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Parental Experience is Linked with Lower Vasopressin Receptor 1a Binding and Decreased Postpartum Androgens in Titi Monkeys

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Abstract

Parenting induces many neurological and behavioral changes that enable parents to rear offspring. Vasopressin plays an important role in this process via its effects on cognition, affect, and neuroplasticity, and in some cases, via interactions with decreased parental androgens. Thus far, the role of these hormones has been primarily studied in rodents. To address this gap, we explored vasopressin receptors and androgens in titi monkeys, a pair-bonding and biparental primate species. In Studies 1–2, we used receptor autoradiography to correlate vasopressin receptor 1a (AVPR1a) binding in the hippocampus (Study 1, $n = 10$) and the rest of the forebrain (Study 2, $n = 23$) with parental status, parental experience, parity, infant carrying, and pair affiliation. We found that parents exhibited lower AVPR1a binding than non-parents throughout most brain regions assessed, with especially strong effects in the hippocampus ($\beta = -0.61$), superior colliculus ($\beta = -0.88$), lateral septum ($\beta = -0.35$), and medial preoptic area ($\beta = -0.29$). The other measures of parental experience also tended to be negatively associated with AVPR1a binding across different brain regions. In Study 3 ($n = 44$), we compared pre- and postpartum urinary androgen levels in parents and non-parents and found that mothers exhibited a sustained androgen decrease across 3–4 months postpartum (relative to 3 months prepartum; β ranged from −0.72 to −0.62 for different comparisons). For males, we found that multiparous fathers exhibited decreased androgen levels at 1–2 weeks postpartum ($\beta = -0.25$) and at 3–4 months postpartum ($\beta = -0.40$) compared to the prepartum, indicating both immediate and long-term reductions with subsequent paternal

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Ethics Statement:
All animal procedures and protocols complied with the standards outlined in the National Institutes of Health guidelines for the care and use of animals and were reviewed and approved by the Institutional Animal Care and Use Committee at UC Davis.

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The authors declare no conflicts of interest.

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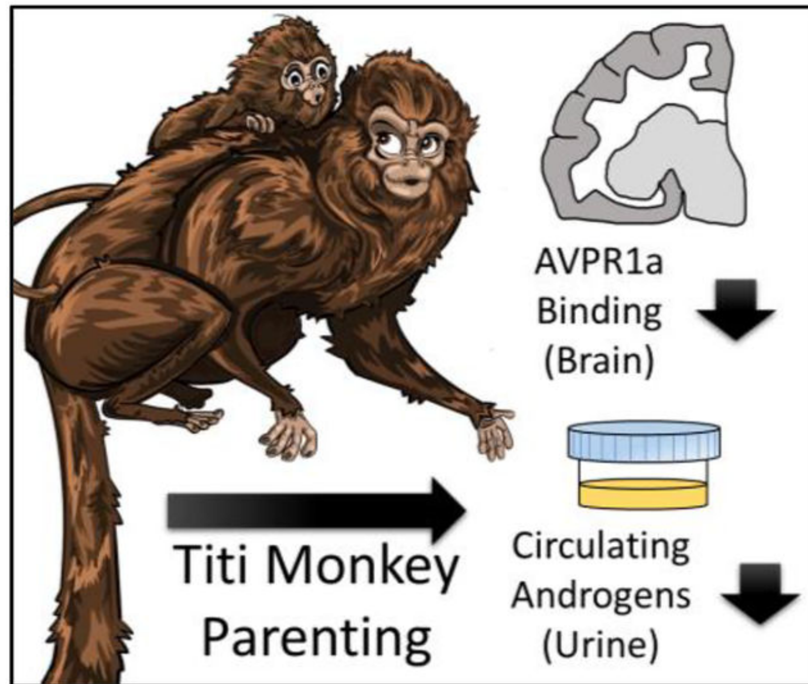
experience. Together, the results of this study suggest that decreases in AVPR1a binding and circulating androgens are associated with parental behavior and physiology in titi monkeys.

Graphic Abstract



We investigated whether parenting was associated with AVPR1a binding (postmortem brain tissue) and circulating androgen levels (postpartum urine samples) in titi monkeys, a biparental primate. In Studies 1–2, parents exhibited globally decreased AVPR1a binding compared to non-parents; reduced AVPR1a binding was also associated with greater parental experience and pair affiliation. In Study 3, parents showed decreased androgen levels across three months postpartum, with different trajectories based on sex and parity.

Keywords

Parental Brain; AVPR1a Binding; Parental Androgen Suppression; Infant Care; Pair Bonding

Introduction

When individuals become parents, they experience many neurological and behavioral changes that enable them to rear offspring[1–3] and act in ways that maximize their offspring's chances for survival[4,5]. Hormones and their receptors play a critical role in this process[6,7]. For example, although virgin females in many rodent species typically attack or avoid pups, the neural circuits underlying these behaviors are hormonally reprogrammed during pregnancy and the postpartum period, enabling females to approach and care for offspring without attacking them[7]. Many other cognitive, affective, and somatosensory brain regions

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contribute to parental care across species[5,8], and there is growing evidence that parenthood can have both short- and long-term effects on the brain and behavior[9–13].

The Roles of Vasopressin and Androgens in Parental Behavior and Neurophysiology

Vasopressin is one of several hormones that regulates parental behavior. For example, in rodents, parental behavior can be facilitated by vasopressin agonists and inhibited by vasopressin antagonists (for reviews, see[14,15]). Peripheral vasopressin levels have also been correlated with various aspects of human parenting[16–18]. Vasopressin may contribute to parenting behavior indirectly by regulating aggression, anxiety, and social recognition, especially via vasopressin receptor 1a (AVPR1a)[19–21]. Additionally, there are many sex differences in AVPR1a expression and regulation[22,23], and in some cases, the effects of vasopressin on parental behavior are dependent on interactions with androgens[24].

Androgens also have a known relationship to parental behavior. For example, meta-analyses show that human men exhibit a relatively robust and long-lasting decrease in androgens when they become fathers[25,26]. Similar trends have been found in other biparental species, including marmosets[27,28], siamangs[29], and a variety of bird species[30]. Decreased paternal androgen levels are thought to enable fathers to bond with their infants and provide paternal care, potentially at the expense of reduced mating effort[30,31]. A similar trade off may occur in females, as a smaller number of studies (relative to studies of males) show that mothers exhibit longer-term parenting-related decreases in circulating androgens (humans:[32,33] marmosets:[34] squirrels:[35]). In support of the trade-off hypothesis[31], many rodent studies have shown that exogenous androgen treatments inhibit parental behavior (for reviews, see[36,37]). However, the effect may not be universal across species, as androgens may facilitate (rather than suppress) parental behavior in certain species (California mice:[38,39]; certain vole species:[40,41]).

Thus far, relatively few studies have investigated how vasopressin receptors and androgens affect parental behavior in nonhuman primates (relative to the number of studies in rodents). One study showed that marmoset fathers exhibited increased AVPR1a immunolabeling in the frontal cortex (but not in the occipital cortex) compared to non-fathers, an effect that coincided with increased neural plasticity[42]. No other studies have investigated the association between parenting and endogenous AVPR1a measures in a primate species (that we know of). Studies in marmosets and tamarins have shown that decreased testosterone is associated with paternal status and fathering behaviors[27,28], and that infant sensory cues acutely reduce circulating testosterone in fathers[43,44]. Marmoset mothers also exhibit a decrease in postpartum androgen levels that correlates with increased infant carrying[34]. To our knowledge, no other studies have investigated the relationship between maternal caregiving and androgen changes in a biparental nonhuman primate species.

The Titi Monkey Animal Model

To address these gaps, we investigated whether different aspects of parental experience were linked with changes in central AVPR1a binding and circulating androgen levels in coppery titi monkeys (*Plecturocebus cupreus*), a monogamous and biparental primate species[45,46]. These characteristics make titi monkeys an excellent model for testing hypotheses about

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parenting-related changes in neurophysiology, especially in fathers, which serve as the primary attachment figure for titi monkey offspring and provide most of the infant carrying[45,47]. Moreover, in a previous study, we found that titi monkey parents exhibited lower hippocampal oxytocin receptor (OXTR) binding compared to non-parents[48], which suggests that OXTR may contribute to socio-cognitive processes that support parental care or that are altered by parental experience (see[9]). In the present study, we explored whether AVPR1a binding (Studies 1–2) and androgens (Study 3) were related to titi monkey parenting.

