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Review

The many faces of progesterone: A role in adult and developing male brain

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Abstract

In addition to its well documented action in female-typical behaviors, progesterone exerts an influence on the brain and behavior of males. This review will discuss the role of progesterone and its receptor in male-typical reproductive behaviors in adulthood and the role of progesterone and its receptor in neural development, in both sexual differentiation of the brain as well as in the development of "non-reproductive" functions. The seemingly inconsistent and contradictory results on progesterone in males that exist in the literature illustrate the complexity of progesterone's actions and illuminate the need for further research in this area. As progestin-containing contraceptives in men are currently being tested and progesterone administration to pregnant women and premature newborns increases, a better understanding of the role of this hormone in behavior and brain development becomes essential.

Keywords: Progesterone; Progesterone receptor; Sexual behavior; Sexual differentiation; Sex differences; Human; Cortex; PRKO

1. Introduction

Progesterone (P), named for its progestational role in maintaining pregnancy, is traditionally regarded as a "female hormone" and the role of P in female-typical behaviors such as lordosis and maternal behavior is well-documented [for reviews see 12,71,79,118]. In females, the ovary (and/or placenta) is the primary source of the dynamic levels of plasma P that exist during the estrous cycle and over the course of pregnancy and lactation. In addition, the role of P in females appears similar in a variety of species, particularly for sexual behavior. However, even in these behaviors which have been studied extensively, there are enigmatic biphasic effects of P. For example, in rat female sexual behavior, P first facilitates, then inhibits lordosis [e.g., 13,56,97,103,151,160, and for review see 13]. At the end of pregnancy in rats, plasma P is at very high levels, but then drops precipitously just prior to parturition [99,100,120]. This dynamic fluctuation in P is essential for maternal behavior to occur and the behavior will not be induced if high levels of P are not subsequently reduced [e.g., 16,98,127,129]. There are numerous other examples of P having biphasic, dual, or differential effects on various physiological and cellular parameters [e.g., 5,57,157,159].

Given the clear role of P in female-typical behaviors and the obvious source(s) of P in females, the idea that P may also play an important physiological role in the brain and behavior of males seems strange. However, adult male rats have circulating P levels in the range of 1.96 ng/ml plasma, the source of which appears to be the adrenal gland (61,102; for comparison, baseline levels in female rats are 3–15 ng/ml and peak levels at proestrus are 25–50 ng/ml). In addition, both the adult and developing male rodent brain express progesterone receptors (PR) in specific areas [17,54,69,70,105,148]. In fact, the idea that P might have a function in male behavior was first explored as early as 1966. P received modest attention as an "anti-androgenic" hormone through the 1970s, but was then virtually ignored until the mid 1990s. A renewed interest in the role of P and its receptor in the male brain, both

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during adulthood and development, has recently emerged. However, the hiatus this topic experienced, in combination with the often differential effects of P depending on timing, dose, species etc., leaves many questions unanswered. This review will examine the work on P in males which spans almost four decades, includes species from lizards to humans and covers the life span from fetal development to adulthood. Specifically, this review will discuss (1) the role of P and its receptor in male-typical reproductive behaviors in adulthood and (2) the role of P and its receptor in neural development, with regard to both sexual differentiation of brain and behavior, as well as the development of "non-reproductive" brain areas and cognitive behaviors. The goal of this review is 2-fold: (1) to attempt to integrate the sometimes inconsistent and contradictory results that exist in the literature on P in males and (2) to illuminate and identify some of the many remaining questions that need to be addressed before we have even a rudimentary understanding of P's actions in the male brain. These questions are more than just academic. Clinical trials on the use of progestins as male contraceptives necessitate a complete understanding of P in the male brain. Additionally, there is a recent resurgence in the administration of progestins to pregnant women in the US to delay or prevent premature delivery and clinical studies in Europe have recently begun in which premature infants are intravenously infused with P (and estradiol) in the initial weeks after birth. In light of these clinical studies, our current lack of knowledge on the potential effects of P on the developing brain is worrisome.

2. Progesterone and progesterone receptors in adult males

2.1. Progesterone and male-typical behaviors

2.1.1. Rodents

The idea that progesterone might influence testosterone-mediated behaviors harkens back to a paper published in 1965, demonstrating that the sterility induced by an injection of 100 μg of testosterone propionate to five-day-old female rats could be prevented when 3 mg of P was administered simultaneously [65]. The conclusion derived from these findings was that P was capable of "protecting" females from the masculinizing effects of testosterone. The so-called "anti-androgenic" effects of P were then extrapolated to androgen-dependent, male-typical behaviors in adulthood. Specifically, the interaction between androgen and P was examined in the display of the testosterone-dependent behaviors, sexual behavior and aggressive behavior. In the majority of these early studies, supraphysiological levels of P (i.e., doses that would greatly exceed the basal levels of adrenally derived P found in normal males) were used. For example, in 1966, Diamond [34] demonstrated that P treatment (50 mg P with 10 mg daily thereafter) significantly impaired male sexual behavior in adult intact guinea pigs, as well as in males that were castrated and given testosterone propionate. Many years later, Connolly and Resko [28] reported similar findings. Daily injections of 1 mg or 10 mg P or 100 μg of the synthetic progestin R5020, impaired sexual behavior in intact male guinea pigs. However, the 10 mg dose produced plasma P levels that were 200-fold higher than levels measured in vehicle treated guinea pigs. Similarly, daily injections of 0.5 mg and 1 mg P, but not 0.25 mg, administered to intact adult male mice, suppressed mounting and intromissive behavior compared to vehicle treated animals, but behavioral differences between the groups did not appear until the third week of treatment [41]. On the other hand, three weeks following the cessation of P injections, differences between P treated mice and controls disappeared, suggesting that the inhibitory effects of P on male sexual behavior in mice were reversible [41]. Notably, P treatment increased genital nosing behavior in this study in a dose dependent fashion. Because this behavior requires vigorous activity, an inhibitory effect of P on male sexual behavior due to a simple anesthetic or sedative effect of P becomes a less likely explanation. In a study using a more moderate dose of P, daily injections of 500 μg P in male hamsters had an inhibitory effect on the restoration of sexual behavior by androgen treatment in long-term castrates [33]. Therefore, in three different species, high doses of P inhibited the facilitatory effects of testosterone on male sexual behavior.

Other studies reported around the same time, were also demonstrating the "anti-androgenic" effects of high doses of P on aggressive behavior in mice. In 1971, Erpino and Chappelle [43] reported that while androgen treatment could restore aggressive behavior in castrated male mice, simultaneous treatment with a high dose of P (a pellet containing 15 mg) could inhibit the restoration of aggressive behavior by both testosterone propionate and androstenedione. However, P alone had no effect on aggressive behavior in castrated male mice. Luttge [76] restored isolation-induced aggression in castrated CD-1 mice with daily injections of 50, 100 or 200 μg testosterone propionate. At lower doses of testosterone, but not at higher doses, the concurrent administration of P (100 μg/day) inhibited aggressive behavior, suggesting that the inhibitory effects of P could be over-ridden with sufficient androgen levels. This, again, argues against a simple sedative effect of P in the reduction of aggressive behavior and instead, suggests a dynamic interaction between testosterone and P in eliciting aggression. Similarly, P treatment (10 mg pellet) attenuated androgen-induced aggression in neonatally androgenized female mice [42]. In this same study, P also reduced the incidence of aggression in intact adult males. In contrast, other non-fighting social behaviors (e.g., tail-rattling, anogenitial nosing, autogrooming, etc.) were not altered by P treatment in either males or androgenized females. The conclusion drawn in each of these studies was that P was capable of exerting "anti-androgenic" effects on male-typical behaviors, in the absence of a sedative effect.

2.2. Proposed mechanisms of anti-androgenic activity of P

Despite the evidence for an anti-androgenic action of progestins in male behavior proposed in the 1970s, our understanding of the mechanisms underlying this action is not much better today than 30 years ago. Over the years, evidence suggesting a variety of mechanisms has been reported. Serum levels of both testosterone and P demonstrate circadian patterns in male rats [61]. Interestingly, testosterone and P patterns are inversely related, suggesting a potential interaction between the two hormones. Serum P levels peak just after the onset of dark, while testosterone levels reach their nadir 2 h later, consistent with the idea that P may induce the catabolism of testosterone [61]. Indeed, Albin et al. [1] observed that while the progestin medroxyprogesterone acetate (MPA) had no effect on 5α-reductase activity in prostate, it significantly increased the activity of this enzyme in liver, suggesting an increased catabolism of testosterone in the presence of MPA. Albin et al. [1] also reported a decrease in the uptake of radiolabelled testosterone by prostate following MPA treatment, consistent with an increase in the clearance rate of testosterone by this progestin. In brain, progesterone treatment decreased the uptake of [^{3}H]-testosterone by the hypothalamus, but not the cerebrum, suggesting that progesterone may decrease the reduction of testosterone to the active metabolite, DHT, within brain regions important for male-typical behaviors [134]. Supporting this idea, the ratio of DHT:T was reduced by progesterone treatment in seminal vesicles (brain was not examined).

Subsequent studies have replicated the findings that progesterone decreases the uptake of androgens by target tissues, but evidence suggests that it does so even in the absence of changes in circulating testosterone. This suggests that progesterone may be directly regulating androgen receptor expression or activity. Using an in vitro binding assay, Connolly et al. [27] demonstrated that daily injections of pharmacological doses of P (10 mg/day) in guinea pigs resulted in a decrease in nuclear androgen receptors (bound with [^{3}H]-DHT) and an increase in cytoplasmic androgen receptors. P treatment did not significantly alter plasma testosterone levels in this study, although it did significantly elevate plasma P levels by 200-fold. Inverse changes in [^{3}H]-DHT binding in nuclear and cytoplasmic fractions are difficult to interpret, particularly if progesterone alters 5α-reductase activity, as suggested by Stern and Eisenfeld [134]. However, very recent evidence, using immunocytochemistry to quantify nuclear androgen receptor [124], suggests that indeed, PR may inhibit the expression of the androgen receptor gene. While Connolly et al. [20] found high doses of P decreased nuclear androgen receptors in the preoptic area, as well as the mediobasal hypothalamus, Schneider et al [124] reported a dramatic increase in the number of androgen receptor immunopositive cells in the medial preoptic nucleus and bed nucleus of the stria terminalis in transgenic mice with an insertional mutation ("knock-out") of the PR gene. These results suggest that PR normally down-regulates the androgen receptor within behaviorally relevant brain areas. However, many questions remain about the role of progesterone in androgen metabolism and androgen receptor function.

