Exploring the Effect of Prenatal Hormone Exposure on Sex-Specific Neuroanatomy, Sexual Maturation, and Maternal Behavior in a Rat Model

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Introduction

Sexual differentiation of the brain may follow a different pattern in transgender individuals than in cisgender individuals, and this may be due to different prenatal hormone environments (Zhou et al. 1995).

While gender is a human construct, we can model aspects of sex differences and the influence of hormones on them in rats.

Male pups, which have higher testosterone levels than female pups, receive more anogenital licking than female pups (Moore 1982), but it is unclear if exogenous testosterone exposure would potentiate or mimic this.

Dams exposed to testosterone while pregnant lick and groom their pups more, which has phenotypic effects on female pups (Borrow et al. 2013); whether the level of testosterone to which pups are exposed impacts this further is unknown.

Prenatal testosterone exposure influences the volume of sexually dimorphic brain areas, like the bed nucleus of the stria terminalis (BNST; Chung et al. 1999), but if this persists into adulthood or into a model exclusive to prenatal exposure remains to be explored.

In the present study, we will in two experiments assess if prenatal testosterone exposure influences phenotypes related to sexual maturity, dam-pup interactions, and BNST volume.

Methods and Materials

General Methods

Experimental Groups
8 pregnant dams injected subcutaneously on gestational day 17 with either:

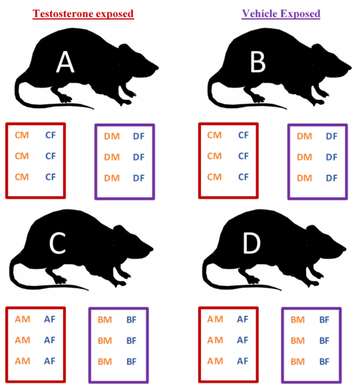
- Testosterone propionate (TP; 2 mg; n=4)
- Vehicle (VEH; 0.1 ml sesame oil; n=4)

Litter Distribution
Pups will be culled to 12 per litter, sexed, marked to indicate sex, and tail-docked to define experimental group

Each dam will receive 3 males and 3 female pups from each group (TP or VEH); none will be their own pups

When applicable, pups will be weaned at PND 21 and housed in same-sex groups of 4

Fig 1: ***Planned*** design for cross-fostering of both sexes of TP &VEH pups to TP&VEH dams.



Experiment 1

Goals: Replicate that TP-exposed dams engage in higher licking behavior than vehicle-exposed dams (Cameron et al. 2008) and assess if this is influenced by pup testosterone status.
Replicate that testosterone-exposed pups exhibit delayed sexual maturation (Borrow et al. 2013) and assess if this influenced by dam testosterone status.

Record interactions between dams and pups
Webcams mounted on shelf above each cage recording for 30-min periods at 4-hour intervals during light cycle on each of postnatal days (PND) 2-8

Dependent Measures & Planned Statistical Analysis
Behaviors scored from videos include maternal-pup contact, licking, and anogenital licking (Moore 1982; frequency & duration in 1st min of each 5-min bin); differences will be assessed across pup TP status, dam TP status, pup sex, and time using 4-way repeated measures ANOVA; planned comparisons will focus on pup sex & dam TP status

Anogenital index (AGI; distance measured from anus to genitals divided by body weight; Borrow et al. 2013) on day of birth (assessed by pup TP status and pup sex using 2-way ANOVA) and on PND 22 (3-way repeated measures ANOVA will be used to include analysis of impact of dam TP status)

Duration until vaginal opening (VO) in females (assessed in female pups across pup & dam TP status using 2-way ANOVA)

Experiment 2

Goal: Replicate that testosterone exposure increases BNST volume in female pups at PND 12 (Chung et al. 1999) and assess if this effect persists into sexual maturity (PND26) and adulthood (PND47)

Perfusions & Histology
Transcardial perfusions will be performed on the pups raised by vehicle-exposed dams to fix brain tissue; brains will be removed & sliced, and the slices stained with cresyl violet

