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Compositional variation in early life parenting structures alters oxytocin and vasopressin 1a receptor development in prairie voles (*Microtus ochrogaster*)

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Abstract

Paternal absence can significantly alter bio-behavioral development in many biparental species. This effect has generally been demonstrated by comparing the development of offspring reared under biparental care with those reared by a single mother. However, studies employing this design conflate two significant modifications to early life experience: removal of father-specific qualities and the general reduction of offspring-directed care. In the socially monogamous prairie vole (*Microtus ochrogaster*), the experience of paternal absence without substitution during development inhibits partner preference formation in adulthood, a hallmark of social monogamy, in females and males. Employing alloparents as substitutes for fathers, our previous work demonstrated that paternal absence affects pair-bond formation in female offspring via reduced *quantity* of care; but it affects pair-bond formation in male offspring by means of a missing paternal *quality* (or qualities). Here, we present evidence that paternal absence (with and without alloparental substitution) may alter the ontogeny of neural oxytocin receptor (OXTR) and/or vasopressin 1a receptor (AVPR1a) distribution in male and female prairie voles. Compared to biparentally reared controls (BPC), male offspring reared in mother only (MON) and maternal-plus-alloparental (MPA) conditions show lower densities of OXTR in the central amygdala; and MPA males show lower densities of OXTR in the caudate putamen and nucleus accumbens. Early life experience was not associated with differences in AVPR1a density in males. However, MON and MPA females show greater densities of AVPR1a in the medial amygdala than BPC; and MPA females show greater densities of AVPR1a in the ventromedial nucleus of the hypothalamus. We also demonstrate with corticosterone concentrations that MON and MPA offspring are not differentially susceptible to a stressor (i.e., social isolation) than BPC offspring. These findings

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suggest that paternal absence, while likely not a salient early life stressor, has neuroendocrine consequences for offspring, some of which may affect partner preference formation.

Keywords

Fathers; Parenting; Paternal Deprivation; Oxytocin; Vasopressin

Introduction:

Paternal care (i.e., parental care demonstrated by fathers) is relatively rare among mammals[1,2]. While the care by mothers is by definition inherent to class Mammalia, there is considerable inter-species variation in the involvement of fathers. In most mammalian species, fathers are absent for the duration of offspring development; or alternatively, fathers are tolerant of offspring but otherwise uninvolved in direct parental behavior. In a small percentage of mammalian species (est. 3–5%)[1,2], fathers demonstrate qualitatively similar (or equivalent) care as mothers. Among rodent species (and likely across class Mammalia), species in which males demonstrate paternal care are typically sexually or socially monogamous and/or cooperatively breeding[1], although exceptions do exist (e.g., *Rhabdomys pumilio*[3]). This connection between monogamy and paternal care has led some to theorize that under certain conditions, maternal care alone was insufficient to protect, provision, and otherwise care for offspring. Furthermore, additional care from fathers (or other alloparents) bridged this gap, improved offspring survival, and improved direct and indirect reproductive fitness of parents and alloparents; thus resulting in a monogamous and/or cooperative mating system[4]. Thus, one explanation for these biparental and monogamous species is that paternal care is not only *helpful*, but *requisite* for the normative biobehavioral development of offspring for whom maternal care alone is insufficient.

Prairie Voles and Partner Preference

The prairie vole is a socially monogamous, biparental, and cooperatively breeding Arvicoline rodent from the American Midwest[5]. The species is perhaps most widely known for their social monogamy, which has been documented extensively through field observations[5] and in the laboratory through the use of the “partner preference test”[6]. In the partner preference test, a subject is paired with a designated mate for some period of time (typically 24-hours) and then assessed for behavioral displays indicative of partner preference formation. The subject is placed in a neutral chamber connected to two other chambers: one containing their partner, the other containing an opposite-sex stranger (Figure 1). A partner preference is said to be formed when the subject spends significantly more time in physical contact with their partner than the stranger. The format of this behavioral assay allows researchers to assess similar behavioral patterns, for example, preference for *proximity* (in addition to or in lieu of contact) to a partner over a stranger, social distancing, and aggression. The neurobiological substrates of partner preference formation and maintenance have been elucidated through the joining of the behavioral outcomes of the partner preference test with correlative and manipulative neuroscientific methods (e.g., autoradiography, immunohistochemistry, and the manipulation of gene expression)[7,8]

as well as the use of comparative studies between socially monogamous and polygynous species[9,10].

Key players in the neuroendocrine process of pair bond formation include the twin neuropeptides, oxytocin (OT) and arginine vasopressin (AVP). Studies of the role of OT and AVP, along with their receptors, has revealed a plethora of means by which OT and AVP operate to facilitate pair-bond formation across disparate species[7,8,11]. An initial comparative study of socially monogamous prairie voles and polygynous meadow voles (*Microtus pennsylvanicus*) demonstrated that prairie voles had significantly greater densities of oxytocin receptors (OXTR) in the caudate putamen and nucleus accumbens, as well as greater densities of arginine vasopressin receptor 1a (AVPR1a) in the medial amygdala, mediodorsal thalamus, and ventral pallidum[9,10]. Subsequent research has expanded the role (and number of sites) of OT and AVP facilitation of pair bonding through continued interspecies comparisons[12] and intraspecific studies[13–15].

Prairie Voles, Paternal Absence, and Consequences for Partner Preference Behavior and Neuroendocrine Development

Paternal absence, at times referred to as ‘paternal deprivation’, significantly alters biobehavioral development in many biparental species. This manipulation of early life experience has generally been studied by comparing the development of offspring reared under various family unit compositions, with the most frequent comparison drawn between offspring reared in conditions of biparental care (i.e., conditions with a mother and father) and those reared under monoparental care (i.e., conditions with a mother alone). As summarized by Bales & Saltzman[16], paternal absence is demonstrated to have wide ranging neurobiological effects on male and female offspring during development in prairie voles, Mandarin voles (*Microtus mandarinus*), and Octodon degus (*Octodon degus*). These effects include changes to serum OT, OT mRNA, and OXTR mRNA, as well as changes associated with the HPA-axis including alterations in corticotropin releasing factor (CRF) – positive cells, CRF receptor 2, and the glucocorticoid receptor. Further recent research expands these findings to include California mice (*Peromyscus californicus)* and suggests that paternal absence contributes to variation in structural and functional neuroplasticity of the hippocampus[17].

In an initial study by Ahern and Young[18], it was demonstrated that both male and female prairie vole offspring reared under conditions of paternal absence demonstrated inhibited partner preference formation. In a subsequent study, they found that prairie vole mothers left to rear offspring without supplemental care from a mate did not compensate for the reduction of care resultant of the father’s absence[19], a finding which has been replicated[20]; although, see Kelly et al.[21], in which mothers compensate for paternal absence when additional environmental challenges are applied. Accordingly, offspring reared under conditions of paternal absence also experienced a general decline in parental investment throughout their pre-weaning development. Thus, studies employing this design conflate two significant modifications to early life experience: removal of father-specific qualities and the general reduction of offspring-directed care.

An alternative approach to the paternal absence paradigm is to instead consider natural variation in biparental care, i.e. variation in the quantity of care received by offspring as a result of individual differences among parents in parenting behavior. This alternative approach, which often contrasts the parents most invested in parental care with those the least invested (i.e., in a top vs. bottom quartile approach), has yielded intriguing results. A collection of work carried out by Perkeybile and colleagues (see Perkeybile and Bales[22] for a more extensive review) demonstrates that natural variation in parental investment has consequences for offspring anxiety-like and social behavior[23] and has implications for offspring brain development. For example, natural variation in the biparental care affects the size of cortical fields in the pup brain[24], intrinsic connections within the primary somatosensory cortex[25], and pup neuroendocrine function[26]. Thus, it appears that a change in quantity of care on its own, while maintaining the biparental dyad, is sufficient to implicate change in offspring biobehavioral development. Therefore, one might consider whether or not paternal absence induces change in offspring biobehavioral development simply through a reduction of care; or, whether or not there is some particularly special paternal *quality* to be missed. Certainly, there can be an interaction of these two approaches; in fact, within the context of the prairie vole parenting dyad, it appears that fathers may play a compensatory role to stabilize the quantity of care received by pups from litter to subsequent litter[27] and potentially within any particular litter's early development[23].