Study 1 Hypotheses

In Study 1, we performed AVPR1a autoradiography on hippocampal brain sections from the same set of parents and non-parents used in our earlier OXTR study[48]. We targeted the hippocampus because a large body of research (primarily in rodents) has shown that parental experience leads to changes in hippocampal neuroplasticity and hippocampal-dependent cognitive abilities[9–11,49,50]. We hypothesized that AVPR1a binding in the hippocampus and other nearby brain regions (see Figure 1) would correlate with dichotomous parental status and other measures of parental experience (total parenting time, whether parents were actively rearing offspring at the time of death), parental behavior (infant carrying), and pair affiliation (see the Methods section for more information on how these variables were coded and defined). Further, we assessed whether AVPR1a binding differed by sex or whether sex moderated the effects of parental status on AVPR1a binding.

The initial analyses for Study 1 showed that parents exhibited relatively global reductions in AVPR1a binding compared to non-parents in both hippocampal and non-hippocampal brain regions (see Results below). We designed Studies 2–3 as follow-up investigations to these findings.

Study 2 Hypotheses

In Study 2, we expanded the Study 1 sample size and used the same autoradiography method to assess AVPR1a binding in the medial preoptic area (MPOA), nucleus accumbens (NAcc), frontal cortex, and other forebrain regions (see Figure 2). We targeted these regions because they play a vital role in the parental brain circuit[3,6], particularly in the context of parental motivation and reward[51]. We hypothesized that parents would exhibit lower AVPR1a binding than non-parents in these regions, and that AVPR1a binding would differ by sex and correlate with active parental status, parity, total parenting time, infant carrying, and pair affiliation. We also used a multilevel hierarchical approach to assess the average effect of parental status (and the other variables considered) on AVPR1a binding across all the brain regions assessed in Studies 1–2.

Study 3 Hypotheses

In Study 3, we assayed urinary androgen levels (a measure that reflects circulating androgen levels released from the gonads and adrenal glands; see[52]) in a different set of subjects than in Studies 1–2, and compared pre- and postpartum androgen levels between primiparous parents, multiparous parents, and non-parents. We hypothesized that fathers and mothers would exhibit reduced androgen levels during the first four months postpartum, and that this

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effect would be stronger in multiparous parents compared to primiparous parents (we based this prediction on previous studies showing that cumulative parental experience may prime greater sensitivity to changes in parental androgen levels[28,53]). Further, we investigated whether urinary androgen levels predicted concurrent levels of infant carrying and pair affiliation across the sampling period.

Methods

All animal procedures complied with the standards outlined in the National Institutes of Health guidelines for the care and use of animals and were approved by the Institutional Animal Care and Use Committee at UC Davis.

Subjects

In Study 1, we assessed AVPR1a binding in 10 adult titi monkeys, including 6 parents (3 females, 3 males) and 4 non-parents (2 females, 2 males). In Study 2, we assessed AVPR1a binding in 23 adult titi monkeys, including 16 parents (9 females, 7 males) and 7 non-parents (3 females, 4 males); these subjects included the 10 subjects from Study 1 plus 13 additional subjects that we selected to increase the sample's size and diversity (in terms of age and parental experience; see Table 1). We note that because we only used subjects with opportunistically available brain tissue (see below), we were unable to have an equal number of parents and non-parents in Studies 1–2. In Study 3, we assessed urinary androgen levels in a separate set of 44 adult titi monkeys, including 27 parents (12 males, 15 females) and 15 non-parents (7 males, 8 females). In all studies, subjects were housed indoors in small family groups that approximated the typical titi monkey social composition in the natural environment[45,46,54] (for more information on subjects' husbandry and care, see[55,56]).

Parental Status, Experience, and Parity

Studies 1–2—In Studies 1–2, we classified subjects dichotomously as parents if they produced at least one surviving offspring (i.e., an offspring that lived at least one full month) or as non-parents if they (i) never produced offspring or (ii) only produced non-surviving offspring (see Table 1 and Supplementary Table 1). All the female subjects (and all but one of the male subjects' pair mates) experienced pregnancy and parturition at least once (in the case of the non-parents, their offspring did not survive), and there were no differences between parents and non-parents in any demographic variable (see Supplementary Table 2). Hence, the key difference between parents and non-parents was whether they had reared offspring. For analyses, we dummy-coded parents as 1 and non-parents as 0.

Within the parent group, we further compared AVPR1a binding between parents that were actively rearing offspring at the time of death ("active parents"; Study 1 $n = 4$, Study 2 $n = 10$) and parents whose offspring had grown and were no longer being reared in the home cage at the time of the parent's death ("retired parents"; Study 1 $n = 2$, Study 2 $n = 6$). Among the retired parents, the "empty nesting" period ranged from 12–48 days in Study 1 and from 6 days to 3.4 years in Study 2. Note, although two of the "retired parents" were "retired" for a relatively short time before death (6–12 days), preliminary analyses showed that even if these subjects were considered active parents, it did not drastically

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change the results of any analyses involving active parental status (results available upon request). In Study 2, we also compared AVPR1a binding between primiparous parents (n = 8) and multiparous parents (n = 8) (we did not analyze parity in Study 1 because there was only one multiparous parent). For analyses, we used separate sets of contrast codes to compare (i) active parents to retired parents and (ii) primiparous parents to multiparous parents (in addition to comparing non-parents to the combined group of parents in each analysis). Additionally, we considered a continuous measure of parental experience ("total parenting time"), calculated as the cumulative number of years that subjects spent rearing at least one offspring in the home cage during their lifetime.

Study 3—In Study 3, we classified subjects' parental status more stringently than in Studies 1–2, based on whether their first live-born offspring survived at least 3 months postpartum (primiparous parents; n = 12 males, 15 females) or not (non-parents; n = 7 males, 8 females). A subset of the primiparous parents were later resampled as multiparous parents (n = 7 males, 6 females) following the birth of a subsequent (a 2nd-12th) surviving offspring (in total, there were 55 unique subject-offspring combinations). For analyses, we dummy-coded parents as 1 and non-parents as 0. Within the parent group, we dummy-coded multiparous parents as 1 and primiparous parents as 0.

Brain Tissue

In Studies 1–2, brain tissue was collected opportunistically from deceased subjects (in all cases, the cause of death or euthanasia was not related to this project and was determined to be non-neurological). Brains were extracted, rinsed with a phosphate-buffer solution, blocked into four coronal hemispheres, and then frozen at −80°C. Brain blocks were sectioned coronally on a cryostat at −20°C and 20 μm thickness and mounted on Fisher SuperFrost-plus glass slides. After sectioning, we stored slides in sealed slide boxes with a desiccant at −80 °C until use in assays.

From the bank of available tissue for each subject, for Study 1 we selected 73 sections spanning the hippocampus (4–10 sections per subject, all from the left hemisphere). For Study 2 we selected 234 tissue sections (3–25 per subject) targeting either the frontal cortex, NAcc, or MPOA (in all cases, the sections from Study 2 were more anterior than the sections from Study 1 and covered a different set of brain regions). Most sections were from the left hemisphere (n = 200), but due to some cases of tissue damage, some sections were from the right hemisphere (n = 34 from three subjects).

^{125}I-LVA Autoradiography and Binding Quantification

We used autoradiography to determine AVPR1a binding in 14 regions of interest in Study 1 and 11 regions of interest in Study 2 (see Figures 1–2 and Table 2) using an established protocol for titi monkey brain tissue[57] (ligand: ^{125}I linear vasopressin antagonist; Perkin Elmer, Waltham, MA, USA) (see Supplementary Note 1 for further explanation).