2.2.1. Primates

The hypothesis that progestins are capable of inhibiting androgen-dependent behaviors has not been restricted to rodents, but extends to primates, both non-human and human. The synthetic progestin, medroxyprogesterone acetate (MPA; Depo-Provera) has been used in humans to reduce sexual motivation in convicted sex offenders. Treatment with this progestin reduces plasma testosterone levels in men by altering testicular function and by regulating metabolic clearance rates [47,116]. It appears to exert the desired behavioral effects by first reducing deviant sexual urges, and ultimately sexual activity.

In Cynomolgus monkeys (*Macaca fascicularis*) MPA treatment, at doses that mimic those used clinically in male sex offenders, reduces sexual activity. Weekly, intramuscular injections of 40 mg MPA to castrated adult males given testosterone to hold plasma testosterone levels constant, decreased sexual behavior and reduced mounting attempts by five to six weeks of treatment [93]. Because previous work in both monkeys and men reported that MPA reduced circulating testosterone levels in intact males, these findings demonstrated that MPA could reduce sexual behavior independent of altered plasma testosterone levels. In fact, previous studies indicated that the effects of MPA on sexual behavior in monkeys were qualitatively different from those of surgical castration, although both treatments quantitatively reduced the incidence of sexual activity [161]. MPA treatment, administered to male monkeys in a regimen sufficient to reduce sexual behavior, significantly reduced the uptake of [^{3}H]-testosterone and [^{3}H]-dihydrotestosterone, whereas it had no effect on the uptake of [^{3}H]-estradiol by hypothalamic, limbic and preoptic regions of the brain known to be important for sexual behavior [92]. These findings suggest that MPA exerts its effects on behavior by reducing the sensitivity of specific brain regions to androgens. However, MPA alters androgen sensitivity indirectly as it appears to act via estradiol-induced progesterone receptors and not directly interfere with androgen receptor activity. Nuclear accumulation of radiolabeled MPA in the arcuate nucleus, the ventromedial nucleus, the medial preoptic nucleus and the anterior hypothalamic area [15] was virtually abolished by pretreatment with P, but was not altered by pre-treatment with dihydrotestosterone. The behavioral effects of MPA on adult male monkeys were reversed with the concurrent administration of the aromatase enzyme inhibitor, fadrazole, [163], suggesting that the behavioral effects of MPA are dependent on estradiol. While MPA treatment alone had no effect on PR immunoreactivity, PR was almost completely abolished in males receiving both MPA and fadrazole.

The role of natural P has also been investigated in the monkey model with the idea that P might serve as an

effective alternative to MPA treatment in human sex offenders, as MPA has many side effects that reduce treatment compliance [88]. To date, studies examining the effects of P treatment on male sexual behavior in monkeys have used supraphysiological doses of P that produce plasma P levels 10 times that of endogenous P levels. Similar to pharmacological doses of P in rodents, supraphysiological doses of P reduced sexual behavior in gonadally intact males [165] and these behavioral effects were independent of alterations in plasma testosterone levels, indicating that the behavioral actions of P do not require changes in testicular secretion of testosterone. Similarly, high doses of P significantly reduced sexual activity and sexual motivation in testosterone-treated castrated male monkeys [164]. P treatment did not alter the plasma testosterone levels produced by the exogenous testosterone treatment, indicating that the metabolic clearance rates for testosterone were not altered by P. Thus it seems that P is capable of altering sexual behavior in monkeys in the absence of changes in circulating testosterone. Interestingly, natural P, unlike MPA [92], did not alter the nuclear accumulation of [^{3}H]-testosterone within the brain, but decreased the accumulation of [^{3}H]-estradiol by 80% within brain tissue containing preoptic area and anterior bed nucleus of the stria terminalis [165]. In monkeys, sexual activity depends, in part, on unmetabolized testosterone and in part, on local aromatization of testosterone to estradiol [162,163,166]. Taken together, these findings suggest that P, at least at high doses, inhibits male sexual behavior in monkeys, possibly by reducing uptake of estradiol within behaviorally important brain areas. The mechanisms by which this might occur remain unknown, although it has been suggested that P may down-regulate estradiol receptors, inhibit aromatization of testosterone to estradiol, or in some other way, interfere with estrogen receptor activity [165]. Thus, from work in non-human primates, as well earlier studies in rodent models, it can be surmised that supraphysiological doses of P exert a functionally "anti-androgenic" (i.e., anti-testicular hormone) action on male-typical behaviors.

2.3. Progesterone facilitation of male sexual behavior in rodents

Following earlier studies on the anti-androgenic effects of supraphysiologic doses of P in rodents, there is an extraordinary gap in the literature on the actions of P in adult males. After almost 15 years of neglect, Witt and colleagues [156] revisited this issue in 1995. However, in a complete turnabout, these authors revealed that physiological levels of P were capable of fully restoring male sexual behavior in castrated male rats, even in the absence of gonadal steroids. Concurrent treatment with the PR antagonist, RU486, completely reversed the facilitatory effects of P, suggesting that P's mechanism of action involved activation of progesterone receptors. Furthermore, only when P supplementation was given, was testosterone treatment able to fully restore sexual behavior in castrated rats. In contrast to early work on P in males, the P capsules used in the Witt et al. [156] study produced plasma P levels in the physiological range. Castrated male rats given P had plasma P levels in 4–6 ng/ml range, whether they were given testosterone or not. Untreated intact males and untreated castrate males had plasma P levels in the 1–2 ng/ml and 2–4 ng/ml range, respectively, consistent with previous work demonstrating that P levels are increased following castration in adult males [102]. While this dose of exogenous P necessarily supplements the endogenous levels of P from adrenal origin, the circulating levels of P produced certainly do not approach levels seen in earlier work. For example, in Connolly et al, [27] plasma P levels exceeded 100 ng/ml following daily injections of 10 mg P. The work of Witt and colleagues, taken together with research from the 1970s using higher doses of P, suggest that P can have both facilitatory effects and inhibitory effects on male sexual behavior depending on circulating levels.

Consistent with the idea that P has dose-dependent, biphasic effects on male sexual behavior, DeBold et al. [33] administered P (500 μg/day) to long-term castrated hamsters, a dose that is arguably closer to physiological levels than the 1–10 mg doses used by others [28,35,41]. This intermediate dose only slightly inhibited the restoration of sexual behavior by androgen replacement. Additionally, 500 μg P administered concurrently with either testosterone propionate or estradiol benzoate, increased the proportion of males continuing to copulate following castration [26], consistent with the findings of Witt et al. [156], although P by itself had no effect in hamsters.

One possible explanation for the seemingly contradictory results of Witt et al. and earlier work is simply the species examined. While Witt and colleagues examined adult male *rats*, none of the earlier work used the rat model and instead examined mice, hamsters and guinea pigs. However, a more likely explanation is the idea that P may exert a biphasic effect on sexual behavior in males. Because at least several of the early studies ruled out sedation effects of high doses of P, it can be argued that P may have dose-dependent effects on male sexual behavior, with low (i.e., physiological) levels facilitating and high (i.e., supraphysiologic, but not sedating) levels inhibiting male behavior. Endogenous P levels in the male rat, arising from the adrenal gland, appear to be under the control of ACTH [102], and fluctuate in a circadian rhythm with levels rising greater than 10-fold from the onset of light to the onset of dark (~50 pg/ml at 0500hr; ~700 pg/ml at 19:30 hours with lights out at 19:00 hours); [61]. This suggests that males may be exposed to natural variations in the levels of plasma P in a behaviorally relevant way. In fact, the onset of male sexual behavior and the rise in circulating P levels are correlated, with P levels beginning to rise about 3–6 h prior to the onset of sexual activity in the rat [61]. In addition, plasma P levels rise significantly following stress in both intact and castrated adult males, presumably as a result of adrenal activation [e.g., 2,117]. Because stress can reduce circulating testosterone levels [e.g., 22,113] and can inhibit sexual behavior in adult

males [e.g., 121,52], the possibility exists that progesterone serves a compensatory role, ensuring that reproductive behavior occurs despite naturally occurring environmental stressors. Conversely, during chronic stress, prolonged elevation in plasma P may be one of the mechanisms by which sexual behavior is disrupted, as P can have anti-androgenic effects as well.

The idea that circulating levels of P can profoundly influence the outcome of testosterone on sexual behavior is supported by research examining the role of P in sexual behavior in various species of lizards. Young et al. [158] suggest that the androgen: progesterone ratio may be a critical factor in determining whether P inhibits testosterone actions or synergizes with testosterone to regulate male sexual behavior. The role of P in lizards has been elegantly reviewed recently [29]. Therefore only a few relevant points will be covered herein. In little striped whiptail lizards (*C. inornatus*), P is capable of fully restoring sexual behavior in some castrated males in the absence of other gonadal hormones [72]. This effect is abolished by the PR antagonist, RU486, and mimicked by the PR agonist, R5020 [73]. This effect is strikingly similar to that reported in rats, in which physiological doses of P could restore sexual behavior in castrated males [156]. However, in green anole lizards (*Anolis carolinensis*), P inhibited sexual behavior in gonadally intact males, but facilitated the ability of testosterone to restore sexual behavior in castrated males [158]. The authors suggest that while the dose of P in both situations was the same, the ratio of P to testosterone differed between the intact males and the castrated males supplemented with a testosterone propionate capsule. P impaired sexual behavior in intact male green anole lizards with endogenous levels of circulating testosterone, but facilitated the effects of exogenous testosterone treatment, which presumably elevated circulating testosterone above endogenous levels, thereby increasing the progesterone: testosterone ratio [158]. Similar effects have also been reported for scent marking behavior in gerbils [51]. In this study, *higher* doses of P inhibited testosterone's actions in restoring scent marking in castrated male gerbils, whereas *lower* doses of P given with the same dose of testosterone, increased the incidence of scent marking in castrated males. Similarly, low doses of the synthetic progestin, cyproterone acetate, facilitated the effects of testosterone on the activity of β-glucuronidase, a glycosidase enzyme in the mouse kidney [96]. Higher doses of P inhibited the androgen-dependent activity of β-glucuronidase. From this type of result in kidney, it has been suggested that progestins can exert androgenic, anti-androgenic and synandrogenic actions, as progestins can mimic, inhibit or potentiate the actions of androgens. Witt et al. [156] provides evidence for P mimicking testosterone's action in male sexual behavior, whereas many of the early studies in mice and guinea pigs suggest that P has anti-androgenic effects on male sexual behavior. Meanwhile, work in lizards and hamsters, as well as the findings of Witt et al. [156] in intact rats, provide evidence for a synandrogenic action of P on male sexual behavior [33,158].