Another alternative source of variation is change mediated through maternal affect. That is, in the generation of the paternal absence condition, the removal of the father may induce an anxiety- or depression-like phenotype in the pregnant mother, thus yielding some form of prenatal stress with the potential to alter maternal behavior into the pre-weaning development of offspring[28]. In related work, prairie vole offspring exposed to prenatal stress who were then cross-fostered into a low-contact environment—which perhaps parallels the environment of a mother-only care situation—demonstrated more anxiety-like behavior and higher circulating corticosterone; while offspring that experienced prenatal stress but high-contact conditions were less anxious, demonstrated lower levels of circulating corticosterone, yet had elevated densities of AVPR1a in the amygdala[29].

Addressing the quantity and quality confound

In a preceding publication[20], we sought to address these apparently confounding variables (i.e., quantity of care vs. a particular paternal quality) by capitalizing on a natural behavior of the prairie vole: alloparental care. Quantity of care is represented as frequency and/or duration of pup-directed care, whereas quality of care consists of the manner in which care is provided along with accompanying behavioral and physiological characteristics of the caregiver. In this case, we used an older female sibling (i.e., a "big sister") to replace fathers. Older sisters provided a quantity of care commensurate with that of fathers, and therefore maintained the quantity of care that would have been provided by fathers while contemporaneously removing any particular paternal quality. Thus, we generated male and female offspring reared with three early life experiences: biparental care (BPC), monoparental care (i.e., mother only, MON), or maternal-plus-alloparental care (MPA) (Figure 1). In accordance with previous work done by Ahern and Young[18], we found that offspring reared under biparental conditions formed pair bonds within a normative period

(i.e., 24-hours); but offspring reared under monoparental conditions did not demonstrate a partner preference. However, we also demonstrated that substitution of paternal care with care from a female alloparent (i.e., an older sister) resulted in typical pair-bond formation in female offspring, but not in male offspring. Thus, paternal absence may affect pair-bond formation in female offspring via reduced *quantity* of care; but it may affect pair-bond formation in male offspring by means of a missing paternal quality (or qualities).

The current study

In the current study, we investigate the neuroendocrine consequences of variation in the parental composition in early life experience. Using brain tissue and blood collected from prairie voles reared in BPC, MON, and MPA conditions, we aimed to determine if the behavioral effects described by Rogers and Bales[20] are reflected in distributions of OXTR and AVPR1a in regions of interest tied to pair bonding; and we correlate OXTR and AVPR1a distributions with our previously reported partner preference behaviors. We also aim to determine whether differences in early life experience correlate with group-level differences in corticosterone concentration following either 48 hours of pairing or 48 hours of social isolation. In doing so, we hope to clarify if paternal absence represents a salient late gestation, prenatal stressor that leaves offspring differentially susceptible to stressors like social isolation. We hypothesize that OXTR and AVPR1a binding in regions associated with pair bonding will differ significantly according to early life experience. As male prairie vole offspring reared under conditions of paternal absence (with or without alloparental substitution) did not show partner preference[20], we predict that male MPA and MON offspring will demonstrate corresponding changes in the densities of OXTR and AVPR1a receptor distributions, which will themselves be distinct from receptor densities in BPC offspring. As female prairie vole offspring reared under paternal absence with alloparental substitution *do* demonstrate partner preferences, but those reared without alloparental substitution do not, we predict that female prairie vole offspring will show similar patterns of OXTR and AVPR1a binding between MPA and BPC females, with a distinctly different pattern in the brains of MON females. Finally, paternal separation during late gestation may represent a stressor to mothers and accordingly a prenatal stressor to offspring; therefore, we hypothesize that offspring reared under conditions of paternal absence (with or without alloparental substitution) may be differentially susceptible to a period of social isolation, as demonstrated through basal corticosterone concentrations. Previous work has connected natural variation in prairie vole parental care to differential susceptibility to stress induced by social isolation[30]. Therefore, we predict elevated levels of corticosterone in MPA and MON individuals (compared to BPC individuals) following a period of social isolation.

Materials and Methods:

Subject Selection and Ethical Considerations

All subjects were recruited from a colony of laboratory-bred prairie voles (*Microtus ochrogaster*) at the University of California, Davis, USA. The prairie vole colony was derived via systematic outbreeding of a wild stock captured near Champaign, Illinois, USA. Room conditions were maintained to approximate summertime conditions in Champaign, Illinois, USA; i.e., all individuals were maintained under a 14:10 light-dark cycle (lights

on at 06:00, lights off at 20:00) with an average room temperature of 70°F (approx. 21°C). Subjects were provided with *ad libitum* access to high-fiber Purina rabbit chow and water. From birth until weaning on post-natal day (PND) 20, all subjects were reared in their respective developmental conditions (described later) in large polycarbonate cages (44 by 22 by 16 cm) with aspen wood bedding (i.e., Sani-Chips) and cotton for nesting material. On PND20, subjects were weaned and housed in same-sex pairs in small polycarbonate cages (27 by 16 by 16 cm) until PND60. As further detailed below, at PND60 some individuals were transferred to a new, small polycarbonate cage with a novel, other-sex conspecific, while others were transferred to a new, small polycarbonate cage and left in isolation. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, Davis.

Treatment Group Formation: Early Life Experience

As more thoroughly outlined in Rogers and Bales[20], all subjects were recruited from the third litter of multiparous prairie vole breeding pairs and reared from birth to weaning (PND20) under three social conditions (i.e., family unit configurations): biparental care (BPC); maternal-plus-alloparental care (MPA; i.e., mother and older sister); and maternal care only (MON). Developmental rearing conditions were established prior to birth on PND20 of each pair's second litter, thus between 0.5 and 2.5 days before expected parturition. Thus, at the time of birth offspring were exposed to a parenting network composed of a mother and father, a mother and an adult older sister (recruited from the parents' first litter), or a mother alone in the BPC, MPA, and MON conditions, respectively. Female alloparents originated from each respective pair's first litter and were maintained with both parents (in lieu of weaning) for the duration of the pair's second litter. Pups were culled at PND1 to establish a maximum litter size of four pups, and when possible even sex ratios (2 female : 2 male). All individuals were weaned on PND20 and rehoused in same-sex sibling pairs; if a same-sex sibling was unavailable, another same-sex and similarly aged weanling (± 3 days) was recruited from the breeding colony as a cage mate. Between PND50 and PND62, one individual in each cage was behaviorally tested in the elevated plus maze (PND52–55), alloparental care testing (PND57–58), and partner preference testing (PND60–62) paradigms (results from which are presented in Rogers and Bales[20]), while the other was left behaviorally naïve. Results of the partner preference test are correlated here with neuroendocrine findings. PND60–62, the behaviorally tested individual was rehoused and paired with an opposite-sex, novel individual (designated to be their mate and partner in partner preference testing). For a two-day period between the range of PND60 and PND62, all paired individuals underwent partner preference testing (also further detailed in Rogers and Bales[20]) (Figure 1). At the time the behaviorally tested sibling was removed for pairing, the behaviorally naïve sibling was left in social isolation for a period of 48-hours before sacrifice. Socially isolated individuals were provided with cotton bedding to improve thermoregulation. All individuals were sacrificed either 48-hours after pairing with a mate or 48-hours after initiation of social isolation, at which time blood and brain tissue were collected.

Blood and Brain Collection

At the time of sacrifice, each individual was removed from their home cage, deeply anesthetized with isoflurane, and euthanized via cervical dislocation followed by a rapid decapitation. Following decapitation, the body was inverted over a conical funnel fitted with a microcentrifuge tube for the collection of trunk blood. The duration of time from original home cage disturbance to collection of blood was ≤ 5 minutes. Collected blood was then immediately centrifuged at 4°C for 12-minutes at 12,000 g. Plasma was then aliquoted from the supernatant and stored at −20°C until the time of the corticosterone assay. Contemporaneously, brain tissue was extracted, flash frozen on dry ice, and then stored at −80°C. All brains were subsequently sliced with a cryostat into 20μm coronal sections, mounted onto Super-frost plus slides, and stored at −80°C until use in autoradiography. Here, results of corticosterone concentration are analyzed and presented for both paired and socially isolated animals. Results of autoradiography are only analyzed and presented for paired animals.