Urinary Androgen Assay

In Study 3, we determined peripheral androgen levels from first-void urine samples collected between 2005–2020 as part of regular colony-wide data collection[58]. From the bank of

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available samples, we selected one prepartum sample from each subject from 3 months before their offspring's birth (early pregnancy; titi monkey gestations typically last 4 months[58]) and 5–6 samples from the 3–4 months after their offspring's birth (approximately 1–2 postpartum samples per month). We chose this range because it captured the period in which offspring are still highly dependent on parental care, with weaning[47] and offspring independence[59] typically occurring at 4–5 months postpartum and maternal postpartum anovulation ending at approximately 6 months postpartum (average interbirth interval = 1 year)[58]. For analyses, we grouped samples based on whether they were obtained prepartum, 1–2 weeks postpartum (0–14 days postpartum), 3–4 weeks postpartum (15–30 days postpartum), 2 months postpartum (31–60 days postpartum), or 3–4 months postpartum (61–119 days postpartum). Although most samples in the 3–4-month postpartum range (n = 58 of 67) fell within 61–90 days postpartum (3 months postpartum), nine samples were from 91–119 days postpartum (4 months postpartum), and so we grouped these samples to create one combined 3–4-month postpartum range for analyses.

We determined androgen levels using an immunoreactive assay that was previously validated to detect testosterone metabolite and other androgen metabolites in titi monkey urine samples[60–62] (hereafter referred to as "androgen levels"). We performed separate assays for males (inter-assay CV = 2.68%, intra-assay CV = 8.35%) and females (inter-assay CV = 4.94%, intra-assay CV = 5.37%).

Infant Carrying and Pair Affiliation

Infant carrying was determined from longitudinal observations conducted approximately 4–5 days a week, 5–6 times a day, every 2 hours between 6:30 AM-4:30 PM. At each scan-sample observation, offspring were marked as being carried by either their *Mother* or *Father*, or as being *Off*. In Studies 1–2, we determined the percentage of each day's observations that each focal parent carried their most recent offspring (during the first two months of life) and calculated a single average for each parent across all daily percentages. In Study 3, we calculated average infant carrying for each parent at each sample point (using observations conducted within the week after each sample was obtained; see below).

Pair affiliation was determined from longitudinal observations conducted at the same frequency as the infant carrying observations. At each observation, pairs were marked as either in *Proximity* (within one arm's length of each other), *Contact*, *Tail Twining* (sitting in close contact with the tails interwoven in a twisted pattern), or *None*. In Studies 1–2, we determined the percentage of each day's observations (from within the last two months of life) that subjects were in *Proximity*, *Contact*, and *Tail Twining*, and calculated a single average for each subject and each affiliation state across all daily percentages. In Study 3, we calculated the average of each affiliation state for each subject at each sample point (using observations conducted within the week after each sample was obtained). Pair affiliation and infant carrying data were available for most subjects in each study (see Table 1).

Data Analysis

We performed all data analysis in R programming (version 4.1.1)[63].

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Studies 1–2—In Studies 1–2, we assessed whether AVPR1a binding was predicted by the following independent variables: parental status, active parental status, parity, total parenting time, infant carrying, pair affiliation (*Proximity*, *Contact*, and *Tail Twining*), and sex. We also tested whether sex moderated the effect of parental status on AVPR1a binding. For each analysis, we used hierarchical linear models (using the *lmer* function from the *lme4* R package[64], version 1.1–27.1) to test whether each variable predicted AVPR1a binding (the outcome variable) across groups of related brain regions (see Table 2), with separate analyses for each predictor and grouping of brain regions. We determined these groupings based on similarities in neuroanatomical structure, connectivity, and function; in some cases, we also based grouping decisions on how strongly AVPR1a binding was correlated between regions (see Supplementary Figures 1–2). For Study 1, we averaged AVPR1a binding across brain sections so that there was only one measure of AVPR1a binding for each subject and brain region (with each subject and region represented as separate rows). To account for subjects and brain regions having multiple measures, we clustered the data using Subject ID and Region and included them as random effects in each model. For Study 2, we included two additional random effects in each model to account for storage and handling effects on AVPR1a binding (from when the brain tissue was sectioned; see Supplementary Note 2 for further explanation) and analyzed AVPR1a binding in a longer format (i.e., not averaged across brain sections). To determine p values for the multilevel estimates, we calculated degrees of freedom as the number of unique subjects in the analysis minus the number of fixed and random effects minus one.

For analyses that were statistically significant across multiple brain regions, we performed follow up analyses by repeating the test separately for each brain region in the grouping. In Study 1, we did this using linear regression (we did not include any random effects in the model, as each subject had only one AVPR1a measure for each brain region). In Study 2, we did this using hierarchical linear models with random effects for Subject ID and the two variables related to storage and handling effects (as subjects had multiple AVPR1a measures for each region in Study 2; we did not include Region as a random effect, as we analyzed only one brain region at a time). To account for multiple comparisons, we applied False Discovery Rate (FDR) correction to each set of p values for each follow up analysis.

In addition to the primary analyses described above, we performed an overall analysis for each predictor to determine the variable’s average association with AVPR1a binding across all brain regions assessed in each study. We also performed additional follow up analyses to control for different covariates related to AVPR1a binding (weight at death), parental status (whether subjects had previously produced non-surviving offspring), infant carrying (parent’s sex), and pair affiliation (parental status and age at pairing). Because most of the original results were consistent across the follow-up covariate analyses, unless otherwise noted in the results, we report these follow up results primarily in the supplementary material (see Supplementary Note 3 and Supplementary Figure 3).

Study 3—In Study 3, we used hierarchical linear models to compare androgen levels within parents and non-parents (and within primiparous and multiparous parents) at the prepartum timepoint with androgen levels at 1–2 weeks postpartum, 3–4 weeks postpartum, 2 months postpartum, and 3–4 months postpartum. In some cases, we additionally compared androgen

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levels between groups at different sampling bins. For the analyses of parity, we only used subjects that were sampled once as a primiparous parent and once as a multiparous parent. For the analyses of infant carrying and pair affiliation (*Proximity*, *Contact*, and *Tail Twining*), we used hierarchical linear models to assess whether these behaviors were predicted by androgen levels, days since birth (a continuous variable coded as sample date minus parturition date), and the interaction between these variables. We explored significant interactions by correlating androgens and pair affiliation/infant carrying at each sampling bin. We also repeated each pair affiliation analysis to control for parental status, but because the results were highly similar to the original analyses, these results are not reported (they are available upon request).

We performed all Study 3 analyses separately for males and females. To account for repeated measures for each subject, we again clustered the data using Subject ID and included it as a random effect in each multilevel model. When applicable, we included Offspring ID nested within Subject ID as an additional random effect to account for repeated measures for primiparous and multiparous parents. For males, we repeated each significant analysis to control for age at sampling (which was negatively correlated with male androgen levels, $r = -0.26$, $p < 0.001$), but because age was not a significant covariate in any analysis, these results are not reported (they are available upon request). Further, because one female non-parent had extreme androgen values at 2- and 3-months postpartum (> 7 SD), we performed sensitivity analyses with these data points excluded to assess whether the results were driven by these values alone.

Results

A summary of the primary analyses is presented below (see Supplementary File 1 for tables containing the results of all analyses performed).

Studies 1–2

Parents Exhibited Lower AVPR1a Binding than Non-Parents—Parents exhibited lower AVPR1a binding than non-parents across all brain regions assessed in Study 1 (overall $\beta = -0.61$, $p = 0.012$). The largest effects were in the hippocampus ($\beta = -0.63$, $p = 0.014$) and midbrain ($\beta = -0.56$, $p = 0.035$) groupings (see Figure 3). Follow up analyses showed that parents exhibited significantly lower AVPR1a binding than non-parents in all hippocampal subregions (β ranged from −0.66 to −0.85, p FDR < 0.038) and the SC ($\beta = -0.88$, p FDR = 0.049) (see Figure 4). In Study 2, parents exhibited lower AVPR1a binding across the basal forebrain grouping ($\beta = -0.25$, $p = 0.044$), including the LS ($\beta = -0.35$, p FDR = 0.040) and MPOA ($\beta = -0.29$, p FDR = 0.013).

