounced diurnal rhythms in progesterone secretion in males and the organizing role of PR in male sexual behavior, suggest that progesterone, like estrogen, is as much a 'male' hormone as it is a 'female' hormone. The apparent plasticity in the ability of neuroendocrine mechanisms to use different signaling molecules contrasts with the remarkable evolutionary stability of the mechanisms themselves [1]. This has implications for predicting the effects of hormones in humans based on diverse animal models and for cross species comparisons in general.

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References

1 Moore, M.C. and Crews, D. (1986) Evolution of mechanisms controlling mating behavior. *Science* 231, 121–125
2 Diamond, J. (1983) Laboratory, field, and natural experiments. *Nature* 304, 586–587
3 Adams, M. *et al*. (2003) The Australian scincid lizard *Menetia greyii*: a new instance of widespread vertebrate parthenogenesis. *Evolution Int. J. Org. Evolution* 57, 2619–2627
4 Fu, J. *et al*. (2000) Allozyme variation patterns and multiple hybridization origins: clonal variation among four sibling parthenogenetic Caucasian rock lizards. *Genetica* 108, 107–112
5 Moritz, C. *et al*. (1989) Genetic diversity and the dynamics of hybrid parthenogenesis in *Cnemidophorus* (Teiidae) and *Heteronotia* (Gekkonidae). In *Evolution and Ecology of Unisexual Vertebrates* (Dawley, R.M. and Bogart, J.P., eds), pp. 87–112, University of the State of New York
6 Tokarskaya, O.N. *et al*. (2004) Instability of (GATA)n microsatellite loci in the parthenogenetic Caucasian rock lizard *Darevskia unisexualis* (Lacertidae). *Mol. Genet. Genomics* 270, 509–513
7 Tokarskaya, O.N. *et al*. (2001) Genetic variation in parthenogenetic Caucasian rock lizards of the genus *Lacerta* (*L. dahli, L. armeniaca, L. unisexualis*) analyzed by DNA fingerprinting. *Mol. Genet. Genomics* 265, 812–819
8 Kearney, M. and Shine, R. (2004) Morphological and physiological correlates of hybrid parthenogenesis. *Am. Nat.* 164, 803–813
9 Kearney, M. and Shine, R. (2005) Lower fecundity in parthenogenetic geckos than sexual relatives in the Australian arid zone. *J. Evol. Biol.* 18, 609–618
10 Crews, D. and Fitzgerald, K.T. (1980) "Sexual" behavior in parthenogenetic lizards (*Cnemidophorus*). *Proc. Natl. Acad. Sci. U. S. A.* 77, 499–502
11 Godwin, J. and Crews, D. (2002) Hormones, brain and behavior in reptiles. In *Hormones, Brain and Behavior* (Vol. 1) (Pfaff, D.W. *et al.*, eds), pp. 545–585, Academic Press
12 West-Eberhard, M.J. (1992) Behavior and evolution. In *Molds, Molecules, and Metazoa: Growing Points in Evolutionary Biology* (Grant, P.R. and Horn, H., eds), pp. 57–75, Princeton University Press
13 Young, L.J. and Crews, D. (1995) Comparative neuroendocrinology of steroid receptor gene expression and regulation: relationship to physiology and behavior. *Trends Endocrinol. Metab.* 6, 317–323
14 Crews, D. *et al*. (1996) Intrahypothalamic implantation of progesterone in castrated male whiptail lizards (*Cnemidophorus inornatus*) elicits courtship and copulatory behavior and affects androgen receptor and progesterone receptor-mRNA expression in the brain. *J. Neurosci.* 16, 7347–7352
15 Woolley, S.C. *et al*. (2001) Evolutionary changes in dopaminergic modulation of courtship behavior in *Cnemidophorus* whiptail lizards. *Horm. Behav.* 40, 483–489
16 Woolley, S.C. and Crews, D. (2004) Species differences in the regulation of tyrosine hydroxylase in *Cnemidophorus* whiptail lizards. *J. Neurobiol.* 60, 360–368
17 Woolley, S.C. *et al*. (2004) Tyrosine hydroxylase expression is affected by sexual vigor and social environment in male *Cnemidophorus inornatus*. *J. Comp. Neurol.* 476, 429–439
18 Dufourny, L. *et al*. (1997) Sex differences in estradiol induced progestin receptor immunoreactivity in the guinea pig preoptic area and hypothalamus. *Neurosci. Lett.* 223, 109–112