Autoradiography and Selection of Regions of Interest

Receptor autoradiography for oxytocin receptor (OXTR) and arginine vasopressin receptor type 1a (AVPR1a) in paired animals was performed with methods validated in prairie voles[31] and outlined briefly here. Selected slides with brain tissue were removed from −80°C storage and brought up to room temperature. The mounted brain tissue was then lightly fixed with 0.1% buffered paraformaldehyde (pH 7.4) for 2 minutes, and then subsequently washed for 10 minutes, twice (i.e., cumulatively 20 minutes) in 50 mM Tris buffer (room temperature, pH 7.4). Slides designated for analysis of OXTR were then incubated for 1-hour in tracer buffer (50 mM Tris buffer + 10 mM MgCl, 0.1% BSA, pH 7.4) containing 50 pM of ^{125}I-Ornithine Vasotocin Analog (^{125}I-OVTA; Vasotocin, d(CH_2)$_5$[Tyr(Me)^2,Thr^4,Orn^8, [^{125}I]Tyr^9-NH_2]-[^{125}I]-OVTA; PerkinElmer). Slides designated for analysis of AVPR1a were incubated for 1-hour in tracer buffer containing 50 pM of ^{125}I-Linear Vasopressin 1a receptor antagonist (^{125}I-LVA; [125I]-Phenylacetyl-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Arg-Tyr-NH_2; PerkinElmer). Following incubation, slides were washed twice in 4°C 50 mM Tris base with 10 mM MgCl (pH 7.4) for 20 minutes total, washed again in room temperature 50 mM Tris buffer with MgCl for 30 minutes with agitation, briefly dipped in deionized water, and then left to air dry. The slides were then exposed to BioMax MR film (Kodak, Rochester, NY) for approximately 96 hours prior to development and analysis.

Regions of interest (ROI) for quantification were selected according to *a priori* hypotheses established in the relevant literature (as cited for each ROI). Of the many possible regions of interest with OXTR, we have decided to focus our attention on 10 regions which have been associated with partner preference formation: the prefrontal cortex (PFC)[8], nucleus accumbens (NAc)[8,11,15,32], caudate putamen (CP)[8], lateral septum (LS)[11], medial preoptic area (MPOA)[12], bed nucleus of the stria terminalis (BNST)[18,32], central amygdala (CeA)[11], insular cortex (IC)[12,15], ventromedial nucleus of the hypothalamus (VMH)[7,12], and the septohippocampal nucleus (SH)[15].

Of the many possible ROIs for AVPR1a, we have decided to focus our attention on eight regions which have been associated with pair-bond formation: LS[7,21], BNST[7], ventral pallidum (VP)[8,11,12,14], CeA[11], medial amygdala (MeA)[12], the mediodorsal thalamus (MDTh)[8], and VMH[7,12]. As done in previous studies[14], we also included the lateral dorsal thalamus (LDThal), which is not associated with the formation of pair bonds but does have noticeably dense binding of AVPR1a.

The quantification of autoradiographs was carried out using the MCID Digital Densitometry Core System (Interfocus Imaging, Cambridge, UK). A calibration curve was generated using microscale standards (American Radiolabeled Chemicals, Saint Louis, MO, USA) and subsequently utilized to extrapolate measurements of optical binding density (OBD; dpg/mg) from autoradiographs. Three representative measurements of OBD were made per ROI per individual and then averaged. Mean values were then normalized intra-individually by subtracting the average OBD associated with a region with non-specific binding (OXTR: primary somatosensory cortex; AVPR1a: Caudate Putamen). In total for OXTR, 28 brains from males and 35 brains from females across the three early life conditions were quantified. In total for AVPR1a, 27 brains from males and 35 brains from females across the three early life conditions were quantified. At times, some breeder pairs yielded all-female or all-male litters, leading to the discrepancy in the number of brains collected between male and female subjects (i.e., 28/27 and 35 brains, respectively). Slides from one male brain were damaged during the process of autoradiography for AVPR1a, therefore resulting in a reduction from 28 to 27 male brains between the assays for OXTR and AVPR1a. Respective to our examined outcomes, we presume all missing data are missing completely at random, i.e., not the result of systematic selection for removal.

Corticosterone Assay

Plasma corticosterone (CORT) was assayed for both paired and socially isolated animals using a radioimmunoassay (MP Biomedicals Corticosterone Double Antibody RIA Kit), which has been previously validated for use in prairie voles[33]. Non-extracted samples were diluted 1:2000 so that all values fell on the standard curve. All samples were run in one assay. Corticosterone concentrations were attained for 100 individuals. Two individuals were excluded: the exclusion rationale for the first individual was that there was no plasma to assay after bringing plasma samples to room temperature; the exclusion rationale for the second individual was that both test tubes associated with this individual were broken and samples subsequently lost during the quantification process. All samples were assayed contemporaneously in duplicate, and mean concentrations were calculated between the two samples associated with each respective individual. Two exceptions were made, with the concentration for two individuals being made according to singular read rather than in duplicate. The rationale for the first exception was that one of the two tubes broke during quantification. The rationale for the second exception was that one of the reads was outside of range, suggesting loss of sample during the assay. The intra-assay coefficient for the 98 samples run in duplicate was 2.17%.

Post-Hoc Behavioral Correlates

From work described in a preceding study[20], a number of behavioral measures were taken from the individuals and the parents of the individuals for whom autoradiographic analyses are presented here. A series of post-hoc, exploratory correlations were made between a selective subset of these behaviors and a subset of autoradiographic measures (OXTR and AVPR1a), i.e. those taken from ROIs for which significant differences in OBD were identified between individuals reared in different early life experiences (i.e., BPC, MON, MPA).

Behavioral measures were selected from partner preference testing and observations of parental care. Each behaviorally tested individual was tested twice in the partner preference paradigm: once after a 30-minute (females) or 60-minute (males) habituation; and again after 24-hours of habituation (both sexes). The partner preference test was carried out in a three-chamber apparatus (Figure 1c) in which the cohabitated partner and an opposite-sex stranger were leashed within two separate chambers connected to a third, "neutral" chamber. During each partner preference test, the test subject was initially placed into the neutral chamber and allowed to move freely between the three chambers and associate freely with both opposite-sex stimuli (i.e., the partner and stranger). From partner preference testing, two primary measures were selected: 1. Time (seconds) spent in contact with the partner; and 2. Ratio of time spent in the same cage as the partner vs. the stranger (calculated as the time in the partner cage divided by the sum of the time in both the partner and stranger cage). A similar ratio of contact preference was not used here, as the distributions of values were extremely biased with most cases equaling 1; this biased distribution was the result of a common behavioral pattern, in which no contact time was made with strangers, thus producing a value of 1 for any animal who demonstrated any contact time with a partner, regardless of how much time was actually spent in contact with the partner. With concern to developmental experience, maternal care, secondary care (i.e., paternal or alloparental), and cumulative care from all caregivers were obtained from and averaged across four, 20-minute focal samples of cumulative, direct-parental care demonstrated in the early post-natal period (PND1–3)[20,23]. These average parental care measures (seconds) were then used as representative measures of experienced *quantity* of parental care.

ROIs for post-hoc correlations were selected under the condition that a significant effect was found between early life experiences. For males, we ran post-hoc correlations between the selected behaviors and OXTR binding in the CeA, CP, and NAc. No correlations were run for AVPR1a binding in males. For females, we ran post-hoc correlations between the selected behaviors and OXTR binding in the CP, LS, MPOA, and NAc; and we ran post-hoc correlations between the selected behaviors and AVPR1a binding in the CeA and VMH.

Statistical Analyses

Before running any statistical analyses, we considered assumptions of normality and homogeneity of variance. Normality was assessed through visual inspection of Q-Q plots fitted with Q-Q lines, visual inspection of histograms, and the use of the Shapiro-Wilk normality test. Homogeneity of variance was assessed through the visual inspection of boxplots as well as the use of the Kruskal-Wallis rank sum test. For analyses in which

multiple individuals from the same litter were used, violations of independence were addressed through the inclusion of random effects for litter. Outliers were identified visually with boxplots in R and confirmed as values a distance of 1.5 times the inter-quartile range above the third quartile and/or below the first quartile (i.e., $Q_1 - 1.5\times IQR$ and/or $Q_3 + 1.5\times IQR$). Outliers were removed to maintain the assumption of normality, as reflected in variable DF in statistical reporting in Tables 1–4. Sample sizes were determined *a priori* and calibrated to find an effect in behavioral testing, results for which are reported in Rogers and Bales[20]; our sample sizes are comparable to those in previous, similar research[18].