2.3.1. Progesterone receptors in adult male brain

While the neural mechanisms underlying P's effects on male sexual behavior are not understood, it is clear that the adult male rat brain is sensitive to P in that, specific regions of the brain, including those integral to male sexual behavior and neuroendocrine functions, express PR. For example, cytosolic binding of [^{3}H]-R5020, is present in the medial preoptic nucleus, the ventromedial hypothalamus and the arcuate/median eminence in adult male rats [17]. PR exists as two isoforms, PR_A and PR_B. These isoforms are encoded by a single gene, but are under the control of different promoters and are regulated differently in male hypothalamus [126]. Both isoforms are capable of binding progestin and driving transcription, but it appears that they may have different functions from one another e.g., [46]. Using in situ hybridization, Lauber et al. [69] demonstrated that PR mRNA was expressed in the preoptic area, arcuate nucleus, the ventrolateral ventromedial nucleus, and the amygdala of intact adult males at levels similar to intact adult females. The expression of PR in the male hypothalamus, appears to consist of more PR-A mRNA than PR-B mRNA, whereas the expression of the two isoforms is equivalent in the preoptic area [54].

Alternatively, P, by way of its neuroactive metabolites, may alter behavior through a more rapid action on GABA receptors in brain. While the details of neurosteroid action go beyond the scope of the present review, many recent reviews on the topic are available [8,9,11,95,123,135]. Surprisingly, very little is understood about the role of this mechanism of P metabolite action in male sexual behavior, although it is clear further investigation might reveal an important interaction between genomic actions of P and effects via neuroactive metabolites in males.

Clearly, the role of P in male sexual behavior is misunderstood at best, and further experiments are critically needed. Specifically, the hypothesis that P has biphasic (or "triphasic") effects on male sexual behavior, depending on dose of P and circulating androgen levels, must be empirically tested within a single species. This becomes increasingly important as the testing of progestins as male hormonal contraceptives becomes more common. Recent studies have demonstrated that injections of MPA (Depoprovera) every three months, in combination with a testosterone implant, suppressed spermatogenesis in men and prevented pregnancy in their female partners [142]. Similarly, treatment with the progestin, levonorgestrel, in combination with testosterone, significantly decreased sperm concentration [45,55]. While these results provide promise for the development of an effective male contraceptive method, they also highlight the deficiency of understanding regarding the effects of progestins on the adult male brain. Further work in this area is obviously important.

2.4. Male sexual behavior in PRKO mice

Given the seemingly inconsistent effects of P treatment in male rodents and non-human primates, a more direct

approach to elucidating the importance of endogenous P levels in male sexual activity is to examine behavior in mice in which the PR gene has been rendered functionless. The generation of a strain of mouse with an insertional mutation within the PR gene, known as PR "knock-out" (PRKO) mice, [77] has made it possible to directly examine the role of the receptor in reproductive function and behavior. Briefly, mice that fail to express functional mRNA for either PR_A or PR_B were created using the embryonic stem cell/gene targeting technique. A neomycin resistance gene was inserted within the PR gene, at a site that results in the premature termination of transcription initiated at both the PR_A and PR_B ATG start codons. Mice homozygous for the mutation lack functional PR. Therefore, increases or deficits in male-typical behaviors in PRKO mice compared to wild-type mice, should directly address the question of whether endogenous P normally inhibits or facilitates sexual behavior. Certainly, female mice that lack functional PR demonstrate numerous reproductive abnormalities and functional deficits [20,77 and for reviews see 25,26]. However, to date, only two papers have examined the sexual behavior of male PRKO mice.

Phelps et al. [101] reported results consistent with the idea that PR is facilitatory in male sexual behavior. These authors examined male sexual behavior in WT and PRKO males and examined the interaction between genotype and the role of experience and gonadal hormones. Only subtle differences were observed between gonadally intact, naïve WT and PRKO mice, with WT males displaying a significant, but small advantage in mount frequency. This subtle difference disappeared with continued behavioral experience. However, when sexual behavior was compared in sexually experienced WT and PRKO males three weeks after castration, the effects were dramatic. While WT males showed little deficit in their sexual behavior after castration, sexual behavior was virtually absent in PRKO males. Nine weeks following castration, after sexual behavior was diminished even in WT males, the effects of testosterone replacement were examined. WT males exhibited increased sexual behavior following testosterone replacement, as expected. However, PRKO males or males that were heterozygous for the insertional mutation, failed to show such an increase. These results suggest that while PR appears to have a small role in sexual behavior when testosterone is present, it may act to facilitate sexual behavior in the absence of testosterone. Yet, this effect appears to be dependent on prior sexual experience. These results are consistent with those of Witt et al. [156]. Taken together, these findings suggest that endogenous P, acting via PR, is capable of *facilitating* sexual behavior in the absence of gonadal hormones.

However, PR, like its ligand P, seems to be a moving target. More recently, Schneider et al. [124] also examined sexual behavior in intact PRKO mice. In seemingly stark contrast to Phelps et al. [101], these authors report that many measures of sexual behavior are *enhanced*, rather than diminished, in PRKO mice. The implications of these findings are that PR normally has an *inhibitory* role in male sexual behavior. In the first two consecutive tests of naïve males, PRKO mice exhibited a significant reduction in the latency to the first mount. Previous studies have demonstrated that basal testosterone levels do not differ between adult PRKO and WT males, ruling out a simple hormonal explanation for the behavioral findings. However, in the Schneider et al. [124] study, PRKO males were not directly compared to their WT littermates, but rather, were compared with males of each of the background strains, C57Bl/6 and 129SvEv. These males had functional PR genes, but were not isogenic with the PRKO mice, nor with the WT mice to which PRKO males were compared in Phelps et al. [101], making a direct comparison between the studies more difficult. However, using a pharmacological approach, in addition to the genetic approach, Schneider et al. [124] observed that treatment with the PR antagonist, RU486, increased the number of mounts and intromissions per test in intact males of the C57Bl/6 background strain. However, the effects of RU486 were only observed on the initial test of naïve males and were not detected in subsequent tests following behavioral experience. Overall, the results of Schneider et al. [124] suggest that the role of PR in male sexual behavior is an *inhibitory* one, but that an interaction between PR and behavioral experience exists. Another report from the same group [125] demonstrates an inhibitory role in another male-typical behavior, aggression toward young and infanticidal behavior.

Interestingly, neither Phelps et al. [101] nor Schneider et al. [124] report a significant effect of P treatment. Schneider et al. [124] treated intact C57 males with P capsules that produced circulating P levels of approximately 40–50 ng/ml and observed no change in male sexual behavior compared to vehicle treated animals. Phelps et al. [101] administered a capsule containing 100 mg P (providing physiological levels of plasma P) to castrated WT, PRKO and heterozygous males and observed no effect on sexual behavior. Therefore, in the presence or absence of gonadal hormones, P treatment failed to facilitate or inhibit male sexual behavior in mice. As both papers suggest, this may simply be due to the fact that the adrenal gland is already producing basal levels of P in both intact and castrated males [102] and that exogenous P administration does not further influence the actions of endogenous P.

The results of Phelps et al. [101] and Schneider et al. [124] appear to be in contradiction. Phelps et al. [101] concluded that PR is able to *facilitate* male sexual behavior, while Schneider et al. [124] conclude that PR plays a substantial role in *inhibiting* components of male sexual behavior. One obvious explanation for the differing results is that the major effects of PR disruption in each study were found under differing hormonal conditions. Phelps et al. [101] reported decreased sexual behavior compared to controls primarily in *castrated* animals, while Schneider et al. [124] reported decreased mount latency in *intact* PRKO males. Certainly, ample evidence exists to suggest that P and androgens interact to influence behavior. Another obvious

difference between the studies lies in the control animals to which the PRKO males were compared. Phelps et al. compared PRKO males to WT males of the same mixed genetic background (C57Bl/6 X 129SvEv), whereas Schneider et al. [124] compared PRKO males to males of the two inbred background strains. WT mice of these three strains (i.e., C57, 129 and C57/129) may differ in their baseline levels of sexual behavior, as genetic strain has been shown to play a significant role in male sexual behavior and, in particular, hybrid strains can demonstrate increased sexual behavior compared to the parental strains e.g., [82–84,136,141]. However, as noted above, the effects of P and its receptor can be enigmatic. P demonstrates biphasic effects, differential effects depending on dose or timing, and dual effects depending on hormonal milieu. Certainly, the possibility exists, that P and its receptor may both facilitate *and* inhibit male sexual behavior, depending on hormonal, experiential, genetic, and other less obvious factors (Fig. 1). Once again, significantly more research is required in this area to parse the many faces of P action in males.

While PRKO mice certainly provide a powerful tool to assess the role of PR in brain and behavior, this model possesses the limitation, as do all knock-out mice, that the protein is not expressed from the moment of conception on. Therefore, it is difficult, when examining adult animals, to determine whether observed effects are attributable to the role of PR in adult brain function, or whether the absence of functional PR during some critical developmental period is the cause for differences in adult behavior. In addition, PR may play a role both during development *and* exert an activational effect on behavior during adulthood. The effects on sexual behavior observed in both the Phelps et al. [101] and Schneider et al. [124] reports could be due, at least in part, to actions of PR during development. The absence of an effect of P administration in adult mice observed in both studies, could be considered consistent with this hypothesis. Therefore, an examination of the role of PR during neural development is essential to a full understanding of the potential role for P and its receptor in male-typical behaviors.