Diverse Measures of Parental Experience Predicted Lower AVPR1a Binding— Active parents exhibited lower AVPR1a binding than retired parents in the Caudate grouping in Study 1 ($\beta = -0.60$, $p = 0.015$) and the Striatum grouping in Study 2 ($\beta = -0.38$, $p = 0.009$) (see Figure 3). Follow up analyses showed that this effect was significant in the Cd. Head ($\beta = -0.56$, p FDR = 0.034), Cd. Tail ($\beta = -0.67$, p FDR = 0.034), Cd. Body ($\beta = -0.47$, $p = 0.015$), and Put. ($\beta = -0.48$, p FDR = 0.007) (see Figure 4). Multiparous parents exhibited lower AVPR1a binding than primiparous parents in the CeA in Study 2 (β

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= −0.35, $p = 0.042$) (see Figure 4). AVPR1a binding was not significantly associated with total parenting time ($p > 0.106$) or infant carrying ($p > 0.092$) across any grouping; however, the estimates for these variables also tended to be negative (see Figure 3).

No effects of Sex on AVPR1a Binding—There were no sex differences in AVPR1a binding ($p > 0.21$; see Figure 3) and no interactions between sex and parental status ($p > 0.095$) in any brain region assessed.

Associations Between AVPR1a Binding and Pair Affiliation—When we controlled for differences in pair affiliation between parents and non-parents, AVPR1a binding was positively associated with *Proximity* ($\beta = 0.39$, $p = 0.038$) and *Tail Twining* ($\beta = 0.28$, $p = 0.046$) across all regions assessed in Study 2 (see Figure 3). The strongest effect sizes were from the Striatum grouping (*Proximity*: $\beta = 0.49$, $p = 0.047$; *Tail Twining*: $\beta = 0.42$, $p = 0.019$). Follow up analyses showed that *Proximity* was significantly associated with AVPR1a binding in the NAcc ($\beta = 0.77$, p FDR = 0.019) and that *Tail Twining* was significantly associated with AVPR1a binding in the Cd. Body ($\beta = 0.52$, p FDR = 0.033) and Put. ($\beta = 0.48$, p FDR = 0.025) (see Supplementary Figure 4).

Study 3

Multiparous Fathers Showed Decreased Postpartum Androgens—Although fathers showed no overall changes in androgen levels from the pre- to postpartum ($p > 0.13$), when we considered paternal parity, multiparous fathers had lower androgen levels at 1–2 weeks postpartum ($\beta = -0.25$, $p = 0.045$) and at 3–4 months postpartum ($\beta = -0.40$, $p = 0.028$) than they did prepartum (see Figure 5). Primiparous fathers showed no changes in pre- to postpartum androgen levels ($p > 0.43$). We note that non-fathers had lower androgen levels at 3–4 weeks ($\beta = -0.13$, $p = 0.033$) and at 3–4 months ($\beta = -0.35$, $p = 0.048$) after the birth and death of their non-surviving offspring than they did prepartum.

Mothers Showed a Sustained Decrease in Urinary Androgen Levels—Mothers had lower androgen levels at all postpartum sampling time points than they did prepartum (β ranged from −0.72 to −0.62; $p < .001$; see Figure 5). This effect had an earlier onset in multiparous mothers (emerging immediately at 1–2 weeks postpartum; $\beta = -0.87$, $p = 0.005$) than it did in primiparous mothers (emerging later at 3–4 weeks postpartum; $\beta = -0.64$, $p = 0.009$). This effect was potentially driven by multiparous mothers having higher prepartum androgen levels than primiparous mothers ($\beta = 0.74$, $p = 0.036$). Non-mothers also showed decreased androgen levels after the birth and death of their non-surviving offspring, but only at 3–4 weeks post-birth ($\beta = -0.54$, $p = 0.004$) and at 2 months post-birth (outliers excluded: $\beta = -0.44$, $p = 0.045$; outliers included: $\beta = 0.02$, $p = 0.94$); the decrease had trending significance at 1–2 weeks post-birth ($\beta = -0.48$, $p = 0.079$).

No Association Between Infant Carrying and Androgen Levels—There were no associations between androgen levels and infant carrying in either mothers or fathers ($p > 0.26$).

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Female Androgens were Differentially Associated with Pair Contact Across the Pre- and Postpartum—For females, days since birth moderated the association between *Contact* and androgen levels (β = 0.35, $p = 0.004$). Follow up analyses showed that *Contact* and androgen levels were positively correlated at prepartum (β = 0.15, $p = 0.035$) and negatively correlated at 1–2 weeks postpartum (β = −0.33, $p = 0.027$) (see Supplementary Figure 5). Although *Contact* was initially associated with androgen levels at 3–4 months postpartum (outliers included: β = 0.61, $p < 0.001$), this association was not significant when the androgen outliers (see Data Analysis section) were excluded (β = 0.13, $p = 0.43$). Androgen levels were not associated with *Contact* in males (β = 0.07, $p = 0.51$) and were not associated with *Proximity* or *Tail Twining* in either males ($p > 0.13$) or females ($p > 0.50$).

Discussion

In this study, we investigated the effects of parental status and experience on central AVPR1a binding and changes in postpartum urinary androgen levels across male and female titi monkeys. In Studies 1–2, we assessed seven groupings of brain regions across 300+ brain sections and found that titi monkey parents exhibited lower AVPR1a binding levels than non-parents across most brain regions assessed, with particularly strong effects in brain regions linked with parental behavior and motivation (the MPOA, LS), parental cognition and neuroplasticity (the hippocampus, LS), and other areas linked with attention (the SC, PSB). AVPR1a binding also tended to be negatively associated with active experience rearing offspring in somatosensory regions (the Caudate and Putamen) and with parity in the CeA (see Figure 3).

These results suggest that reduced AVPR1a binding (presumably from exposure to offspring) is a mechanism that recruits various cognitive and affective processes that are necessary for parental care in different contexts. For example, one of the largest effect sizes for parental status was for the SC (a region involved in attentional orienting[65]), which could suggest that reduced AVPR1a binding in this region plays a role in orienting parents' attention towards infants and/or away from other competing social stimuli. A similar process may occur in the hippocampus, a brain region that shows substantial changes in neural plasticity and organization with parental experience (rodents:[9,10,49]) and a region in which AVPR1a binding has previously been shown to modulate social recognition (mice:[66,67], however, see[68]) and anxiety (mice:[69])—processes that could be relevant to parental behavior. The effect size was especially large for the DG, a part of the hippocampus that shows substantial neural plasticity and reorganization in response to parental experience[10]. Further, we found that multiparous parents exhibited lower AVPR1a binding than primiparous parents in the CeA, a brain region that is broadly involved in threat response[70,71] and in which AVPR1a binding modulates maternal aggression (rats:[72]).

Reduced AVPR1a binding could also have direct effects on parental behavior via its effects in the MPOA, a region that is broadly implicated in regulating parental behavioral neuroendocrinology[6] in part via AVPR1a binding (rats:[73]), and the LS, a region that is implicated in paternal care (voles:[74]) and that contributes more broadly to regulating mood, anxiety, aggression, social recognition, and a variety of motivated behaviors[75–77]. Further,

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the LS is associated with pair bonding across different species see[78], and several studies from our laboratory have implicated it (and other brain structures) in titi monkey pair bond formation[79–81], separation distress[82], and "jealousy" responses towards simulated infidelity[83]. Our analyses of pair affiliation suggest that AVPR1a binding in dopaminergic regions in the striatum (the NAcc, Cd. Body, and Put.) may also contribute by modulating changes in dyadic affiliation when pair mates become parents[59] (see Supplementary Note 4 for further discussion of the pair affiliation results). Taken together, the results for Studies 1–2 suggest that AVPR1a binding may have both direct and indirect effects on parental behavior (potentially by modulating changes in parental cognition, affect, and/or somatosensation). However, an alternative possibility is that parental behavior was a cause (and not a consequence) of reduced AVPR1a binding or that these variables were bi-directionally linked (e.g., see[8]).

