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SHORT COMMUNICATION

Circulating plasma testosterone during early neonatal life in the socially monogamous and biparental prairie vole (*Microtus ochrogaster*)

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Summary Adult male prairie voles (*Microtus ochrogaster*) are highly gregarious and socially monogamous, which is dissimilar to most other male rodents but reminiscent of many primates, including humans. This has resulted in prairie voles becoming a premier model in which to study the neural and hormonal basis of complex social behaviors, as well as the atypical development of these behaviors. This research is impeded by the complete lack of knowledge about the gonadal steroid environment during early development in this species. In many other animals, testicular hormones released during perinatal life permanently organize the neural substrates underlying later social behaviors in males, so knowledge about the presence or possibly even absence of testosterone in male prairie vole pups would provide important insight into their neurobehavioral development. In this study, male and female prairie vole pups were sacrificed 1, 2, 3, 4 or 5 days after birth and radioimmunoassay used to determine plasma levels of testosterone. We found that testosterone was detectable in both sexes on all days and that levels were significantly higher in males than females. Testosterone in neonatal males was sometimes as high as breeding males sampled one day after the birth of a litter. This study is the first to examine circulating testosterone in neonatal prairie voles, or any other species of *Microtus*, and the results indicate similarity to other rodents. This is surprising because some previous studies manipulating gonadal hormones in neonatal prairie voles have found limited effects on their neurobehavioral development, suggesting the existence of some unique, yet-to-be-revealed aspects of their neuroendocrine profile during early life.

The scientific literature studying the neuroendocrinological basis of social behaviors in all mammals, including humans, demonstrates the critical importance of perinatal exposure to steroid hormones, or lack of exposure, for sex-appropriate behavioral displays during adulthood (Arnold, 2002; De Vries and Simerly, 2002). This literature has provided a wealth of information useful for understanding some aspects of human brain and behavior development. However, the far majority of experimental studies have been conducted on laboratory rodents that are not ideal models for understanding human sociality because most rodents do not parallel the gregariousness, long-term social bonding, and biparental

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care typical of human societies. Because of this, the socially monogamous prairie vole (*Microtus ochrogaster*) has become the preeminent model in which to study the neuroendocrine basis of complex social behaviors (Young et al., 2011). Their usefulness from either a clinical or non-clinical perspective, though, is impeded by the fact that nothing is known about circulating gonadal hormones during early life in this species.

The gonadal steroid profile of developing prairie voles might be similar to many other developing mammals, with male prairie voles having higher circulating testosterone than females, but previous research indicates that this might not be the case. Although neonatal castration demasculinizes male prairie voles' extra-hypothalamic arginine—vasopressin (AVP) (Lonstein et al., 2005) and parental behavior (Lonstein et al., 2002), treating neonatally castrated males with exogenous testosterone does not maintain masculinity of these traits. Furthermore, prolonged perinatal testosterone treatment of females does not masculinize their adult AVP expression or parental behavior (Lonstein and De Vries, 2000; Lonstein et al., 2002, 2005). Similarly, Roberts et al. (1997) reported the unusual findings that neonatal castration does not demasculinize later sexual behavior in male prairie voles and that neonatal testosterone treatment does not masculinize sexual behavior in either sex of prairie vole, but instead demasculinizes it. Given these results, testicular secretion of high levels of testosterone may not occur during neonatal life in male prairie voles and thus cannot contribute to the masculinization of their brain and behavior. To evaluate this, we measured circulating plasma testosterone in male and female prairie voles during early neonatal life, which is part of the perinatal critical period for when gonadal and other steroid hormones affect neurobehavioral development in prairie voles and many other mammals, including primates (Mann and Fraser, 1996).

1. Methods

1.1. Subjects

Subjects were 50 young litters and 20 adult F5 and F6 generation prairie voles (*M. ochrogaster*) born and raised in our colony, as previously described (Northcutt and Lonstein, 2008). Litters were derived from primiparous mothers and their mates. Adults from which plasma samples were taken were also primiparous and previously parentally inexperienced. Starting 21 days after mating, cages were checked multiple times daily between 1000 and 1700 h for pups. The first observation of pups was designated as postnatal day 1. Cages were changed once a week, but not after birth of a litter. Procedures were in accordance with the standards for use of animals in research by the National Institutes of Health and Michigan State University.

1.2. Blood sampling

Between 13:00 and 16:00 h, home cages containing breeding pairs and their 1—5 day old litters were individually brought to a nearby sampling room. Pups were removed from the nest or gently removed from a teat if attached. Pups were quickly decapitated and trunk blood from each pup collected in separate heparinized 1.5 ml mini-centrifuge tubes that were immediately placed on ice. Blood sampling for an entire litter typically took <4 min. Tubes were centrifuged at 15,000 rpm for 15 min and the plasma removed and frozen at −80 °C until all samples in the study were collected. Prairie vole litters contain only 2—3 pups/sex (see below) and newborn prairie voles weigh ~2 g; because each pup could provide only ~25—30 μl of whole blood, plasma samples were later briefly thawed, pooled within sex for each litter, and then shipped on dry ice to the French laboratory at the University of Nebraska. Postmortem abdomens were examined for testes using a dissecting microscope to determine pup sex. Each postnatal day examined involved between 5 and 8 separate pools of plasma for each sex.

Blood was also obtained from 10 breeding pairs sacrificed within 36 h after the dam gave birth to her first litter. Adults were removed from their home cage, overdosed through an IP injection of ketamine and xylazine, and rapidly decapitated. Adult samples were treated identically as pup samples, with the exception that pooling was unnecessary.

1.3. Radioimmunoassay

Samples were extracted three times and evaporated, and testosterone then determined by a single radioimmunoassay using commercially available I^{125}-labeled double-antibody RIA reagents according to the manufacturer's recommended protocols (MP Biomedicals, Irvine, CA). The assay had a lower limit of sensitivity of 0.2 ng/ml. Adult samples were analyzed in duplicate, but the litter samples were analyzed in singleton using 25 μl of the pooled plasma, which was the majority of the sample for each sex within each litter. Intra-assay variability for the adult samples was 6.6%.

1.4. Data analyses

Of the 50 litters generated, nine were discarded because they did not contain enough pups to provide the sufficient plasma required by the assay, or the samples were lost. Litter data were analyzed with analysis of variance (ANOVA) using sex and postnatal day at time of sacrifice as factors. These data were also analyzed with separate ANOVAs using litter size or litter sex ratio as factors. Circulating testosterone from the adult breeding pairs was analyzed with paired *t*-tests. Statistical significance was indicated by $p \leq 0.05$.

2. Results

The 41 litters used in the study contained 5.6 ± 1.6 pups per litter (range 3—9 pups) and had an average sex ratio of 53% males to 47% females (range 14—100% males). There was very high parallelism between diluted samples of vole plasma and the RIA standards (Fig. 1). Testosterone was detectable in plasma of both sexes of pups on all days and was significantly higher in males than females ($F(1,56) = 4.45$, $p \leq 0.04$; Fig. 2a). There was no significant main effect of postnatal day ($F(4,56) = 0.51$, $p \geq 0.7$) or sex by postnatal day interaction ($F(4,56) = 0.11$, $p \geq 0.9$) on pups' plasma testosterone. There was no significant effect of litter size or litter sex ratio on testosterone levels in either sex of pup or when collapsed across sex (all *p*s ≥ 0.15). Adult breeder males had