3. P and PR in sexual differentiation

3.1. Role of P in the sexual differentiation of behavior in rodents

The idea in the late 1960s and 1970s that P could have anti-androgenic activity on sexual behavior in adult males gave way to the hypothesis that P may also be capable of attenuating the masculinizing effects of testosterone during the perinatal period to alter subsequent adult behaviors. In support of this notion, two papers published in the mid 1960s suggested that injections of P were capable of reducing the incidence of sterility induced by a single injection of testosterone propionate in five day old female rats [3,65], suggesting that P could have anti-androgenic effects during sexual differentiation, as well as in adulthood.

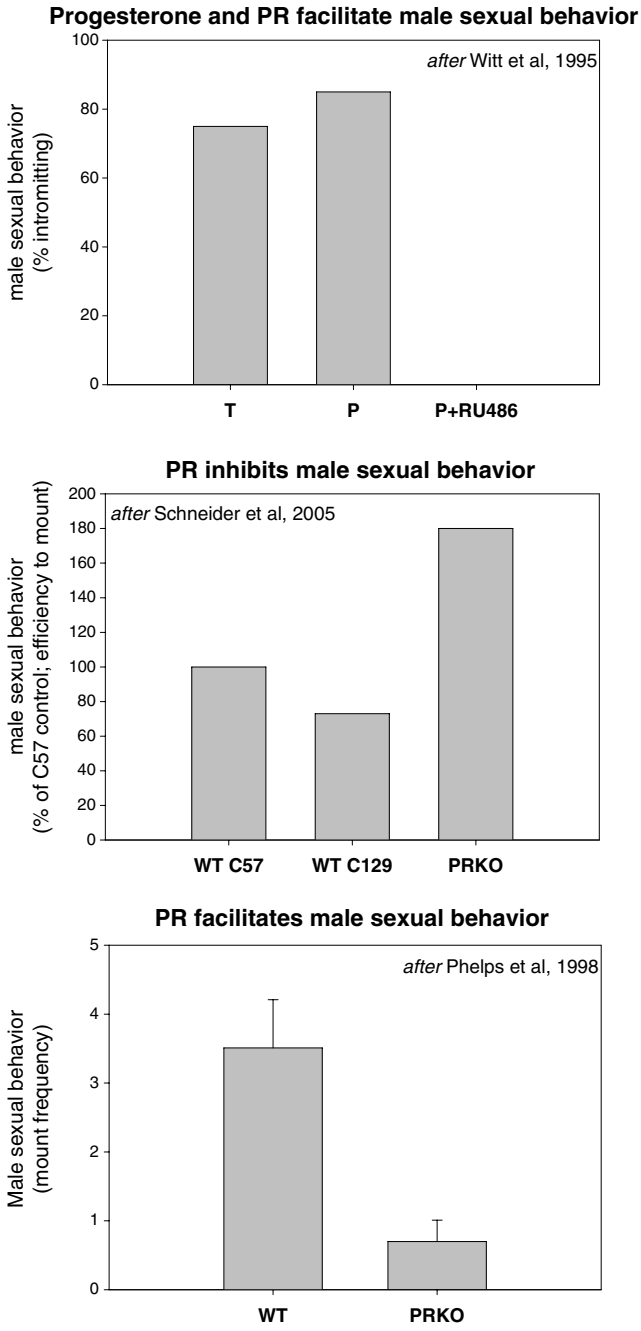

Fig. 1. Progesterone (P) and its receptor can have both facilitatory and inhibitory effects on male sexual behavior depending on physiological context (e.g., dose, timing, hormonal milieu, behavioral experience, species etc.). Top panel: P was capable of fully restoring male sexual behavior in castrated male rats in the absence of testosterone. The effect of P was completely abolished by the progesterone receptor (PR) antagonist, RU486. These results suggest that P is facilitatory and can mimic the effects of testosterone in reinstating male sexual behavior (after Ref. [156]). Middle panel: Naïve, intact, male mice lacking functional PR (PR "knock-out" (PRKO) mice) mounted more efficiently (i.e., reduced mount latency) compared to wild type mice of the two background strains (C57Bl/6J and C129SvEv), suggesting that PR normally plays an inhibitory role in male sexual behavior (after Ref. [124]). Bottom panel: Sexual behavior (i.e., mount frequency) was significantly reduced in PRKO male mice compared to wild-type mice of the same C57/C129 mixed strain three weeks following castration. These results suggest that PR normally facilitates sexual behavior in the absence of gonadal testosterone (after Ref. [101]).

Several studies directly tested this idea with regard to the sexual differentiation of male sexual behavior. One of the earliest studies, published in 1973, reported that an injection of 5 mg of P directly to male neonatal rats on the third day of life, reduced both ejaculatory behavior and intromissions in intact males in adulthood [35]. These results are consistent with the idea that high doses of P during development can exert "demasculinizing" effects on subsequent adult behavior. It is noteworthy, however, that lower doses of P (1.25 mg) *increased* mounting behavior in adulthood. While still a relatively high dose of P for a neonate, this finding suggests that, like in adulthood, different doses of P may exert differential effects on neural development and behavior. In keeping with these early findings, injections of 3.3 mg/kg of P to lactating mothers from postnatal day 2 through 14, significantly impaired sexual behavior in the male offspring as adults, in response to replacement with either testosterone propionate or estradiol benzoate following castration [58,59]. McEwen et al. [81] administered the synthetic progestin, R5020 directly to neonatal males from postnatal day 2 through 8. In adulthood, males were castrated and treated with estradiol benzoate and P in a regimen that induces lordosis in female rats. Males that had been neonatally exposed to R5020 displayed significantly increased levels of lordosis compared to control males. In each of these cases, the findings are consistent with the notion that P has "anti-androgenic" actions during periods of sexual differentiation of the brain. It reduced the incidence of male-typical behaviors and increased the incidence of female-typical behaviors.

Lest we think for a moment that P exerts predictable effects on brain and behavior, further studies examining the effects of the PR antagonist, RU486, during sexual differentiation provide results in direct juxtaposition to those studies on P exposure. Treatment of male neonatal rats with RU486 for about the first two weeks of life, dramatically decreased subsequent adult sexual behavior in males that were castrated as adults and given testosterone replacement [74,146]. In addition, RU486 treatment for the first two weeks, or as little as the first three days, of life, increased the incidence of lordosis in adult castrated males given estradiol benzoate and P [146,153], referred to by the authors as the "antidefeminization" of behavior by RU486 [153].

In a nutshell, exposure to either P or RU486 during neonatal development similarly reduced male sexual behavior and/or increased female sexual behavior in adult male rats. It may be that there is an optimal level of steroid hormone receptor activity that is necessary during neonatal life for normal behavioral development, such that too much or too little receptor activity is detrimental for later sexual behavior in males. This idea is supported by a comparison of the results of Lonstein et al. [74] and those of Hull et al. [59] and Hull [58] in which blocking PR activity or activating PR activity for prolonged periods of development similarly disrupted male sexual behavior. These findings are reminiscent of the counterintuitive ability of neonatal testosterone administration to impair later sexual behavior in males rats [35] and ferrets [7], which is similar to the effects observed after perinatal treatment with either anti-androgens or aromatase inhibitors e.g., [35,137].

The similar effects of neonatal treatment with RU486 or P on sexual behavior in male rats may also be related to the timing of manipulations in PR activity, particularly with respect to the perinatal rat's natural pattern of exposure to P. In the experiments of Hull and colleagues, postnatal elevations in P exposure extended from postnatal days 2–14 [58] or from birth through 28 days of age [59]. Although maternal and fetal P levels are high during middle to late gestation [53,152,154] maternal levels drop sharply before birth and remain very low for the first three days postpartum [53]. Exposure to pharmacological levels of P during early neonatal life, a time when pups normally ingest milk with very low levels of this hormone, may have contributed to changes in adult behavior. Giving high doses of P may mask the natural fluctuations in timing of P exposure that are critical for normal sexual differentiation. In contrast, blocking the actions of endogenous P with RU486 only blocks P action during times when it would normally have its effects. It is well known that the timing of P exposure can be critical in either facilitating or inhibiting a variety of measures, including maternal behavior [e.g., 16,98,127,129], female sexual behavior [e.g., 14,56,97,103,151,160 and for review see 13], as well as spine density in the hippocampus [157], tyrosine hydroxylase activity in the forebrain [57], the concentration of cAMP in the hypothalamus [159], and the estrogen-induced secretion of luteinizing hormone [5]. Since endogenous levels of P may be quite dynamic during gestation and neonatal life, it is not difficult to imagine that P may exert similar biphasic effects during neural development.

In an apparent example of just such a hypothesis, P treatment has been shown to have differential effects on brain monoamine oxidase (MAO) activity depending on the precise timing of the treatment during the perinatal period. In these studies, pregnant rats were injected with 3.3. mg/kg P beginning on gestation day 7 [132]. The activity levels of both MAO_A and MAO_B were measured in homogenates of fetal brain on gestational days 14, 17, 20 and 22. P treatment significantly increased MAO_A activity on E20 compared to controls, whereas P treatment significantly reduced MAO_A activity just 48 h later, on E22, by preventing an apparent developmental increase of MAO_A activity observed in controls. MAO_B was significantly increased by P treatment on E17 and E20, but did not differ between treated animals and controls on E22. While the functional consequences of P regulation of MAO activity are still not understood, these results demonstrate once again, the complex, dynamic and biphasic properties of P action. In a similar study [44], prenatal P treatment did not significantly alter MAO_A or MAO_B activity on the day of birth, when P treatment was terminated five days before parturition. Postnatal P exposure, beginning on the day of birth, significantly increased both MAO_A and MAO_B activity by postnatal day 7. Interestingly, both prenatal and postnatal P

treatment combined resulted in higher levels of MAO activity than postnatal P alone, even though prenatal treatment alone had no significant effect on MAO. The authors suggest that prenatal P exposure may in some way "prime" the brain enhancing the effects of later postnatal P exposure [44].