19 Hull, E. *et al.* (2002) Male sexual behavior. In *Hormones, Brain and Behavior* (Vol. 1) (Pfaff, D.W. *et al.*, eds), pp. 1–138, Academic Press
20 Argiolas, A. (1994) Nitric oxide is a central mediator of penile erection. *Neuropharmacology* 33, 1339–1344
21 Hull, E. *et al*. (2004) Dopamine and serotonin: influences on male sexual behavior. *Physiol. Behav.* 83, 291–307
22 Mani, S.K. *et al*. (1994) Nitric oxide mediates sexual behavior in female rats. *Proc. Natl. Acad. Sci. U. S. A.* 91, 6468–6472
23 Sanderson, N. *et al*. (2005) Nitric oxide synthase inhibition suppresses male-like pseudocopulatory behavior in whiptail lizards. *Brain Res.* 1052, 236–239
24 Cartledge, J. *et al*. (2001) The role of nitric oxide in penile erection. *Expert Opin. Pharmacother.* 2, 95–107
25 Kalra, P.S. and Kalra, S.P. (1977) Circadian periodicities of serum androgens, progesterone, gonadotropins and luteinizing hormone-releasing hormone in male rats: the effects of hypothalamic deafferentation, castration and adrenalectomy. *Endocrinology* 101, 1821–1827
26 Kalra, P.S. and Kalra, S.P. (1980) Progesterone stimulates testosterone secretion in male rats. *Neuroendocrinology* 30, 183–186
27 Kage, A. *et al*. (1982) Diurnal and ultradian variations of plasma concentrations of eleven adrenal steroid hormones in human males. *Klin. Wochenschr.* 60, 659–666
28 Mauvais-Jarvis, P. *et al*. (1975) Progesterone administered by percutaneous route: an antiandrogen locally useful. *Ann. Endocrinol. (Paris)* 36, 55–62
29 Opstad, K. (1994) Circadian rhythm of hormones is extinguished during prolonged physical stress, sleep, and energy deficiency in young men. *Eur. J. Endocrinol.* 131, 56–66
30 Vermeulen, A. and Verdonck, L. (1976) Radioimmunoassay of 17β-hyroxy-5α-androstan-3-one, 4-androstene-3, 17-dione, dehydroepiandrosterone, 17-hydroxyprogesterone and progesterone and its application to human male plasma. *J. Steroid Biochem.* 7, 1–10
31 Witt, D.M. *et al*. (1994) Progesterone and sexual behavior in males. *Psychoneuroendocrinology* 19, 553–562
32 Witt, D.M. *et al*. (1997) Intrahypothalamic progesterone regulates androgen-dependent sexual behavior in male rats. *Soc. Neurosci. Abstr.* 23, 1357
33 Phelps, S.M. *et al*. (1998) Regulation of male sexual behavior by progesterone receptor, sexual experience and androgen. *Horm. Behav.* 34, 294–302
34 Jennings, D.H. *et al*. (2004) Role of the adrenal gland in early post-hatching differentiation of alternative male phenotypes in the tree lizard (*Urosaurus ornatus*). *Gen. Comp. Endocrinol.* 135, 81–89
35 Moore, M.C. *et al*. (1998) Hormonal control and evolution of alternative male phenotypes: generalizations of models for sexual differentiation. *Am. Zool.* 38, 133–151
36 Quadros, P.S. *et al*. (2002) Regulation of sex differences in progesterone receptor expression in the medial preoptic nucleus of postnatal rats. *J. Neuroendocrinol.* 14, 761–767
37 Quadros, P.S. *et al*. (2002) Progesterone receptors and the sexual differentiation of the medial preoptic nucleus. *J. Neurobiol.* 51, 24–32

38 Kudwa, A.E. *et al*. (2004) Estrogen receptor β modulates estradiol induction of progestin receptor immunoreactivity in male, but not in female, mouse medial preoptic area. *Endocrinology* 145, 4500–4506
39 Crews, D. and Sakata, J.T. (2000) Evolution of brain mechanisms controlling sexual behavior. In *Sexual Differentiation of the Brain* (Matsumoto, A., ed.), pp. 113–130, CRC Press
40 Rand, M.S. and Crews, D. (1994) The bisexual brain: sex behavior differences and sex differences in parthenogenetic and sexual lizards. *Brain Res.* 663, 163–167
41 Godwin, J. and Crews, D. (1999) Hormonal regulation of progesterone receptor mRNA expression in the hypothalamus of whiptail lizards: regional and species differences. *J. Neurobiol.* 39, 287–293