Overall, the results of Studies 1–2 are consistent with an established literature showing that AVPR1a binding and vasopressin more broadly regulate different aspects of parental care[3,14,21]. For example, our results for the hippocampus are consistent with a previous study of male California mice in which fathers showed reduced levels of hippocampal AVPR1a expression by the end of the first month postpartum[84]. Our findings for the LS are consistent with two previous studies (in voles) showing a negative association between AVPR1a binding in the LS and parenting (voles:[85,86]); however, one study showed a positive association between parental behavior and AVPR1a binding in the LS (female mice:[87]), and another study showed no association (male prairie voles:[88]). Our findings for the MPOA, SC, and CeA also stand apart from several previous studies that found no significant associations between parenting and AVPR1a binding/expression in these regions (rodents:[85,86,88,89]). These differences may possibly reflect a species difference in AVPR1a regulation and distribution between rodents and primates[90], or differences in how AVPR1a was measured in the different studies (i.e., autoradiography versus gene expression). Another important difference was that the general direction of our results (i.e., parental status and experience were associated with *lower* AVPR1a binding) was in the opposite direction of the results from a previous study in marmosets, in which AVPR1a expression *increased* in the prefrontal cortex of fathers rearing offspring[42] (moreover, there were no significant effects of parental status on AVPR1a binding in the frontal cortex in the present study). As with the rodent studies discussed above, the difference in direction could potentially reflect differences in how vasopressin signaling is related to parental behavior in different species or AVPR1a measurement (i.e., autoradiography across gross brain regions in the present study versus immunolabeling of individual dendritic spines in the marmoset study). Further, it is important to consider that AVPR1a binding can have excitatory or inhibitory effects on neuronal signaling, and that reduced AVPR1a binding could potentially be a compensatory response to increased levels of vasopressin signaling (i.e., reduced AVPR1a binding may reflect increased AVPR1a signaling). Hence, it is possible that our study and the marmoset study[42] both reflect a similar underlying process (i.e., changes in AVPR1a signaling with parental experience) but at different levels of analysis. Further research is needed to explore these different possibilities across different species.

In Study 3, we assessed urinary androgen levels in 370+ urine samples obtained from over 40 subjects and found that parents exhibited decreases in postpartum urinary androgen

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levels that differed by sex and parity. For fathers, there were both immediate and gradual decreases in postpartum androgens, but only for multiparous fathers. The specificity of our findings to multiparous fathers is consistent with other studies of paternal androgen changes in both humans[53] and marmosets[28], which showed that multiparous fathers exhibited a greater postpartum decrease in androgen levels compared to primiparous fathers. This could indicate that the initial onset of fathering behavior is less dependent on androgen suppression in male titi monkeys, which show relatively high tolerance for infants even prior to mating[91]. An alternative explanation could be that multiparous fathers' reduced androgen levels were a secondary consequence of reduced opportunities for mating due to the demands of caring for offspring. Somewhat unexpectedly, male non-parents also showed decreased androgens at 3–4 weeks postpartum and at 3–4 months postpartum (relative to at the prepartum). We speculate that this result could also be related to changes in mating patterns and/or female receptivity, especially if the female pair mates became pregnant again (which could potentially have occurred within the 3–4 month postpartum study period, as female titi monkeys typically resume cycling shortly after parturition if their offspring does not survive[58]). Nonetheless, without direct measures of mating frequency and sexual receptivity, further research is needed to explore how changes in androgen levels contribute to mating and parenting behavior in male titi monkeys.

For females, mothers and non-mothers both showed an initial decrease in postpartum androgens (potentially due to hormonal changes during late pregnancy and/or parturition); however, the decrease was stronger and only sustained in mothers rearing offspring (and began earlier in multiparous mothers compared to primiparous mothers). Given that adult female titi monkeys typically avoid carrying infants except when nursing[47] and may even avoid their male mate while he carries the infant[59], these results suggest that androgen suppression may play a role in the onset of maternal care. The results of the pair affiliation analyses support this interpretation and suggest that decreased maternal androgens may be related to increased tolerance for affiliative contact with the pair mate (and the infant he carries) during the immediate postpartum period. Alternatively, these findings could reflect postpartum anovulation (which lasts approximately 6.5 months post-parturition in mothers of surviving offspring; see[58]), a mechanism that ensures mothers do not get pregnant again while they are still nursing. Further research is needed to disentangle these possibilities.

Taken together, the results of Studies 1–3 suggest that, in titi monkeys, AVPR1a binding and androgens affect (or are affected by) parental behavior. Although we hypothesized that these biological factors would have different effects in males and females, we found that parental status had consistent effects on AVPR1a binding in both fathers and mothers, and there was no evidence of any overall sex differences in AVPR1a binding. These results were somewhat surprising, given that many previous studies have shown that vasopressin has sexually dimorphic effects on a variety of parental and non-parental behaviors in other species, in many cases via interactions with sex hormones[19,74,75,92]. One possibility is that reduced AVPR1a binding facilitates parental behavior in both male and female titi monkeys, and that any sexually dimorphic effects depend on interactions with changing postpartum androgen levels (which showed different trajectories in fathers and mothers). This interpretation is supported by rodent studies showing that androgen manipulations modulate AVPR1a binding[93,94] and that AVPR1a binding manipulations modulate parental behavior[73,95,96].

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However, parental behavior is likely determined by many complex and multidirectional interactions between these and other factors, and because we did not experimentally manipulate these hormones, we ultimately cannot determine causality. Another important caveat is that we were unable to directly link AVPR1a binding and urinary androgen levels in the same set of subjects in the present study, nor were we able to include a comparison group of subjects which had never experienced pregnancy or parturition, and therefore we were unable to assess how these experiences might affect AVPR1a binding. Further, some of the subgroup analyses were limited by a small sample size (especially the analyses of infant carrying in Study 1, which had only 6 parents with available data). Moreover, we measured central AVPR1a binding and peripheral urinary androgen levels at different time points (i.e., the postmortem versus the postpartum) and therefore could not evaluate concurrent parenting-related changes in these measures or interactions between them.

Conclusion

In the current study, we found that parental status and experience were associated with AVPR1a binding and postpartum urinary androgen levels in a large sample of both male and female titi monkeys. Our results for Studies 1–2 are based on one of the largest nonhuman primate autoradiography samples to date and suggest that parental status and experience have relatively global effects on AVPR1a binding. We found that parents had lower AVPR1a binding than non-parents across diverse brain regions, including regions that are directly involved in regulating parental behavior and regions that may indirectly contribute via their role in learning and memory, attention, social recognition, somatosensory processing, executive motor control, fear and anxiety, and protective aggression. The results of Study 3 showed that titi monkeys experienced complex changes in postpartum androgen levels that depended on sex and parity level, which may potentially interact with changes in central AVPR1a binding to modulate parental behavior and affiliation between pair mates. Overall, our findings are consistent with studies showing that parental experience can have relatively long-lasting and bi-directional effects on the brain and behavior, and continued research is needed to explore how androgens and vasopressin modulate (and are modulated by) different cognitive, behavioral, and affective components of parenting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability Statement:

The data and R analysis code that support the findings of this study are openly available on the Open Science Framework (OSF) at https://osf.io/738mp/?view_only=3e9e097978164920be994f4f654c79bc.