3.2. PR in developing brain

Yet another dynamic in the role of P in the sexual differentiation of the brain, lies in the sensitivity of the fetal and neonatal brain to P. In other words, expression of PR provides clues as to which brain regions are sensitive to P during specific windows of development. Additionally, differential sensitivity of males and females to P during critical periods of sexual differentiation could provide a rather functional mechanism for P to influence the sexes differently, as plasma P levels do not differ between males and females, either during fetal or early neonatal life [108,154]. PR *mRNA* can be measured in fetal brain at least two days before birth (the earliest date examined) [62] while *in vitro* PR binding assays were unable to detect progestin receptor *binding* in female fetal hypothalamus either on day 19 or day 21 of gestation [63]. Both P binding and PR mRNA have been detected in the whole hypothalamus of neonatal rats [62,63] and the expression of PR is developmentally regulated. In females, during postnatal life, progestin binding and PR mRNA levels in preoptic area/hypothalamus increased during the first two weeks of life [62,63]. However, the lack of anatomical resolution inherent to the techniques used in these studies does not permit a more detailed description of which hypothalamic nuclei express PR.

Utilizing the cellular level resolution of immunocytochemistry to detect nuclear PR protein in specific regions of the developing brain [148,149], our laboratory has reported that numerous regions of both the fetal and neonatal rat forebrain express high levels of PR immunoreactivity (PRir) including, but not limited to, the AVPv, the bed nucleus of the stria terminalis (BST), the MPN, the central amygdala, the lateral hypothalamus, the ventromedial nucleus (VMN), and the neocortex [150]. Preliminary studies from our laboratory using in situ hybridization demonstrate the presence of PR mRNA in these regions as well (unpublished observations). In addition, we have recently extended these findings to the midbrain. We observe relatively high levels of PRir in regions such as the substantia nigra, ventral tegmental area, the retrorubral field, as well as the rhombic lip [109].

3.3. Sex differences in PR expression: hormonal regulation

As mentioned, the levels of circulating P do not differ between perinatal males and females [108,154]. Yet, another way in which P may influence sexual differentiation of the brain and behavior is if differential sensitivity to P exists between the male and female brain. Indeed, as early as 1984 it had been demonstrated that progestin receptors (as measured by in vitro binding assays) were present in the male hypothalamus/preoptic area immediately after birth [64] and sex differences in progestin binding were observed in the neonatal brain as early as 1988 [155]. In homogenized hypothalamic/preoptic area tissue males had slightly higher concentrations of cytosolic progestin receptors as measured by binding of [^{3}H]-R5020 to the cytsolic fraction. Additional studies have demonstrated that it is specific regions of the brain that display sex differences in PR expression, particularly regions involved in male sexual behavior and gonadotropin secretion. A dramatic sex difference exists in the expression of PRir in the medial preoptic nucleus (MPN) of rats and mice during development (Fig. 2) [148,149]. From E19 to ~PD10, the male MPN expresses high levels of PRir [104,105,148] and mRNA (unpublished observation), whereas the female MPN expresses very little PR [104,105,148]. Before E19, neither males nor females express detectable levels of PRir in the MPN [148]. Sometime around P10, females begin to express PR, thereby dramatically reducing, but not eliminating, the sex difference [105]. These finding suggest that there may be a developmental window (E19 through ~P10) during which the MPN of males is more responsive to equivalent levels of circulating P than the female MPN. This is different from testosterone's action during perinatal development, in which surges of steroid secretion from the fetal and neonatal testes produce short-lived, but dramatic sex differences in circulating testosterone levels and, if anything, the male MPN may be equally, or even less sensitive to testosterone's metabolite estradiol, with regard to steroid receptor expression [38].

In fact, the prenatal surge in testosterone that occurs around E18/19 [154] is responsible for the induction of PR expression in the male MPN. Prenatal treatment of females with testosterone (from E17 or E19 through the day of birth) significantly increased PR expression in the MPN on E22, compared to control females [105]. The aromatization of testosterone to estradiol is responsible for PR expression in males, because PR expression is increased in the MPN of females treated prenatally with the synthetic estrogen, DES, but not in those treated with the androgenic metabolite of testosterone, dihydrotestosterone [105]. Additionally, prenatal treatment with the aromatase enzyme inhibitor, ATD, but not the antiandrogen, flutamide, reduced PR expression in males by E22 [105].

The sex difference in PR expression in the MPN, which also exists in mice, is abolished in transgenic mice lacking a functional estrogen receptor α gene [149]. Castration of male rats on the day of birth, significantly reduced the levels of PRir in the MPN by P4 [104], suggesting that estradiol induction of PR exhibits plasticity and indicates that PR expression is not permanently altered by prenatal steroid hormone exposure. In this regard, estradiol appears to be exerting an "activational" and not an "organizational" effect during development. Induction of PR expression in the MPN of females after the first postnatal week is

prevented by removal of the ovaries on postnatal day 4, prior to the onset of steroidogenesis [104].

Similar to the MPN, neurons of the AVPv also express PR (Fig. 2) and there is a dramatic sex difference in the expression of PR mRNA and protein in both rats and mice, with males expressing much higher levels of PR than females [105, unpublished observations]. In rats, this sex difference begins on about embryonic day 19, before which time neither sex expresses detectable levels of PR in AVPv, and is dramatically reduced or abolished by P8-10 when females begin to express PR. The expression of PR in the AVPv is regulated by estradiol both pre- and postnatally virtually identical to the regulation of PR in the perinatal MPN, as described above [104,105, unpublished observations]. In mice, PR expression in the AVPv is abolished in males lacking a functional ERα gene [149].

However, even what seems like a clear and straightforward sex difference in PR expression during development, when examined further, illustrates once again, the complexity of elucidating the role of PR in the brain. In mice, PR expression in the ventrolateral subdivision of the ventromedial nucleus (VMN-vl) shows a sex difference similar to those described above; in effect, males express high levels of PR in the VMN during development, whereas females express very little PR in this region (Fig. 2) [149]. In stark contrast, the sex difference in PR expression is absent, or possibly even reversed, in the VMN of the rat (Fig. 3) [107]. These findings illustrate several important points. One, PR expression in the developing brain is complex, demonstrating region-specific, sex-specific and species-specific regulation. Two, an important point that cannot be forgotten, particularly in light of the explosive literature on transgenic mice, is that mice are not little rats. Similarities between even these two seemingly close species cannot be assumed.

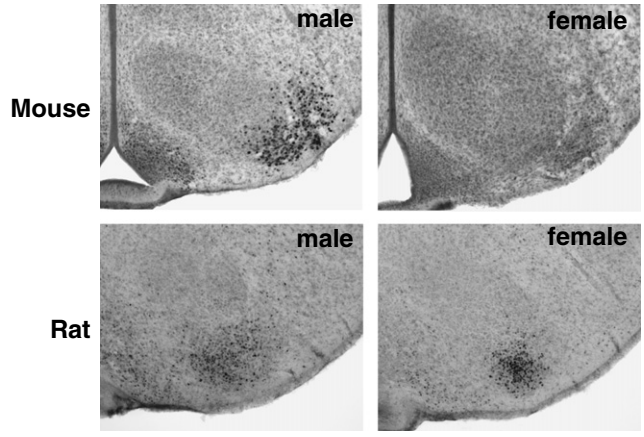

Fig. 3. Sex differences in progesterone receptor (PR) expression in the ventromedial nucleus (VMN) of the hypothalamus are species-dependent. PR immunoreactivity in the VMN of male and female mice (top panel) and rats (bottom panel) at one week of age. In mice, males express higher levels of PR in the VMN compared to females. In contrast, the sex difference is absent, or even reversed, in rats of the same age.

3.4. Role of PR in the sexual differentiation of the brain

The function of the sex difference in PR expression during development is not clear and in fact, the function of virtually all major sex differences in the rodent CNS remains elusive. However, the studies described above suggest that either P administration or inhibiting PR function with RU486 can

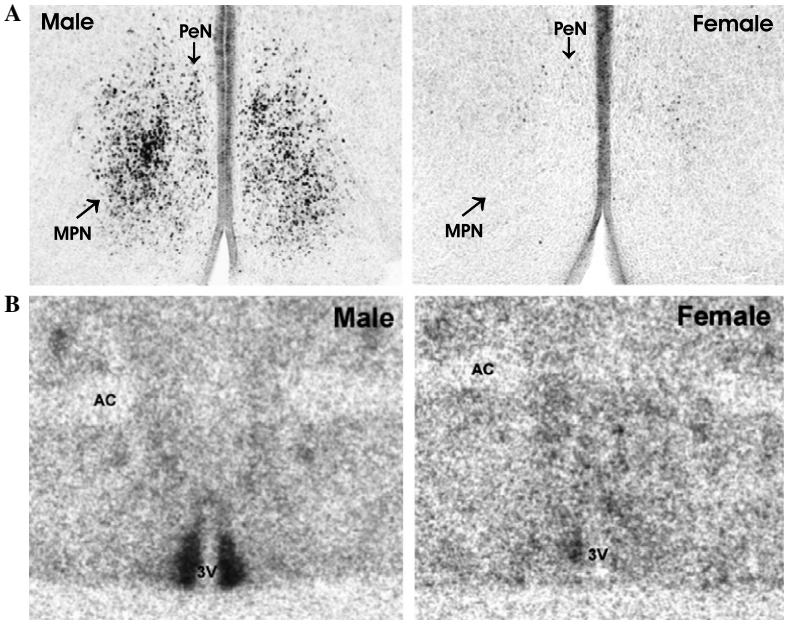

Fig. 2. Dramatic sex differences in progesterone receptor (PR) expression exist within the preoptic area of neonatal rats, in which males express significantly higher levels of PR than females. (A) PR immunoreactivity in the medial preoptic nucleus (MPN) and the periventricular nucleus (PeN) of male and female rats on the day of birth (from Ref. [148] with permission. Copyright 1998, The Endocrine Society). (B) PR mRNA, detected by in situ hybridization, in the anteroventral periventricular nucleus (AVPv) of male and female rats on the day of birth. 3V, third ventricle; AC, anterior commissure (Wagner, unpublished observations).

disrupt male sexual behavior [35,58,59,74,146], a behavior that is dependent on the MPN. The MPN is sexually dimorphic in several characteristics, most notably in its morphology. Originally coined the sexually dimorphic nucleus of the preoptic area (SDN-POA) [49], the volume of the central component of the MPN (MPNc) is several times larger than in the adult female [48]. The sex difference in MPNc volume is attributable to the differential exposure of males and females to the gonadal steroid hormone testosterone and its subsequent aromatization to estradiol during specific periods of development. Estradiol exerts its effects by decreasing the incidence of apoptosis or programmed cell death [21,32]. However, the cellular events that are triggered by estradiol to alter the molecular cascade of programmed cell death are not well understood. Because estradiol induces PR expression in the MPN during development and the masculinization of the MPNc is dependent on estradiol, the possibility exists that this hormone alters MPN development, at least in part, through the induction of PR. In fact, PR is expressed within the MPNc of neonatal males [106].