One-way, between-subjects analysis of variance (ANOVA) was used to compare effects of early life experience (i.e., BPC vs. MON vs. MPA) on optical binding density. Only in cases where ANOVA yielded significant results ($p < .05$), post-hoc comparisons were completed from within the ANOVA with false discovery rate (FDR) correction. One ANOVA and subsequent post-hoc analyses (if applicable) were run per ROI per receptor type, and analyses by ANOVA were run in males and females separately. The process of pair-bond formation occurs at different rates in males and females[34] and implicates different brain regions[8]. Post-hoc exploratory correlates were run with Pearson's product-moment correlation. Linear mixed models were fit by restricted maximum likelihood (REML) to consider the effects of early life experience, experience of pairing vs. social isolation, and biological sex on circulating corticosterone levels at the time of sacrifice. The level of statistical significance for each test was set at $p = .05$. Analyses were completed in R Studio (version 1.3.959). Graphs were made in R using ggplot2 and in GraphPad Prism (version 8.4.3).

Results:

Quantitative differences in OXTR binding according to early life experience

OXTR by early life experience in males—^{125}I-OVTA binding differed significantly amongst males from different early life experiences (BPC, MON, and MPA) in the CeA, CP, and NAc; but no significant differences according to early life experience were identified in the BNST, IC, LS, MPOA, PFC, SH, or VMH (Table 1; Figure 2). In the CeA, ^{125}I-OVTA binding was significantly greater in BPC males than MON males ($t(24) = 2.83$, $p = .028$, $d = 1.52$), as was binding between BPC males and MPA males ($t(24) = 2.33$, $p = .043$, $d = 0.99$); but binding between MON and MPA males did not significantly differ ($t(24) = 0.99$, $p = .334$, $d = 0.53$). In the CP, ^{125}I-OVTA binding was significantly greater in BPC males than MPA males ($t(23) = 2.82$, $p = .029$, $d = 1.26$); but binding did not significantly differ between BPC and MON males ($t(23) = 1.45$, $p = .241$, $d = 0.75$), nor did it significantly differ between MON and MPA males ($t(23) = -0.99$, $p = .331$, $d = 0.51$). In the NAc, ^{125}I-OVTA binding was significantly greater in BPC males than MPA males ($t(23) = 3.27$, $p = .010$, $d = 1.47$); but binding did not significantly differ between BPC and MON males ($t(23) = 1.60$, $p = .184$, $d = 0.81$), nor did it significantly differ between MON and MPA males ($t(23) = -1.24$, $p = .227$, $d = 0.65$).

OXTR and post-hoc behavioral correlations in males—In regard to partner preference behavior in adulthood, ^{125}I-OVTA binding in the CeA was significantly

positively correlated with time spent in contact with a partner (r = 0.42, p = .037), and it was significantly positively correlated with preference to spend time in a cage with a partner over that with a stranger (r = 0.40, p = .045). ^{125}I-OVTA binding in the CP and NAc did not significantly correlate with time spent in contact with a partner nor was it significantly correlated with preference to spend time in a cage with a partner over that with a stranger (Table 5). Moreover, ^{125}I-OVTA binding in the CeA, CP, and NAc did not significantly correlate with the quantity of combined care received from mothers and other caregivers in the early prenatal period, nor did it significantly correlate with the quantity of care received from mothers or secondary in the same developmental period (Table 5).

OXTR by early life experience in females—^{125}I-OVTA binding differed significantly amongst females from different early life experiences (BPC, MON, and MPA) in the CP, LS, MPOA, and NAc; but no significant differences according to early life experience were identified in the BNST, CeA, IC, PFC, SH, or VMH (Table 2, Figure 3). In the CP, ^{125}I-OVTA binding was not significantly different between groups following FDR correction (Table 2). In the LS, ^{125}I-OVTA binding was significantly greater in BPC females than in MPA females (t(29) = 2.68, p = .018, d = 1.07), and binding was significantly greater in MON females than in MPA females (t(29) = −3.40, p = .006, d = 1.6), but binding between BPC and MON females did not significantly differ (t(29) = −1.10, p = .279, d = 0.53). In the MPOA, ^{125}I-OVTA binding was significantly greater in BPC females than in MPA females (t(31) = 2.93, p = .017, d = 1.13), and binding was significantly greater in MON females than in MPA females (t(31) = −2.69, p = .017, d = 1.28), but binding between BPC and MON females did not significantly differ (t(31) = −0.32, p = .754, d = 0.14). In the NAc, ^{125}I-OVTA binding was significantly greater in BPC females than in MPA females (t(28) = 2.85, p = .025, d = 1.16), but binding was not significantly different between MON females and MPA females (t(28) = −0.36, p = .720, d = 0.19), nor was binding significantly different between BPC and MON females (t(28) = 2.02, p = .080, d = 0.97).

OXTR and post-hoc behavioral correlations in females—^{125}I-OVTA binding in the LS significantly correlated with the quantity of care received from secondary caregivers (r = −0.35, p = .049) in the same developmental period. However, ^{125}I-OVTA binding in the CP, LS, MPOA, and NAc did not otherwise significantly correlate with the quantity of combined care received from mothers and other caregivers in the early prenatal period, the quantity of care received from mothers, or from secondary caregivers in the same developmental period; nor ^{125}I-OVTA binding in any of these regions correlate significantly with time spent in contact with a partner or preference to spend time in a cage with a partner over that with a stranger (Table 5).

Quantitative differences in AVPR1a binding according to early life experience

AVPR1a by early life experience in males—^{125}I-LVA binding did not significantly differ amongst males from different early life experiences (BPC, MON, and MPA) in any of the selected ROIs (i.e., BNST, CeA, LDTh, LS, MDTh, MeA, VMH, or VP) (Table 3, Figure 4). Accordingly, no post-hoc behavioral correlations were run.

AVPR1a by early life experience in females—^{125}I-LVA binding differed significantly amongst females from different early life experiences (BPC, MON, and MPA) in the CeA and VMH; but no significant differences according to early life experience were identified in the BNST, LDTh, LS, MDTh, MeA, or VP (Table 4, Figure 5). In the CeA, ^{125}I-LVA binding was significantly lower in BPC females than in MPA females ($t(31) = -2.31$, $p = .042$, $d = 0.89$), and binding was significantly lower in BPC females than in MON females ($t(31) = -3.16$, $p = .011$, $d = 1.46$), but binding between MON and MPA females did not significantly differ ($t(31) = -1.22$, $p = .232$, $d = 0.57$). In the VMH, ^{125}I-LVA binding was significantly greater in MPA females than in BPC females ($t(32) = -2.57$, $p = .045$, $d = 0.97$), but binding between MPA females and MON females did not significantly differ ($t(32) = 2.07$, $p = .070$, $d = 0.97$), nor did binding between BPC and MON females ($t(32) = -0.01$, $p = .994$, $d = 0.00$).

AVPR1a and post-hoc behavioral correlations in females—^{125}I-LVA binding in the CeA and VMH did not significantly correlate with the quantity of combined care received from mothers and other, nor did it significantly correlate with the quantity of care received from mothers or the quantity of care received from secondary caregivers in the same developmental period. In regard to partner preference behavior in adulthood, ^{125}I-LVA binding in the CeA and VMH was not significantly correlated with time spent in contact with a partner, nor was it significantly correlated with preference to spend time in a cage with a partner over that with a stranger (Table 5).

Quantitative differences in circulating corticosterone to early life experience, acute pre-sacrifice experience, and biological sex

Sampled individuals were distributed such that there were 57 females and 43 males; and there were 42 individuals in the BPC condition, 37 individuals in the MPA condition, and 21 individuals in the MON condition. In the 48-hours prior to sacrifice, 59 individuals were paired with a mate, while 41 individuals were left in social isolation.