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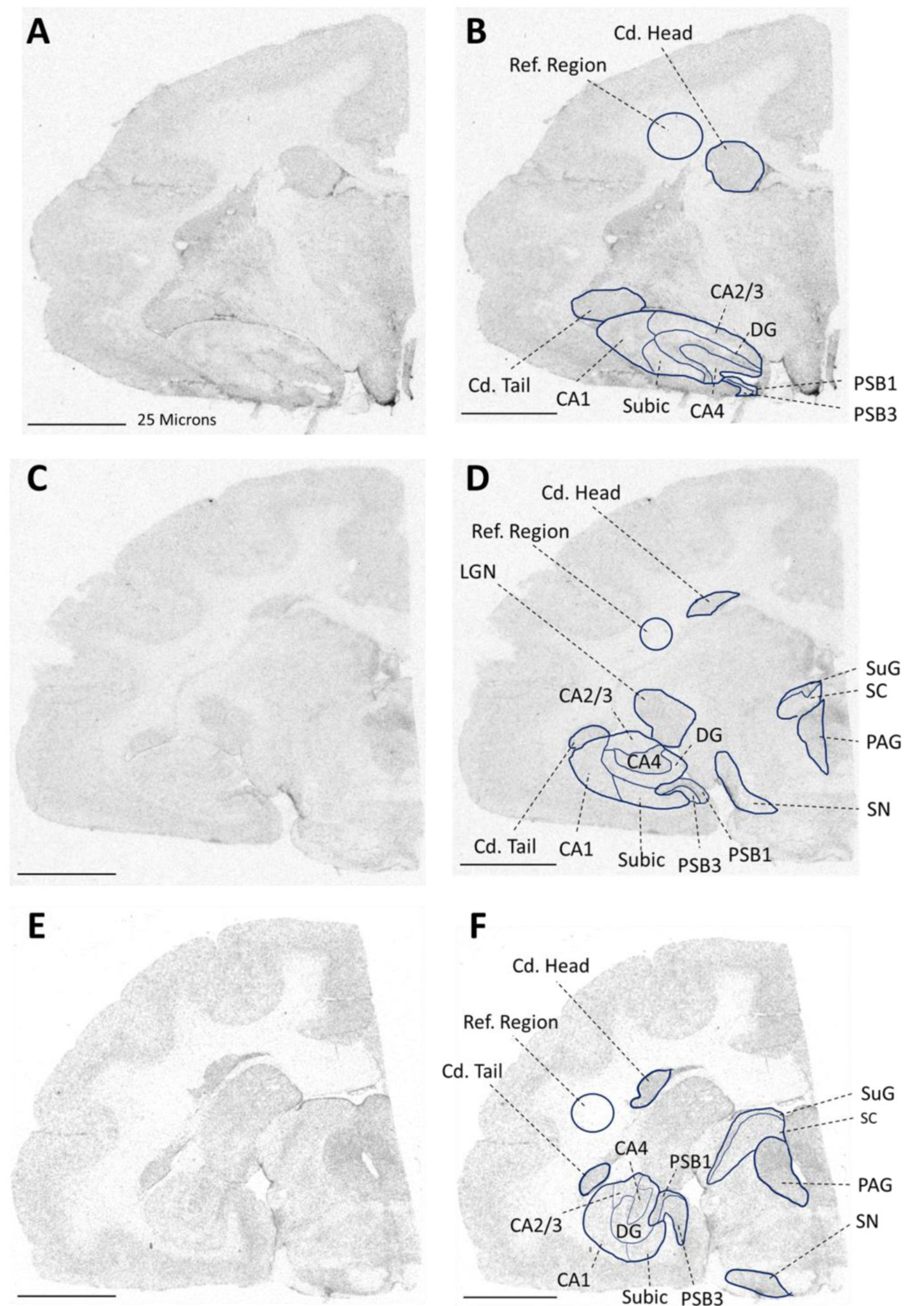

Figure 1.

Brain Regions Quantified in Study 1.

The figure shows the brain regions quantified in Study 1 on three representative AVPR1a autoradiograms (the image on the left side shows the unlabeled autoradiogram, and the image on the right shows the same autoradiogram with the regions of interest that we quantified). Ref. Region (reference region) indicates the approximate segment of white matter that we sampled to correct AVPR1a binding density values from each section for background binding levels. The black line at the bottom left corner of each panel indicates

25 microns. See Table 2 for a full list of abbreviations and for how the regions were grouped for analyses.

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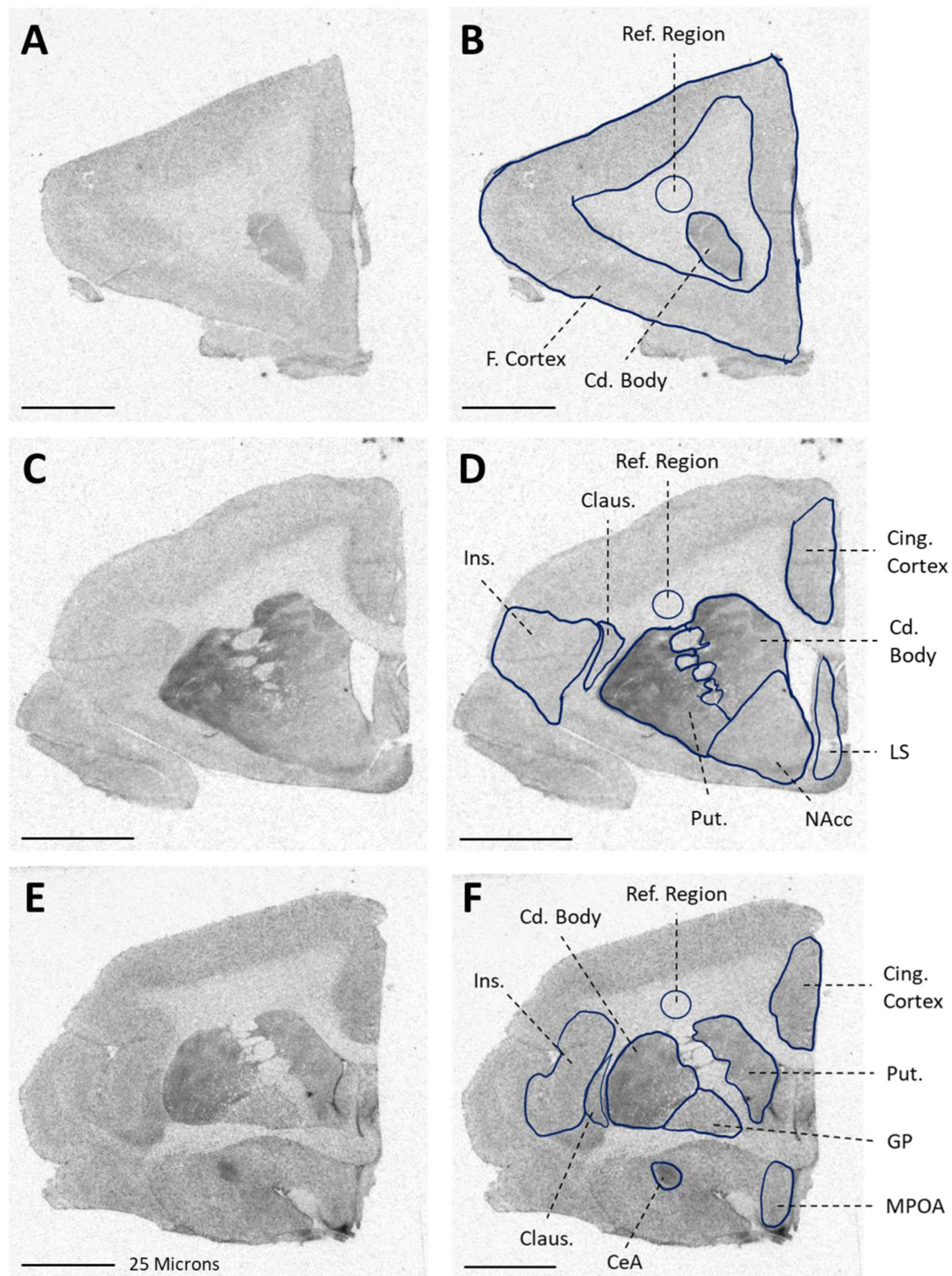

Figure 2.

Brain Regions Quantified in Study 2.

The figure shows the brain regions quantified in Study 2 on three representative AVPR1a autoradiograms (the image on the left side shows the unlabeled autoradiogram, and the image on the right shows the same autoradiogram with the regions of interest that we quantified). Panels A and B correspond to a section targeting the frontal cortex, and panels C and D correspond to a section targeting the NAcc. Panels E and F correspond to a section targeting the MPOA. Ref. Region (reference region) indicates the approximate segment of white matter that we sampled to correct AVPR1a binding density values from each section

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for background binding levels. The black line at the bottom left corner of each panel indicates 25 microns. See Table 2 for a full list of abbreviations and for how the regions were grouped for analyses.