Quadros et al. [106] investigated the role of PR activity in the sexual differentiation of MPNc volume. Needless to say, the role of PR in this process is not a predictable, straightforward one, as the effects of RU486 were dependent on sex, regardless of postnatal testosterone exposure. Females were treated with testosterone propionate from postnatal days 1–8, which has been shown to masculinize the volume of the MPNc [32,37,114,115] and to induce PR in the MPN of females [105, unpublished observations] or were treated with the oil vehicle. In addition, females received either RU486 or the vehicle concurrent with the testosterone treatment. RU486 treatment attenuated the masculinizing effects of testosterone on MPNc volume in females, suggesting that indeed, testosterone and its metabolite, estradiol, act to masculinize the MPNc, at least in part, through the induction of PR (Fig. 4) [106]). From these findings it would be reasonably predicted that RU486 would similarly attenuate the masculinizing effects of endogenous testosterone in neonatal males. In stark contrast, however, the same RU486 treatment in males castrated on the day of birth and given the identical dose of testosterone propionate as given to the females, significantly *increased* the volume of the MPNc over and above untreated males [106]. In effect, RU486 "hypermasculinized" MPNc volume in males (Fig. 4).

The most parsimonious explanation for the differential effects of RU486 in intact males and testosterone treated females is sex differences in the exposure to testicular hormones prenatally. The MPNc is already sexually dimorphic on the day of birth [60] although the difference is relatively small compared to the large difference observed in adulthood. A single injection of TP before birth is sufficient to increase MPNc volume in females compared to control females [115], suggesting that although the MPNc undergoes significant differentiation postnatally, prenatal hormone exposure alone can significantly alter the development of MPNc. Additionally, the sex difference in

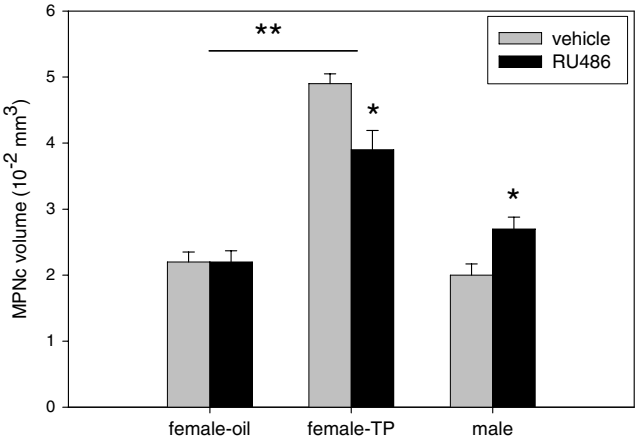

Fig. 4. Progesterone receptor (PR) mediates the actions of testosterone in the sexual differentiation of the central component of the medial preoptic nucleus (MPNc; SDN-POA). Neonatal treatment with testosterone propionate (TP) significantly increased the volume of the MPNc in females, as expected. However, concurrent administration of the PR antagonist RU486 attenuated the masculinizing effects of TP, suggesting that testosterone's metabolite, estradiol, may alter the development of the MPNc, at least in part, through the induction of PR. Counter-intuitively, neonatal administration of RU486 to intact males, did not reduce the effects of gonadal steroid hormones, but augmented the effects of testosterone on MPNc volume, resulting in a "hyper-masculinized" MPNc. Taken together, these results suggest that progesterone and PR may have differential effects on sexual differentiation dependent on previous hormone exposure prenatally (from Quadros et al. [106]). *significantly different from vehicle group ($p < 0.05$); **significantly different from oil treated females ($p < 0.01$).

PR expression is present as early as E19. The males in this study presumably had functional PR in the MPN a full four days in utero prior to the onset of RU486 treatment. Therefore, the MPN may be sufficiently differentiated by the day of birth such that the response to RU486 differs in males and females even in the presence of comparable postnatal hormone treatment. These results suggest that neonatal females treated with testosterone and neonatal males may not be identical models of sexual differentiation. These findings also point to the potential biphasic effects that P may exert in developing brain. P levels in mothers are extremely high at the end of pregnancy and fetal levels are correlated with maternal levels [108]. In contrast, P levels neonatally, are relatively low (unpublished observations). The prenatal actions of endogenous P may "pave the way" for differential effects of P during neonatal development.

3.5. Role of P exposure in human sexual development

During the 1960s and 1970s, progestins were prescribed to pregnant women, sometimes in combination with estrogens, during the first trimester for the prevention of recurrent miscarriages or to relieve the early symptoms of pregnancy, including pre-eclampsia. The practice of treating women early in pregnancy with progestins was abolished when it became evident that some of the girls born to these women had ambiguous genitalia requiring corrective surgery [94]. This was, in hindsight, attributed to the fact

that some of the progestins used in the pregnant women were androgen derived and/or had relatively strong androgenic properties due to an affinity for androgen receptors. Numerous studies were performed attempting to assess the behavioral outcome of progestin exposure in the offspring of treated women. However, due to numerous methodological limitations, (such as an often poor choice of controls, limited numbers of subjects or the need to pool subjects that were exposed to various types of progestins for various periods of time during gestation and who may or may not have also been exposed to exogenous estrogen), have made it difficult to draw general conclusions. Some reports [80] measured more "male-like" behaviors (by 1970s criteria; e.g., an increase in tomboyism, athletic interests and skills, preference for functional clothing rather than traditional feminine clothing and a priority for career over marriage) in exposed girls compared to controls. It was concluded from these findings that in addition to masculinizing the genitalia of the female offspring, the androgen-derived progestins to which they were exposed had a masculinizing effect on the brain as well. One could argue by today's standards, that rather than being "masculinized", it is possible these girls were simply more independent than their counterparts of the time. Indeed subsequent studies (discussed below) observed that girls exposed to progesterone prenatally scored as more self-assured, independent and confident on a personality assessment [111].

Nonetheless, additional studies exist in which the behavior of offspring exposed to non-androgen-derived progestins was examined, thereby effectively removing the confound of androgenic effects. Zussman et al. [as reported in 90] examined teenage boys and girls who had been exposed in utero to exogenous P, which had been administered to the mothers for the relief of the early symptoms of pre-eclamptic toxemia. P exposure in boys was negatively correlated with physical activity in childhood and with heterosexual activity in adolescence. In exposed girls, P exposure was negatively correlated with tomboyism and positively correlated with traditional feminine activities in childhood. From this report, the conclusion was made that prenatal P exposure has "anti-androgenic" effects on brain and behavior in humans. In other studies, in utero exposure to the progestin, medroxyprogesterone acetate (MPA; Depo-Provera) was examined in adolescent boys and girls [39,89]. Significantly fewer hormone-exposed girls were rated as tomboys during childhood and more of them showed a consistent preference for feminine clothing. This was reported, once again, as consistent with the idea that progestin had an anti-androgenic effect on brain development. However, no effects of MPA exposure were observed in boys, making a conclusion difficult. The conclusion that is clear from these types of studies is that our understanding of the effects of P on human brain development is far from clear. Today, P is commonly prescribed during early pregnancy for luteal phase dysfunction and in conjunction with ovulation stimulation drugs. In fact, a recent study demonstrated that mothers of male infants born with hypospadia, a deviation in the normal masculinization of the genitalia, were significantly more likely to have taken P between 4 and 14 weeks after conception [19]. It is not clear what effects this P treatment might be having on the masculinization of the brain. Furthermore, as both prenatal and postnatal P administration for the prevention and treatment of premature birth increases, this issue may need to be revisited sooner, rather than later (discussed further below).

3.6. Progesterone and sexual differentiation of the brain: a working model

Existing evidence from both brain and peripheral tissues, supports a working model in which there is a complex and dynamic interaction between fluctuating levels of testosterone and P, as well as cross-regulation between steroid receptors, both in terms of regulation of steroid receptor expression or in the regulation of transcriptional activity of receptors (Fig. 5). This working model, largely based on work done in rats can be described as follows: The testes release a surge of testosterone on E18/19 and again just shortly after birth [154]. Testosterone, once in the fetal brain, can be converted to estradiol by the enzyme aromatase or to dihydrotestosterone by 5α-reductase. Estradiol activates ERα, which drives the transcription of the PR gene directly by interaction with ERE's that exist within the promoter regions of the PR gene. This increase in PR expression occurs in the male MPN only, presumably incurring sensitivity of the region to P in one sex but not the other. P, possibly of maternal and/or adrenal origin, or even synthesized within the brain de novo, activates PR. As the levels of P may be very dynamic, particularly if they are of maternal origin [85,99,120], the timing of PR activation may be critical to normal neural development in males. In addition, the expression of ER may be down-regulated by P under some conditions [4,12,18] creating a situation in which P could indirectly down-regulate its own expression. Furthermore, PR may be capable of altering the transcriptional activity of ERα [67,68]. Moreover, PR_A may regulate the transcriptional activity of PR_B e.g., [68,147]. From these findings, one can begin to create a working model of P action during the development of the male brain. This model proposes a dynamic developmental process in which the players exert their effects in what seems like a well choreographed ballet that ensures proper masculine development of the brain. Surely this is a model that will require significantly more research before its complexity is fully elucidated.