A mixed-effects model was run to determine if basal corticosterone concentration differed according to the fixed effects of sex, condition, or life experience in the 48-hours prior to sacrifice. A random effect of litter was included to control for shared variance between siblings. There was a significant effect of life experience in the 48-hours prior to sacrifice on corticosterone concentration, such that individuals who lived with a mate of the opposite-sex showed significantly lower corticosterone concentrations than those living in social isolation ($b = -459.30$, CI95% = [−819.12. −59.73], $SE = 212.86$, $t(87) = -2.16$, $p = .034$) (Figure 6). There were no main effects for early life experience ($p = .696$) or sex ($p = .886$).

Discussion:

The current study examined whether or not paternal absence in early life development, with or without alloparental substitution, generated significant differences in distributions of OXTR and AVPR1a in regions of the brain associated with partner preference formation. We also sought to tie any such neural receptor binding differences to behavioral phenotypes of monogamy. Moreover, we considered if individuals reared in conditions of paternal absence, with or without alloparental substitution, demonstrated a phenotype of differential

susceptibility to social isolation, as explored through basal corticosterone. This study builds upon a line of work explored by our research team[20], other researchers exploring paternal absence in prairie voles[18,19,21,35], and those studying paternal absence in other biparental species[17,36–39].

OXTR, Variation in Early Life Experience, and Pair-Bond Formation

In male subjects, we identified significant group differences in OXTR distributions in the CeA, CP, and NAc. Specifically, MPA males demonstrated significantly less OXTR binding than biparental controls in these three ROIs; MON males also demonstrated significantly less OXTR binding than biparental controls in the CeA. This result stands in contrast to previous work, which also examined OXTR in the CeA of BPC and MON males and found no differences[18]. This difference could be due to experience prior to sacrifice; i.e., whereas Ahern and Young[18] examined sexually naïve animals, we examined animals 48-hours after pairing. Thus, it is possible that the process of pair bonding (or not pair bonding, in the case of MON animals and MPA males) yields our observed effect that is not otherwise present prior to pair bonding. Like Ahern and Young[18], we also found no differences in OXTR binding in the BNST, LS, and MPOA of male subjects. In a previous study[20], we identified that male MON and MPA offspring exhibited inhibited partner preference formation. Intriguingly, both MON and MPA males also show less binding of OXTR in the CeA, which we also found was positively correlated with time spent in contact with a partner as well as preference for proximity to a partner in the partner preference test. Given that this research design did not directly test a causal role of reduced OXTR in the CeA in inhibited pair-bond formation, a future study would need to utilize a more direct manipulation of OXTR in the CeA to determine causality. Hypothetically, the increased availability of OXTR in the CeA of biparentally reared males may reduce anxiety-like behavior[40], and it is possible that with reduced OXTR in the CeA, MON and MPA males have reduced ability for socio-affective inference[41], which could be important for pair-bond formation. Increased OXTR in the CP and NAc is associated with monogamous behavior[8,11,15,32], therefore there is more prior evidence to support that reduced OXTR in the CP and NAc of MPA males may, in part, explain the inhibition of partner preference formation amongst MPA males. Notably, MON males, which also have inhibited partner preference formation, do show a lower average level of OXTR binding in the CP and NAc, but not significantly so; although, it is possible that with a larger sample size in the MON group, this effect would have become evident. Alternatively, particularly reduced OXTR in the CP and NAc could in fact be unique to the MPA condition and could be the result of an interaction of paternal absence and high-contact conditions, as MPA animals experienced the highest levels of care in the pre-weaning period[42].

In female subjects, we identified significant group differences in OXTR distributions in the LS, MPOA, and NAc. In all three ROIs, MPA females had lower binding density of OXTR than biparental controls. In the LS and MPOA, MPA females also had lower binding density of OXTR than MON females. We found no significant differences in OXTR binding between MON and BPC females, which is consistent with previous research[18]. Findings from Rogers and Bales[20] suggest, however, that MPA females *do* form partner preferences whereas MON females *do not*. Therefore, it is unclear how these observed

differences in binding densities between BPC/MON and MPA females might be reflected in affective, behavioral, and/or cognitive outcomes beyond those observed in the present context. Notably, Rogers and Bales[20] identified that MPA females did receive significantly more care than their conspecifics; however, we found no significant correlations between parental investment (at the maternal, paternal/alloparental, or combined effort levels) and OXTR binding, with one exception being a marginally significant ($p = .049$) negative correlation between paternal/alloparental care and OXTR binding in the LS, which may be driven by the increased quantity of care received by MPA females from their older sisters[20].

AVPR1a, Variation in Early Life Experience, and Pair-Bond Formation

In male subjects, we identified no significant group differences in AVPR1a distributions in any of the eight ROIs considered, which is consistent with previous research[18]. In female subjects, we identified differences in densities of AVPR1a binding in the CeA and VMH. Specifically, MPA and MON females had significantly more binding of AVPR1a in the CeA than biparental controls; and MPA females had significantly more binding of AVPR1a in the VMH than biparental controls. In regard to increased AVPR1a binding in the CeA amongst MPA and MON females, elevated AVPR1a binding in the amygdala has been reported in high contact prairie vole offspring which experienced prenatal stress and were subsequently cross-fostered into the care of high contact parents[29]; although for reasons discussed in the following section, it is unlikely that paternal absence represents a salient stressor to pups. Moreover, given that MPA females *do* form partner preferences whereas MON females *do not*, it remains unclear what the similarly elevated levels of AVPR1a binding in the CeA could imply for behavioral outcomes related to pair bonding. Similarly, given the similarity in AVPR1a binding in the VMH of BPC and MON individuals, yet dissimilarity in their pair bonding behaviors[20], it is unclear what might be represented by an increase in AVPR1a binding in the VMH of MPA females.

Corticosterone and Adult Social Experience

Given that social isolation represents a significant stressor for social species, it is perhaps unsurprising that we identified increased levels of circulating corticosterone in isolated individuals compared to newly paired individuals. In contrast to the adverse conditions of social isolation, exposure to a novel, opposite-sex conspecific can reduce levels of corticosterone in prairie voles[43]. However, we found no significant differences in circulating corticosterone with regard to early life experience or biological sex. Previous work has demonstrated that prairie vole offspring reared by high-contact parents are differentially susceptible to social isolation when compared to low-contact offspring. In other stress paradigms, prairie vole offspring exposed to prenatal stress who were then cross-fostered into the care of a low-contact parenting dyad showed more anxiety-like behavior and increased levels of circulating corticosterone[29].

Because pups reared under MPA conditions experienced significantly increased care and pups reared under MON conditions experienced significantly lower levels of care[20], we might have expected to see a replication of effects arising from high-contact and low-contact parental care, respectively. Yet we found neither altered anxiety-like behavior[20] nor increased circulating corticosterone in either the MPA or MON group. Several studies across

three rodent models of biparental care (i.e., prairie voles, mandarin voles, and California mice) have also shown little to no effect of paternal absence on anxiety-like behavior in adulthood (Table 6). Others fail to find differences in corticosterone concentration in MON vs BPC prairie voles[18], and variation in the neuroendocrine substrates of the HPA-axis are either null or suggest a more resilient phenotype in MON prairie voles[18,35]. Similarly, paternal absence does not result in increased serum corticosterone in adult California mice[44]. Early in development (i.e., PND8–14), mandarin vole pups show elevated levels of serum corticosterone and ACTH[45]; however, findings from Jia et al.[46] show that MON mandarin vole pups are attended to less that BPC pups (as also seen in prairie voles[19,27,47]), therefore it remains unclear if the elevated CORT and ACTH are indicators of stress or metabolic processes related to thermoregulation. In adulthood, serum CORT and ACTH are elevated in female MON mandarin voles, but not in males[48]. Both male and female mandarin voles show decreases in hippocampal glucocorticoid receptor and brain-derived neurotrophic factor, while only females show similar declines in the dentate gyrus[48].