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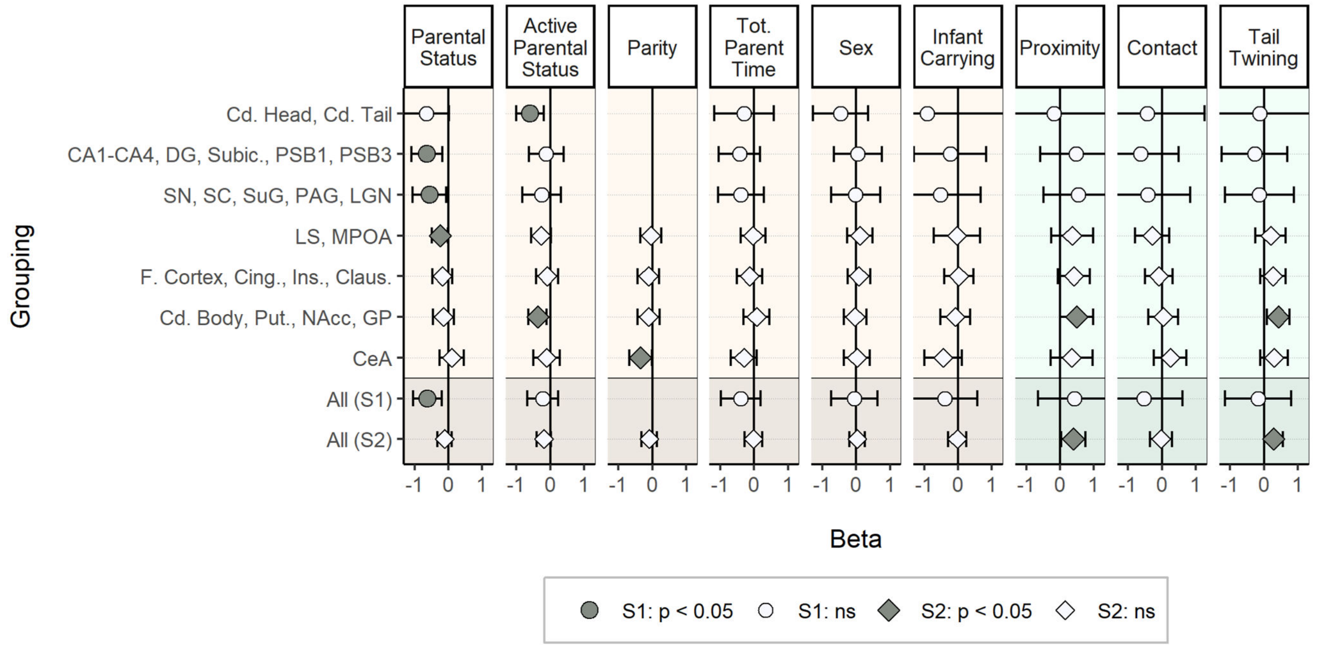

Figure 3.

Beta Estimates of Primary AVPR1a Binding Analyses (Studies 1–2).

The figure shows the overall negative relationship (indicated by the standardized beta estimates on the x axis) between parental status (the first panel) and AVPR1a binding in each grouping of brain regions (the y axis). The results for active parental status (active parents vs. retired parents), parity (multiparous vs. primiparous parents), total parenting time, sex, infant carrying (yellow columns), and each pair affiliation state (controlling for parental status; the green columns) are also shown. Negative estimates indicate lower AVPR1a binding in parents (for parental status), active parents (for active parental status), multiparous parents (for parity), and males (for sex). Estimates from Study 1 (S1) are depicted with circles and estimates from Study 2 (S2) are depicted with diamonds. The overall effect sizes for each study are shown at the bottom of each panel (the darker grey segments of the panels). The error bars represent the 95% confidence intervals of each estimate. The solid black vertical line indicates a beta estimate of 0 (no association); confidence intervals that overlapped this line were not significant (*ns*, indicated by white points) and confidence intervals that did not were significant ($p < .05$, indicated by dark grey points).

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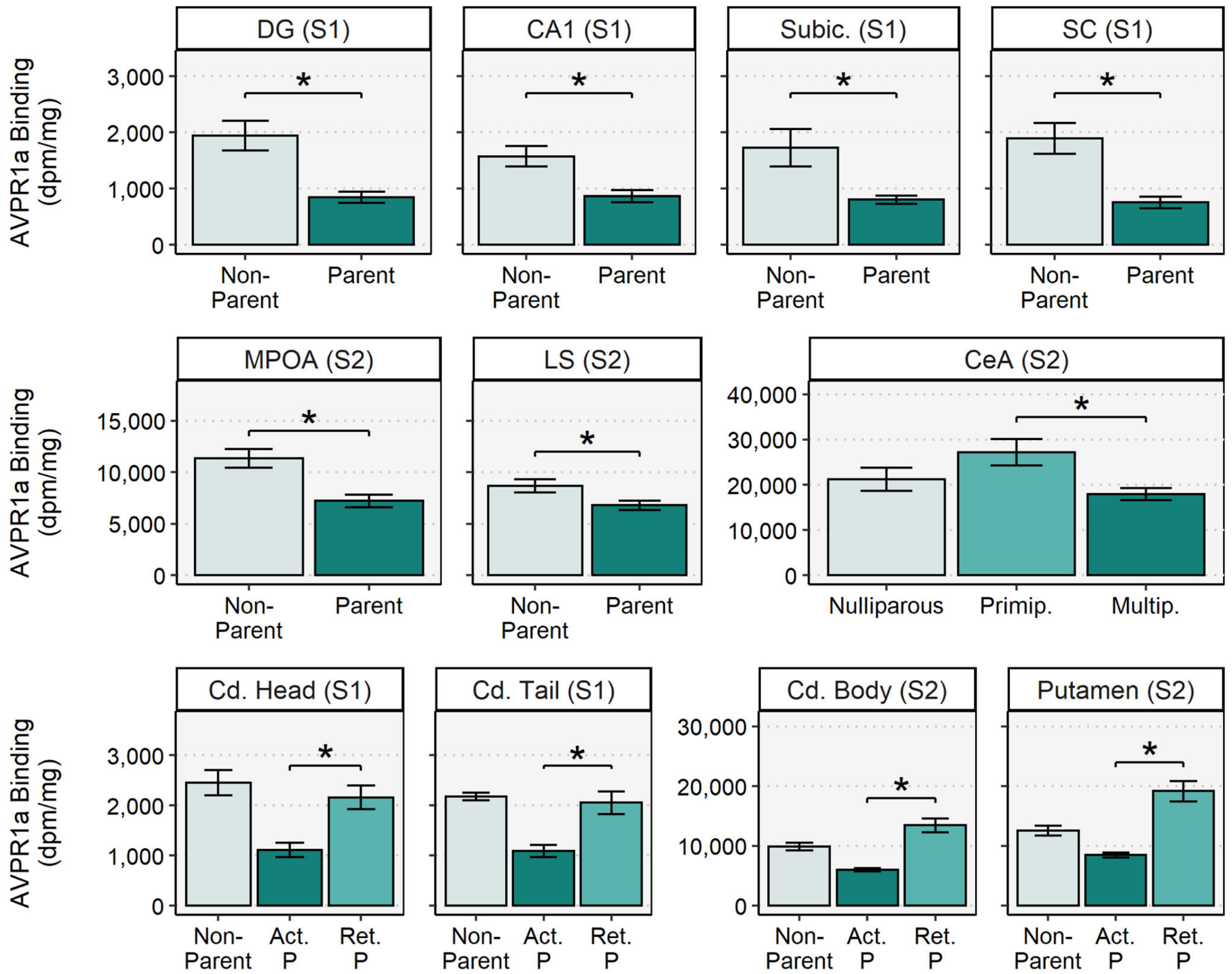

Figure 4.

Effects of Dichotomous Parental Status, Parity, and Active Parental Status on Lower AVPR1a Binding (Studies 1–2).

The graph shows the effects of dichotomous parental status, parity, and active parental status (labeled on the x axis) on lower AVPR1a binding (on the y axis) for selected brain regions of interest (indicated in the panel titles) in Study 1 (S1) and Study (S2). Darker-colored bars indicate the group with the most parental experience (i.e., parents, multiparous parents, and active parents). Error bars indicate ± 1 standard error. Asterisks (*) indicate the comparison was significant at $p < 0.05$. Abbreviations: *Primip.* Indicates primiparous parents, *Multip.* Indicates multiparous parents, *Act. P* indicates active parents, *Ret. P* indicates retired parents. The data can be further visualized using the following interactive R Shiny dashboards for Study 1 (https://alexander-baxter.shinyapps.io/Titi_AVPR1a_Baxter2023_Study1/) and Study 2 (https://alexander-baxter.shinyapps.io/Titi_AVPR1a_Baxter2023_Study2/).