4. P and PR in the development of non-reproductive function

In addition to evidence suggesting a role for P and its receptor in reproductive behaviors and sexual differentiation of the rodent brain, evidence suggests that P and its receptor may exert a greater influence on the development of non-reproductive functions than originally appreciated. Reports from the 1970s, which remain controversial,

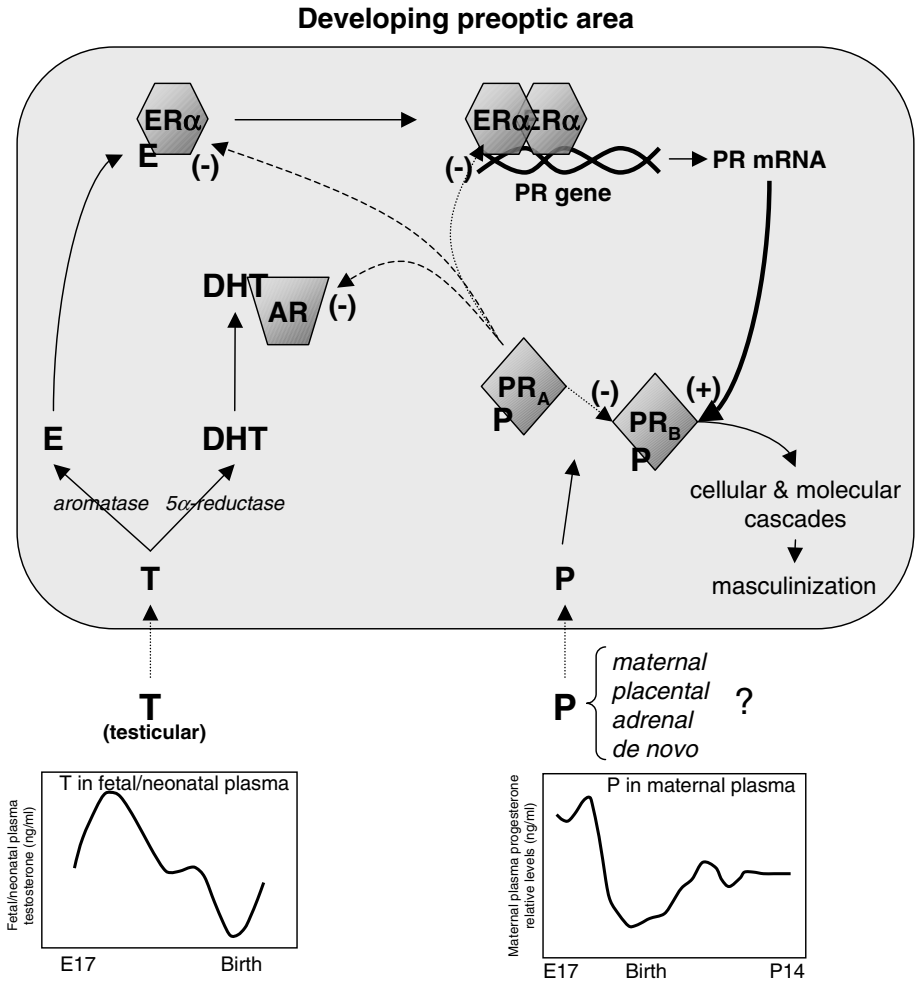

Fig. 5. A schematic representation of the perinatal preoptic area demonstrating the potential interactions between testosterone, its metabolites and progesterone that may occur during sexual differentiation of this area. In addition, induction or inhibition of steroid receptor expression may occur, as well as regulation of transcriptional activity across receptors or receptor subtypes. In this working model, testosterone (T) secretion from the fetal and neonatal testes peaks around E18/19 and again on P1 (from Weisz and Ward [154]). Once in the brain, T can be aromatized to estradiol (E) or reduced to DHT. DHT activates androgen receptors (AR), whereas E activates estrogen receptor alpha (ERα; and possibly ERβ (not shown)), which results in the increased transcription of the progesterone receptor (PR) gene. The result is an increase in PR expression in males, but not females, by E19/20. Progesterone (P) in maternal circulation reaches extremely high levels and undergoes dynamic changes during the end of pregnancy and lactation [85,108,130,154, unpublished observation]. Progesterone in the fetus is correlated with maternal P prenatally, suggesting a maternal source ([108]. P may also be derived from adrenal glands, the placenta (prenatally) or synthesized de novo within the brain. P, regardless of the source, activates PR_A and PR_B which may initiate a cascade of cellular and molecular events resulting in male-like brain development. Additionally, as P and T levels rise and fall over the course of development, PR may regulate ERα expression [4,12,18] and/or influence the transcriptional activity of ERα [67,68]. Furthermore, PR may regulate the expression of AR [124]. Depending on the relative expression of the PR isoforms, PR_A may inhibit the transcriptional activity of PR_B [68,147] thereby modifying the cascade. In this model, there exists a dynamic and interactive relationship between gonadally derived steroids, steroids of maternal/adrenal origin and the activity of their cognate receptors; a model in which each component can be empirically tested. This model, based primarily on research done in rats, proposes a well-choreographed hormonal "ballet" during development which results in the proper masculinization of brain regions critical for male sexual behavior and neuroendocrine regulation.

suggested that humans exposed to progestin prenatally exhibited an enhancement in cognitive ability. Work in rodents suggests that exposure to P during development can alter subsequent learning behavior and that PR is expressed in cortical cells during development.

4.1. Perinatal progesterone exposure affects learning in rodents

In 1980, Hull and colleagues, examined the effects of perinatal P exposure on subsequent maze performance in rats [59], partly in response to controversial findings in humans on P and cognition (discussed below). In their first experiment, pregnant females received 4 mg, 8 mg or 12 mg P pellets on day 6 of gestation. Pups remained with the P treated mothers following birth, presumably resulting in a prolonged period of perinatal P exposure in the offspring. In adulthood, males and females that had been exposed to P or control capsules were tested in the Lashley III maze. Males performed better in the maze than females, with males having faster latencies to complete the maze. The high dose of P significantly impaired performance of males

compared to controls and low dose P. However, P had no effect on the performance of females. In a follow-up experiment, [131], offspring were exposed to P perinatally via maternal injections of P during pregnancy (3.3 mg/kg from days 8–18 of gestation) and during lactation (3.3 mg/kg from postnatal days 2–21). Beginning on day 19 of life, offspring were tested in an active avoidance task. Progesterone treated pups exhibited faster latencies in performance for the entire task, in terms of both improved acquisition of the avoidance response, as well a greater resistance to extinction. No sex differences in performance were observed with or without progesterone treatment. Taken together, these papers suggest that while perinatal P exposure clearly facilitated active avoidance behavior, it also impaired maze performance. This suggests that P exerts a more complex effect on the developing brain than a simple facilitation of learning and also suggests that its effects are not merely an alteration of sexual dimorphisms in this behavior. In other words, P is not simply "masculinizing" or "demasculinizing" cognitive behaviors (i.e., having an "antiandrogenic" or "synandrogenic" effects as discussed above). Rather, P may exert significant effects on the development of brain regions mediating non-reproductive functions in a manner completely independent of gonadal hormones.

4.2. Expression of PR in developing cerebral cortex

Dating back to 1980, evidence existed that cells of cortex expressed PR in developing rats and mice. For example, using in vitro binding assays, MacLusky and McEwen [78] demonstrated that high affinity [^{3}H]-R5020 binding in cortex of neonatal female rats was detectable on postnatal days 1-3, increasing by P3-5 with peak levels on P8-10. Binding decreased between P15-17 and P23-25, suggesting that progestin binding is transiently elevated during the first two weeks of life. Using steroid hormone autoradiography in mice, it was reported that nuclear accumulation of [^{125}I]-R5020 was found in deep and intermediate layers of cortex at birth and then intermediate and more superficial layers by postnatal days 2 and 8, decreasing by postnatal day 12 [128]. Kato and colleagues used reverse transcriptase-PCR to measure levels of PR mRNA in cerebral cortex of females over the course of development. PR mRNA for both A and B isoforms combined was detectable by two days prior to birth, but increased by the day of birth with an even further increase by postnatal day 2. However, $PR_{A\&B}$ mRNA increased dramatically by postnatal day 4 reaching peak levels by postnatal day 8 and remaining relatively high through postnatal day 18 [62]. When PR mRNA for the B isoform only was measured, the pattern was very similar, reaching peak levels on postnatal day 8. However, there was a sharp decline in PR_B mRNA between days 8 and 12 with PR_B mRNA reaching relatively low levels by postnatal day 18. Work by this same group had previously demonstrated that binding of [^{3}H]-R5020, presumably a measure of PR protein, reached peak levels around postnatal day 8, but declined significantly at later ages, [63], suggesting that PR_A mRNA is untranslated and that functional PR is expressed transiently in cortex during development. Using subtractive logic, it appears that the majority of PR mRNA expression in the cortex during neonatal life consists of the PR_B isoform, whereas later in life, the PR_A isoform is predominant.

The techniques used previously to examine PR expression in cortex have lacked the anatomical resolution to determine precisely where in cortex PR is expressed. The neocortex is a laminated structure with the adult cortex divided into six lamina that are anatomically and functionally distinct [145]. Using immunocytochemistry to detect PR protein (A and B), which permits cellular level resolution, preliminary work from the Wagner lab has demonstrated PR immunoreactivity is first detected in cortex a few days before birth. It is expressed in cells of layer 5 beginning on about postnatal day 1 and then in layers 2/3 beginning on about postnatal day 7 [75]. This pattern of PRir disappears sometime between postnatal days 14 and 28.

Interestingly, the pattern of PR_B mRNA expression closely mimics the detection of PR protein, as measured by in vitro binding assays [63] as well as immunocytochemistry [75]. Fig. 6 schematically illustrates the patterns of PR_B mRNA, PR immunoreactivity and PR binding over the course of postnatal life in the rat. These three reports, spanning 20 years time and utilizing three different techniques, suggest that PR is transiently expressed in developing cortex, with peak levels occurring around the end of the first week of life and raise the very interesting idea that P may exert effects on fundamental aspects of cortical development. Perhaps surprisingly, cognitive behavior in PRKO mice has not yet been fully explored and a detailed description of the expression of PR in mouse developing neocortex has not been reported, thus highlighting a need for future studies.