In light of the behavioral findings summarized in Table 6, it is unclear if these differences translate into increased experience of stress and/or anxiety. It is less likely that the stress associated with mate loss in late gestation is equivalent to other forms of prenatal stress that would hypothetically otherwise leave offspring differentially susceptible to stressors like social isolation. Moreover, while it is possible for pups to experience stress contagion via stressed mothers, it may be that stress experienced by mothers following the loss of a mate is insufficiently salient to alter the stress physiology of her offspring. Thus, it may generally be unsuitable to use the paternal absence paradigm as an alternative to other models of prenatal or early life stress with well demonstrated effects[29,49,50]. This is not to say that in some contexts paternal absence is not an adverse or disadvantageous experience (e.g., survival rate[17]). Moreover, this does not make the paternal absence paradigm less interesting. The paternal absence paradigm consistently drives variation in social behavior in ways not demonstrated in stress paradigms, but rather more akin to the variation due to environmental change (e.g., see Roberts et al.[51]).Thus, one might hypothesize that in the natural habitat paternal absence acts as an indicator of broader environmental circumstances (e.g., predation, population density, resource scarcity, etc.) and drives variation in social behavior accordingly. This distinction also provides an opportunity to combine conditions of paternal absence with more salient stress paradigms in unique combinations that might be interactive rather than additive (for example see Agarwal et al.[44]).

Conclusions and Future Directions

We identified a number of differences between early life experiences in the distributions of OXTR and AVPR1a, some of which correlate with behaviors associated with partner preference formation (i.e., OXTR distribution in the CeA of males correlates with time spend in physical contact with a partner and preference for proximity to a partner). However, many of these differences do not appear to correlate with partner preference behaviors. Moreover, our results demonstrate that while MPA animals have not experienced a deficit in the quantity of care they received in the preweaning period, they nevertheless have divergent patterns of OXTR and AVPR1a. This may further support a hypothesis that the care of fathers carries a unique set of qualities important for typical offspring development

in biparental, socially monogamous species. It remains unclear what, if any, affective or behavioral differences may result from these neuroendocrine differences. Nevertheless, the collection of affective and behavioral assays thus far applied within this paradigm is far from exhaustive, and the application of new assays within this paradigm may yet reveal significant affective and behavioral differences which are driven by variation in oxytocin and vasopressin receptor densities. Moreover, it should be reiterated that a growing number of studies of paternal absence in prairie voles (with and without alloparental substitution) demonstrate consistent behavioral consequences (e.g., inhibition of partner preference formation). In the absence of a clear connection between these behavioral consequences and OXTR and AVPR1a receptor densities, there are opportunities for new routes of investigation, particularly as novel methods are becoming available for use in prairie voles and other non-traditional laboratory species. Notably, while many of our neuroendocrine findings in comparisons between BPC and MON offspring replicate those of previous findings[18], our novel findings, drawn from comparisons of MPA females to their BPC and MON conspecifics, help narrow potential routes of investigation and illustrate new ways in which variations in early life social structure may influence offspring neuroendocrine development. New evidence suggests that BPC males experience an increase in dopamine turnover in the NAc above that in MON males[47]. Given that both the CeA and NAc were repeatedly implicated in our findings, and given pair-bond formation requires the integration of social memory and reward[7], we suggest that future directions should include a deeper exploration of the mesolimbic pathway, including a deeper assessment of dopaminergic projections to the CeA and NAc, as well as additional structures throughout the hippocampus. Alternatively, it may be useful to consider a broader approach (e.g., RNA-sequencing) to generate new targets of interest. In any of these routes of investigation, inclusion of our novel MPA condition improves study design and should further elucidate the mechanisms that drive the developmental consequences of paternal absence in biparental species.

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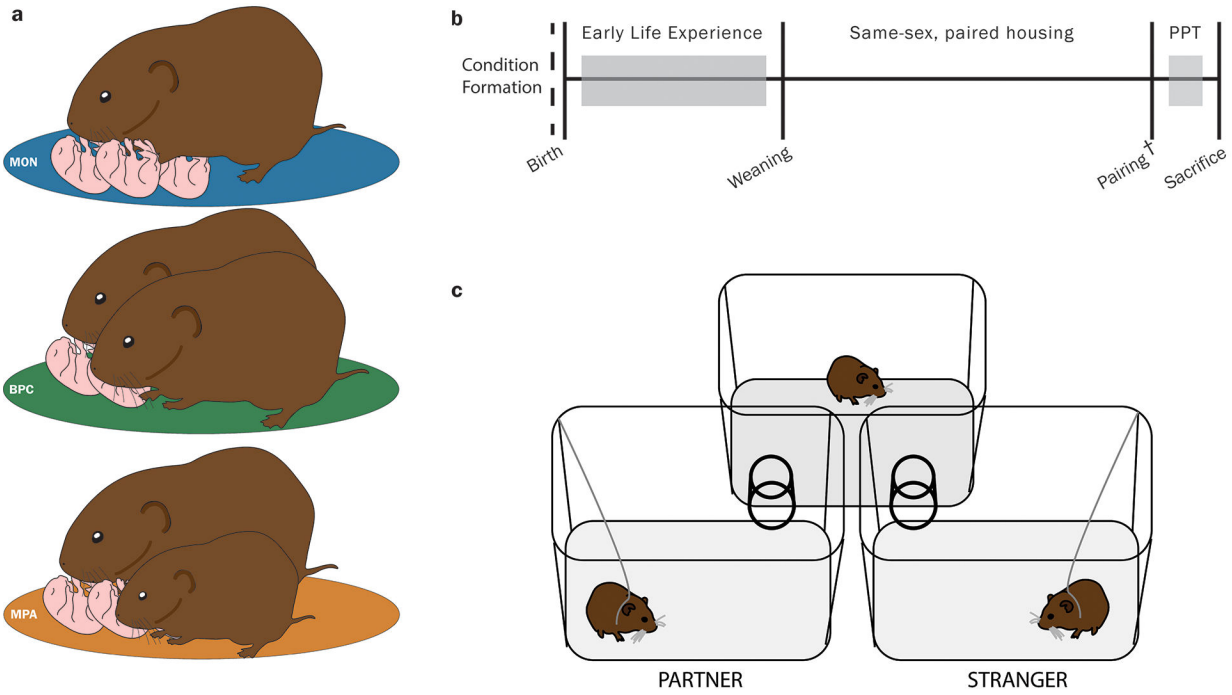

Figure 1: Study Design.

(**a**) Subjects were reared under three possible conditions biparental care (BPC), monoparental care (MON), and maternal-plus-alloparental care (MPA). (**b**) These three variants in early life experience were established with the removal of the father and/or experienced alloparent (maintained from litter 1) on PND20 of the preceding second litter, i.e. in late gestation just prior to parturition of the litter of interest. Conditions were maintained between birth and weaning (PND21), at which point individuals were re-housed with a same-sex cage mate. Subjects were paired with an opposite-sex partner on PND60/61 and tested over a two-day period in the partner preference test, which was followed by sacrifice (48-hours post-pairing) and tissue collection. (**c**) Partner preference testing was completed in an apparatus composed of three shoebox cages connected with tubes, with one "neutral cage" connected to a cage housing a designated partner and another cage housing a stranger. † Some individuals (i.e., same-sex, sibling cage mates not used in behavioral testing) were left in social isolation prior to sacrifice, in lieu of experiencing pairing and undergoing partner preference testing.