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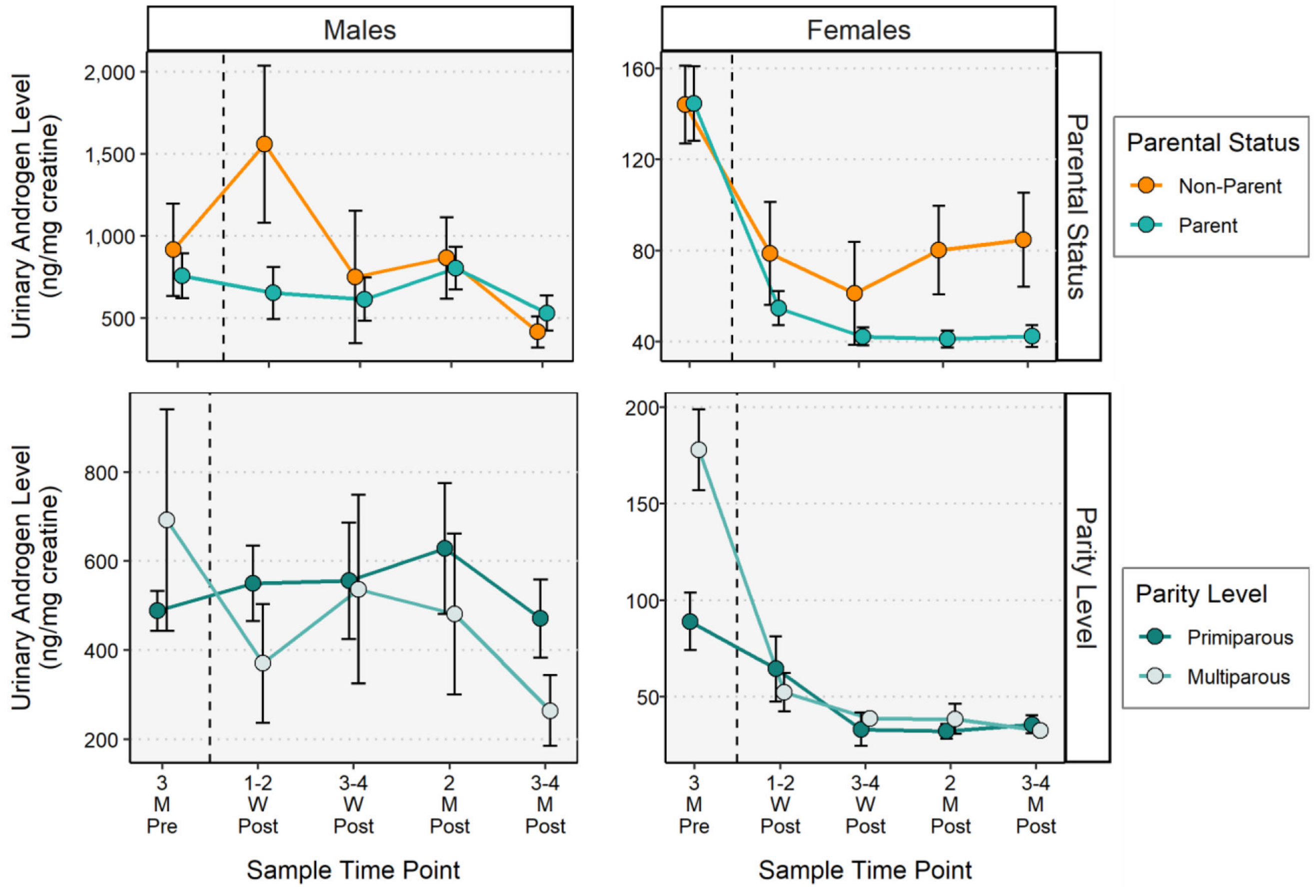

Figure 5.

Urinary Androgens in Parents and Non-Parents Across the Pre- and Post-Partum (Study 3). The graph shows changes in average urinary androgen levels (on the y axis) across sampling time point bins (on the x axis) by parental status (the top two panels) and by parity level (the bottom two panels). Data for males are shown in the left column and data for females are shown in the right column. Error bars indicate ±1 standard error. The black vertical dashed line delineates the prepartum sampling time point from the postpartum sampling time points. For the analyses of parental status, data from all subjects were used (the parent group included both primiparous and multiparous parents; however, the non-parent group did not include the two high androgen outliers for the female non-parents at the 2 month and 3–4-month time points). For the analyses by parity, only data from subjects that were sampled once as a primiparous parent and once as a multiparous parent were used. Note, the error bars shown on the graph do not necessarily reflect how the standard errors were calculated in the multilevel analyses (which included random effects for Subject ID and Offspring ID). This is why some of the significant comparisons reported in the results section have overlapping error bars in this graph, and why some of the comparisons that have non-overlapping error bars in this graph were not significantly different in the analyses. See the Results section for a full summary of which comparisons were significantly

different. Abbreviations: M indicates months, W indicates weeks, Pre indicates prepartum, Post indicates postpartum.

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Table 1.

Sample Sizes and Study Demographics (Studies 1–3)

Variable	Study 1	Study 2	Study 3
Study Parameters			
Biological Measure Assessed	AVPR1a Binding	AVPR1a Binding	Urinary Androgens
Target Brain Regions	Hippocampus	Frontal Cortex NAcc MPOA	-
Total Biological Samples	73 brain sections	234 brain sections	374 urine samples
Sampling Period	Post-mortem tissue	Post-mortem tissue	3–4 months prepartum- 3–4 months postpartum
Sample Size			
Total Number of Subjects	10	23	42 (55[†])
By Available Data			
Parents with Available Infant Carry Data[*]	6	13	26 (38[†])
Subjects with Available Affiliation Data[*]	8	18	41 (53[†])
Demographic Characteristics			
Age Range	4.0–7.5 y	4.0–18.8 y	1.9–15.7 y
Among Parents			
Number of Surviving Offspring Reared	1–2	1–12	1–12
Oldest Offspring Reared	2–14 m	2–61 m	3 m
Among Non-Parents			
Number of Non-Surviving Offspring Birthed	1–2	1–6	1
Oldest Non-Surviving Offspring	0 d	0–12 d	0–8 d

The table shows summary information about each study sample. Note, the demographic information for Studies 1–2 pertain to subjects' entire lifetime, whereas the information for Study 3 pertains to subjects at the time of urine sampling (the first 3–4 months postpartum). For an expanded summary of Studies 1–2, see Supplementary Table1.

[*] In Studies 1–2, some subjects were missing infant carrying and/or pair affiliation data because they died before colony-wide data collection on these measures began.

[†] The values in parentheses indicate the number of unique subject-offspring combinations for Study 3.

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Table 2.

Brain Regions and Groupings in Studies 1–2.

Grouping & Brain Region	Abbreviation
Study 1: Hippocampus	
CA field 1 of hippocampus	CA1
CA field 4 of hippocampus	CA4
CA fields 2/3 of hippocampus	CA2/3
Dentate gyrus	DG
Subiculum	Subic.
Presubiculum layer 1	PSB1
Presubiculum layer 3	PSB3
Study 1: Midbrain	
Superficial grey layer of the superior colliculus	SuG
Superior colliculus (not including the SuG)	SC
Lateral geniculate nucleus of the thalamus	LGN
Periaqueductal grey	PAG
Substantia nigra	SN
Study 1: Caudate	
Caudate head	Cd. Head
Caudate tail	Cd. Tail
Study 2: Cortex (and Claustrum)	
Frontal cortex	F. Cortex
Cingulate cortex	Cing. Cortex
Insula	Ins.
Claustrum	Claus.
Study 2: Striatum	
Caudate body	Cd. Body
Putamen	Put.
Nucleus accumbens	NAcc
Globus pallidus	GP
Study 2: Basal Forebrain Regions	
Lateral septum	LS
Media preoptic area of hypothalamus	MPOA
Study 2: Amygdala	
Central nucleus of the amygdala	CeA

The table lists the full names and abbreviations used for each brain region quantified in Studies 1–2, as well as the grouping that the brain region was included in for analyses. See Supplementary Figures 1–2 for a summary of how strongly AVPR1a binding was correlated between regions and for further explanation of why we grouped the brain regions in this way.