The source of the ligand for PR in cortex is not known, but several possibilities exist. Although the perinatal ovary is quiescent until the second week of life [50,110,122], there appear to be at least two alternate sources of P: the maternal ovary and/or de novo synthesis within the developing brain itself. Maternal P levels are high, not only during gestation, but also during lactation [100,120] and P may pass to neonates through mother's milk [10,138]. In addition, the perinatal rodent brain expresses all the enzymes necessary for the *de novo* synthesis of progesterone from cholesterol [23,24,66,119,143,144,167], potentially producing locally high concentrations of P. Thus it is likely that the postnatal brain, and therefore, the developing neocortex, may be exposed to significant amounts of P.

4.3. Prenatal exposure to progestins and cognition in humans

The idea that exposure to P during perinatal life plays a role in cortical development and/or cognitive behavior is not unique to the rodent literature. As mentioned above,

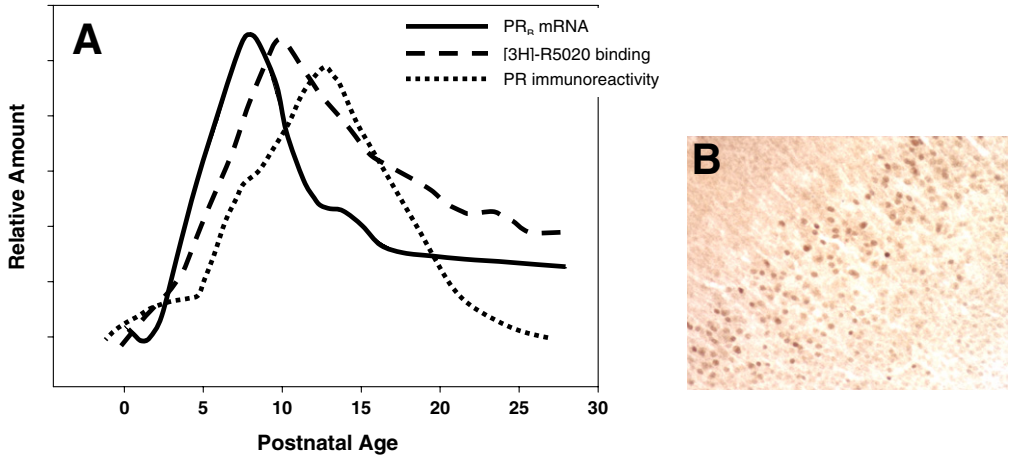

Fig. 6. (A) A schematic representation of the relative levels of the progesterone receptor B (PR_B) mRNA [62], [^{3}H]-R5020 binding ([63] and PR immunoreactivity ([75] within the neocortex during the first month of life in the rat. Despite three different technical approaches and the span of several decades, the results are remarkably consistent and suggest that there is a transient expression of PR within the cortex during critical periods of development. (B) PR immunoreactivity within cells of the cortex in a postnatal day 7 male rat [75].

during the 1960s and 1970s, progestins and P were prescribed, sometimes in combination with estrogens, to pregnant women during their first trimester for the prevention of recurrent miscarriages or to relieve the early symptoms of pregnancy. In addition to some of the psychosocial and psychosexual behavioral effects described above, it was initially reported that girls exposed to progestins prenatally had higher than average IQ scores [40]. The observation was also made that children whose mothers received prenatal P were more likely to be standing and walking on their first birthday compared to controls and that they had greater academic achievement at 9–10 years of age [30]. Subsequent studies demonstrated that numerical ability was the most notably facilitated by P exposure, with a more modest, but significant facilitation in mechanical ability and spatial ability [Zussman et al, 1975 as reported in 31]. Interestingly, it was reported that academic success showed a dose-dependent effect, with academic performance being highest in those whose mothers had received over 5 g of P. Further, P administered before the 16th week of gestation produced the most robust effects on educational success. These controversial findings generated a wave of publications attempting to replicate these reports. Several studies failed to replicate the findings of Dalton, leaving the original findings somewhat in question [39,91,112]. However, these studies proved to be only pseudo-replications, in that they were performed on offspring whose mothers had received synthetic progestins, rather than natural P as in the Dalton study. Furthermore, mothers in the replication studies had, in general, received lower doses of progestin than the mothers of the children in the Dalton study. Therefore, to this day, the question remains open.

Many of the follow-up studies to the original finding, utilized performance on an IQ test as the measure of cognitive ability and found no significant differences in IQ scores between progestin treated offspring and controls. The conclusion was drawn, that the original Dalton studies were flawed. However, a relatively well-controlled study published in *Nature* in 1977 [111] examined offspring exposed exclusively or mostly to progestins (very low estrogen; progestin group), offspring exposed to exclusively or mostly estrogens (very low progestin; estrogen group) and their untreated siblings. All hormone treatment was begun in the first trimester of pregnancy and lasted at least four weeks. In this study, as in others, exposure to progestins with or without exposure to estrogens, had no effect on IQ, as measured by the Wechsler Intelligence Scale. However, subjects' responses on the Cattell personality questionnaire revealed that offspring exposed to progestins were significantly more independent, sensitive, individualistic, self-assured and self-sufficient compared to estrogen exposed subjects or control siblings. Separate studies have demonstrated that these traits are positively correlated with school achievement. Therefore, it is possible that findings demonstrating higher educational attainment in progestin-exposed offspring may be attributable to progestin's effects on complex cognitive traits and not strictly on "IQ." Needless to say, the question of whether prenatal progestin exposure influences cognitive ability remains in desperate need of more rigorous assessment.

Despite this, it has become increasingly common in recent years, to administer progestins to women late in pregnancy to prevent premature birth. Typically, 17α-hydroxyprogesterone caproate is given to women with a history of preterm birth, via daily injections beginning between 16 and 20 weeks of gestation and administration is stopped at 36 weeks gestation or until delivery [87]. In a clinical trial conducted for the National Institute of Child Health and Human Development, it was reported that this treatment significantly reduced the chances of delivery before 35 weeks of gestation and significantly lowered the rates of intraventricular hemorrhage and the need for

supplemental oxygen in the infants, compared to a similar group given placebo injections. However, in several recent comprehensive papers discussing this practice [36,86,87,133] almost nothing is mentioned about the need for follow-up in the infants born to P treated mothers.

Furthermore, clinical trials in which premature infants are treated with P and estradiol have begun in recent years. The logic behind this relatively new practice is that plasma levels of estradiol and P increase in both the mother and fetus by a factor of 100 during pregnancy. Therefore, infants born prematurely are presumably denied exposure to the maternal hormones of pregnancy, which may aid fetal development. In studies conducted in Germany, premature infants born before 29 weeks gestation and having a birth weight less than 1000 g were administered intravenous 17β-estradiol and P continuously for the first six weeks of life at doses that mimic intrauterine levels of these hormones. Improved postnatal bone development and lower rates of chronic lung disease were noted in treated infants compared to controls [140]. Interestingly, when follow-up testing was done on treated premature infants at 15 months (corrected age), the Psychomotor Developmental Index was normal in treated infants, whereas it was below average in untreated premature infants [139]. This suggests that steroid treatment had a beneficial effect on the developing brain. Of course, from these studies, the effects of estradiol versus P cannot be separated. However, before this practice becomes routine, further investigation elucidating the role of neonatal exposure to these two steroids is certainly warranted.

Additionally, progestin-only containing contraceptives are routinely prescribed to lactating women as a "safer" form of birth control compared to estrogen containing contraceptives. One of the progestins commonly used in this type of contraceptive, levonorgestrel, (LNG) is found in mother's milk and can be detected in the serum of breastfed infants [10,138]. Additionally, thyroid stimulating hormone levels are elevated in breastfed infants whose mothers used LNG containing contraceptives compared to breastfed infants whose mothers used a non-hormonal means of birth control [6], suggesting that this progestin in maternal circulation may be capable of altering infant physiology. While exposure of fetuses and infants to P seems to be on the rise, our understanding of the neural and cognitive consequences of this practice remain, remarkably unexplored.

5. Conclusions

If it is possible to draw a single conclusion from the literature reviewed above, it is that P is much more than a "progestational" hormone and indeed, much more than simply a "female" hormone. P can facilitate, inhibit or mimic the actions of testosterone in male-typical behaviors in species ranging from lizards to humans. From rodent models, it appears that the actions of P and its receptor may be dependent on, not only the circulating levels of P, but on the milieu of circulating gonadal hormones, previous behavioral experience, the species examined and possibly, genetics. Dramatic sex differences (male $\gg$ female) in the expression of PR during fetal and neonatal development occur in brain regions critical for reproductive behavior and function. Taken together with findings that perinatal exposure to P or the PR antagonist, RU486, can alter the sexual differentiation of the brain and behavior, this strongly suggests that P may play a unique role in the development of the male brain. Yet, once again, P appears to have differential effects depending on species, hormonal milieu and perhaps most critically, the specific time point during gestation or neonatal development during which exposure occurs.

In addition to effects on reproductive parameters, P and its receptor may play an under-appreciated role in cortical development and cognition. Early work in humans hinted that exposure to P prenatally might alter cognitive characteristics, and converging evidence over several decades suggests that indeed, the cortex may be transiently sensitive to P during critical periods of development. This adds to the growing notion that steroid hormones not only exert effects in the development of reproductive capacities, but may play an integral role in fundamental developmental processes within brain regions not classically considered to have reproductive function.

Wading through the scant and sporadic literature on the role of P in the male, it can be easily recognized that P is not a 'one trick pony.' Rather, P appears capable of exerting very different effects depending on the physiological context. On the one hand, the literature appears to be wrought with contradictions, inconsistencies and paradoxes. On the other hand, these ostensible problems can be viewed as clues to the complexity of P's action and future research can use these clues to parse out the specific parameters that enable P and its receptor to exert its dynamic and multifaceted effects on the adult and developing male brain. These questions become more than academic in light of increased interest in progestins as contraceptive agents in adult men, despite very little consensus regarding the actions of progestins in the male brain. These questions become clinically urgent in light of the increasingly common administration of progestins to women in late pregnancy for the prevention of premature delivery and the emerging administration of P to premature infants to aid maturation, despite virtually no understanding of the actions of progestins on the developing brain.

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