- A male and female from each condition (TP or vehicle) will be perfused on PND 12, 26, 47 (n=4 per litter and n=8 per PND in each phase)

Dependent Measures & Planned Statistical Analysis
The BNST will be identified, and its volume measured stereologically; differences will be assessed at each time point between pup TP status & sex using 2-way ANOVA

Expected Results

Experiment 1

Licking and Anogenital Licking (A)

TM TD > TM VD = VM TD = TF TD > TF VD = VF TD = VM VD > VF VD

Contact (B)

TM TD = TF TD = VM TD = VF TD = TM VD = TF VD = VM VD = VF VD

Fig 2: ***Hypothesized*** differences in the amount of (a) overall and anogenital licking and (b) contact time during postnatal day (PND)2-8 received by male (M) and female (F) pups exposed prenatally to testosterone propionate (T) or vehicle (V) and cross-fostered to testosterone- or vehicle-exposed dams (D). We expect to find that 1) testosterone-exposed dams will execute more licking than vehicle-exposed dams (as per Cameron et al. 2008) but this will not impact overall contact (as per Champagne et al. 2003) and 2) licking (especially anogenital licking) will increase as a function of testosterone (endogenous or exogenous; as per Moore 1982).

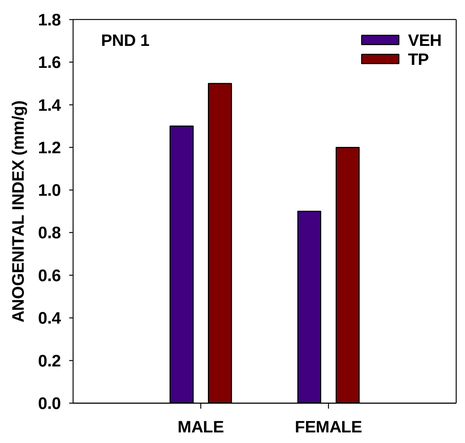


Fig 3: ***Hypothesized*** anogenital index (AGI; distance measured from anus to genitals divided by body weight) at postnatal day (PND) 1 in male and female pups exposed prenatally to testosterone propionate (TP) or vehicle (VEH) (cross-fostered to either TP- or VEH-exposed dams just after measures). We expect to find that larger AGI based on how much testosterone (endogenous or exogenous) that the pup is exposed to (e.g., TP-males > VEH males = TP females > VEH females). Differences at PND22 (not shown) are expected to be further compounded by dam behavior based on dam TP-exposure status. Y-axis scales and hypothesized means based on Borrow et al. (2013).

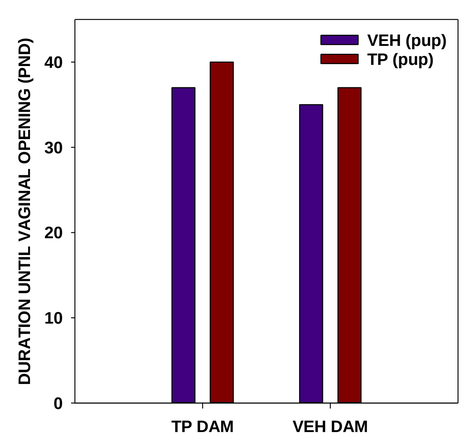


Fig 4: ***Hypothesized*** duration until vaginal opening in female pups exposed prenatally to testosterone propionate (TP) or vehicle (VEH) and cross-fostered to either TP- or VEH-exposed dams. We expect to find a longer duration until vaginal opening in TP females, and that this will be exacerbated by increased licking behavior executed by TP-exposed dams (e.g., time until vaginal opening of TP female pups raised by TP dams > TP females raised by VEH dams = VEH females raised by TP dams > VEH females raised by VEH dams). Y-axis scales and hypothesized means based on Borrow et al. (2013).