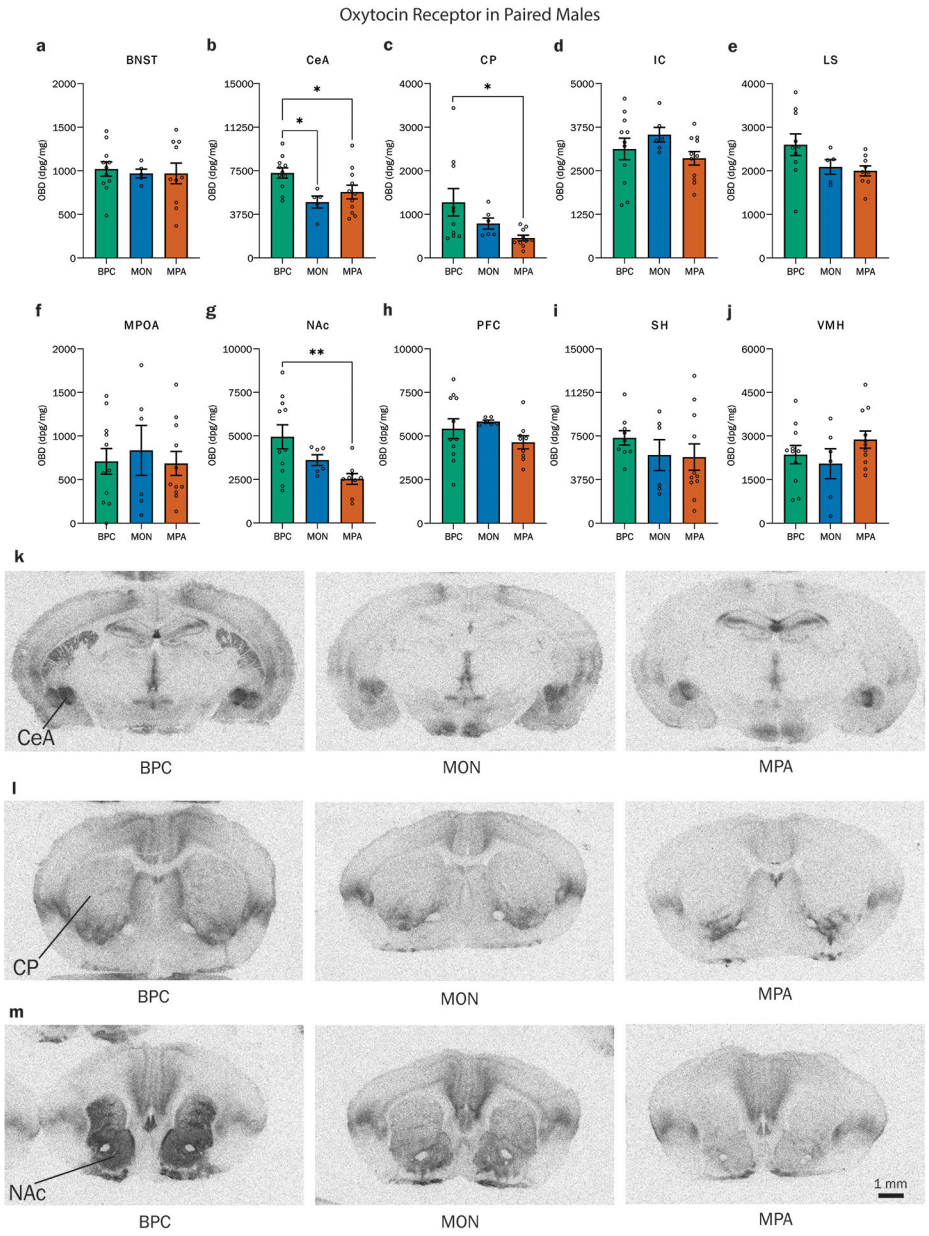

Figure 2: Oxytocin receptor binding in paired males.

OXTR binding was quantified in paired males reared under biparental care (BPC), monoparental care (MON), and maternal-plus-alloparental care (MPA) conditions in 10 regions of interest: (**a**) BNST, (**b**) CeA, (**c**) CP, (**d**) IC, (**e**) LS, (**f**) MPOA, (**g**) NAc, (**h**) PFC, (**i**) SH, and (**j**) VMH. Bar graphs depict mean and standard error of the mean. Significant differences were identified according to early life experience in the CeA, CP, and NAc, as illustrated by representative film autoradiographs in (**k**), (**l**), and (**m**), respectively. ROI Abbreviations: bed nucleus of the stria terminalis (BNST); central amygdala (CeA); caudate putamen (CP); insular cortex (IC); lateral septum (LS); medial preoptic area (MPOA); nucleus accumbens (NAc); prefrontal cortex (PFC); septohippocampal nucleus (SH); and ventromedial nucleus of the hypothalamus (VMH).

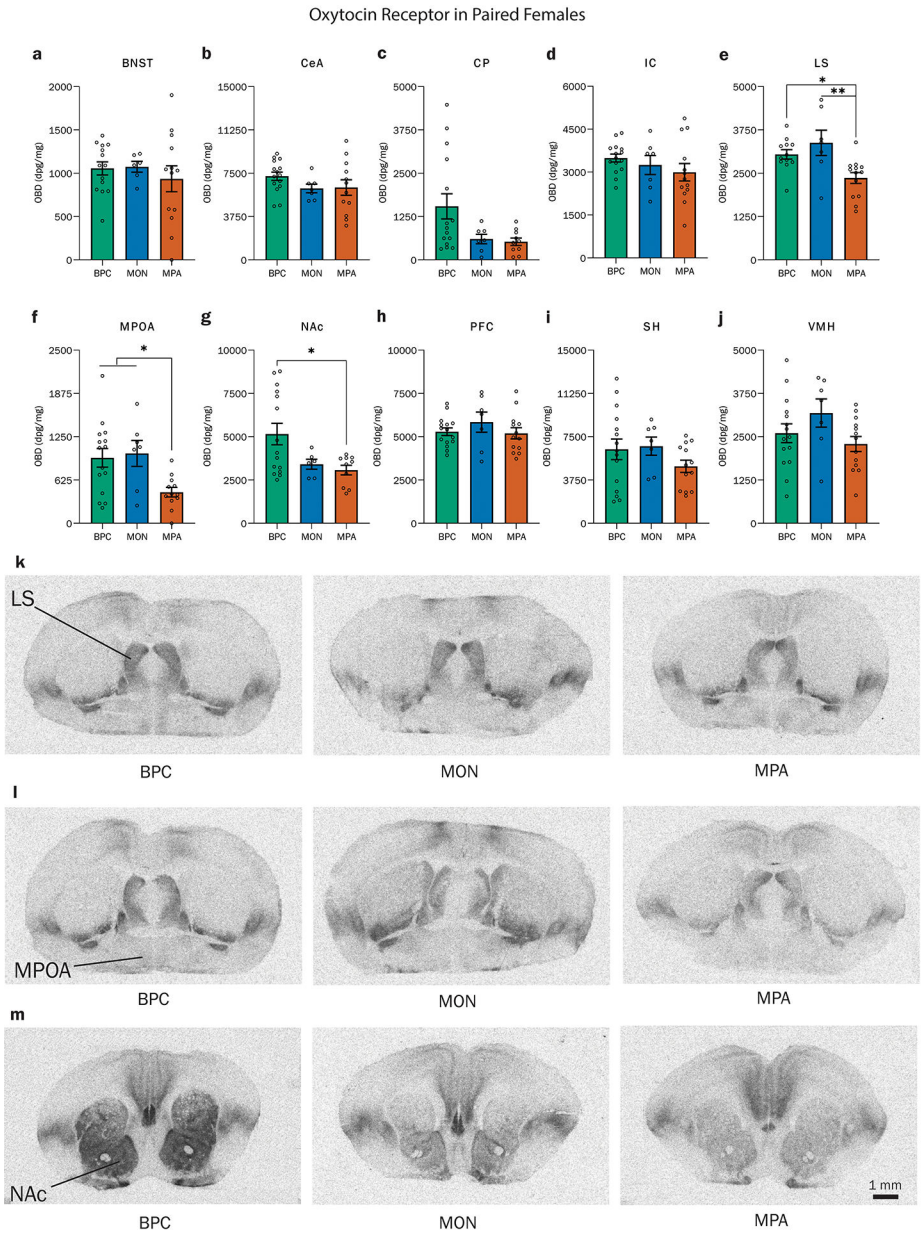

Figure 3: Oxytocin receptor binding in paired females.

OXTR binding was quantified in paired females reared under biparental care (BPC), monoparental care (MON), and maternal-plus-alloparental care (MPA) conditions in 10 regions of interest: (**a**) BNST, (**b**) CeA, (**c**) CP, (**d**) IC, (**e**) LS, (**f**) MPOA, (**g**) NAc, (**h**) PFC, (**i**) SH, and (**j**) VMH. Bar graphs depict mean and standard error of the mean. Significant differences were identified according to early life experience in the LS, MPOA, and NAc, as illustrated by representative film autoradiographs in (**k**), (**l**), and (**m**), respectively. ROI Abbreviations: bed nucleus of the stria terminalis (BNST); central amygdala (CeA); caudate putamen (CP); insular cortex (IC); lateral septum (LS); medial preoptic area (MPOA); nucleus accumbens (NAc); prefrontal cortex (PFC); septohippocampal nucleus (SH); and ventromedial nucleus of the hypothalamus (VMH).