Experiment 2

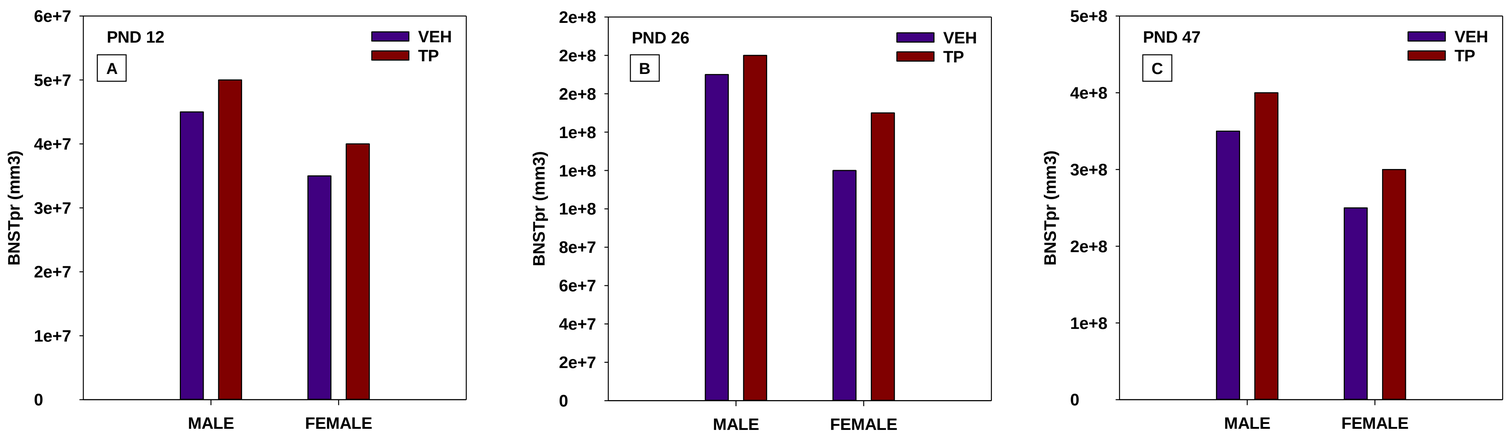


Fig 5: ***Hypothesized*** mean volume of the principle nucleus of the bed nucleus of the stria terminalis (BNSTpr) in male and female pups exposed prenatally to testosterone propionate (TP) or vehicle (VEH) (all cross-fostered to VEH-exposed dams) at (a) postnatal day (PND)12 (to replicate Chung et al. 1999), (b) PND26 (to represent early adolescence), and (c) PND47 (to represent sexual maturity). We expect to find that the BNSTpr of 1) VEH males will be larger than that of VEH females; 2) TP males will be larger than that of VEH males, and 3) TP females will be larger than that of VEH females, and that these findings will persist from PND12 into adulthood. Y-axis scales (*note different scales)* and hypothesized means based on (a) Chung et al. (1999), (b) Weathington et al. (2013), and (c) del Abril et al. (1987)

Discussion

If TP-exposed dams lick their cross-fostered pups more than VEH dams, and if this leads to a longer duration until vaginal opening in female pups, we would replicate Borrow et al. (2013) and Moore (1982).

- Failure to replicate would suggest that a single TP dose provides insufficient influence

If these measures are compounded by pup testosterone status, it would suggest that maternal behaviors and resulting pup phenotypes are interactively influenced.

- No interaction would suggest a drive by internal rather than external factors

If prenatal TP increases anogenital index at birth and BNST volume at PND12 and beyond, we would replicate Borrow et al. (2013) and Chung et al. (1999), respectively.

- Failure to replicate would suggest that the single dose of TP is insufficient to influence these measures or that the effect on the BNST does not persist past PND12

Prenatally TP-exposed female rats possessing 1) larger anogenital distance, 2) delayed vaginal opening, and 3) intermediate BNST volume could serve as a model of aspects of transgender brains and offer possible insights into this occurrence.

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