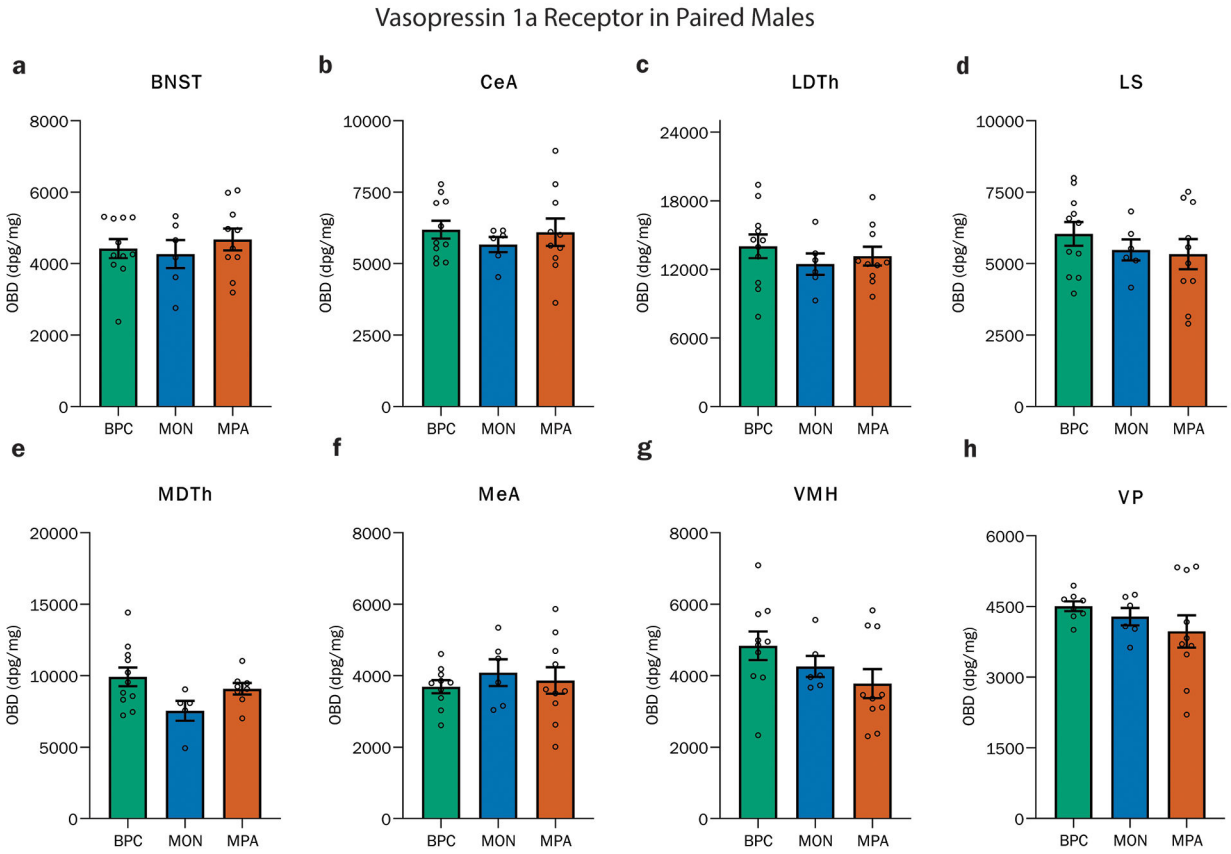

Figure 4: Vasopressin 1a receptor binding in paired males.

AVPR1a binding was quantified in paired males reared under biparental care (BPC), monoparental care (MON), and maternal-plus-alloparental care (MPA) conditions in 8 regions of interest: (**a**) BNST, (**b**) CeA, (**c**) LDTh, (**d**) LS, (**e**) MDTh, (**f**) MeA, (**g**) VMH, and (**h**) VP. Bar graphs depict mean and standard error of the mean. Significant differences in AVPR1a binding were not identified according to early life experience. ROI Abbreviations: bed nucleus of the stria terminalis (BNST); central amygdala (CeA); lateral dorsal thalamus (LDTh); lateral septum (LS); mediodorsal thalamus (MDTh); medial amygdala (MeA); ventromedial nucleus of the hypothalamus (VMH); and ventral pallidum (VP).

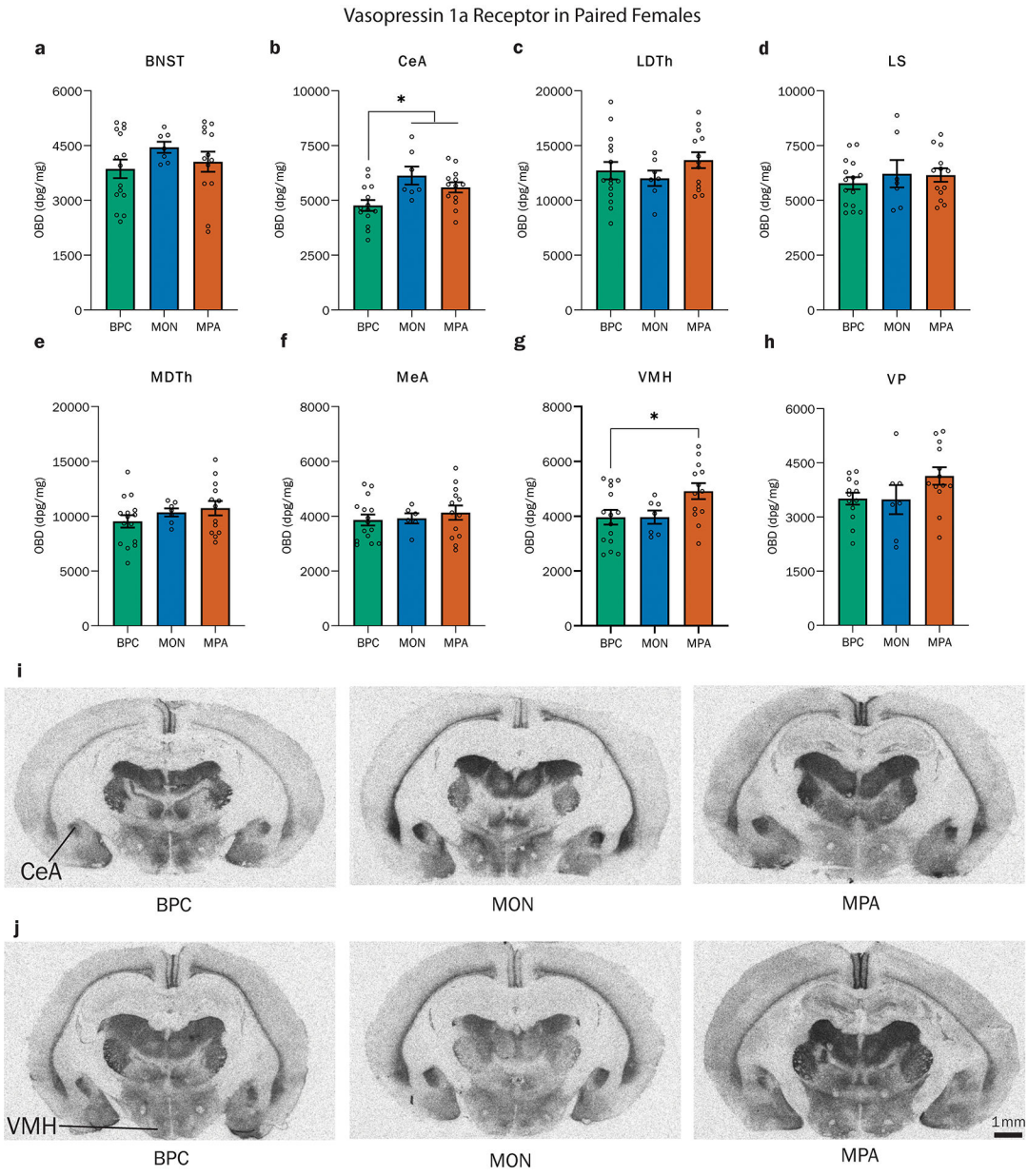

Figure 5: Vasopressin 1a receptor binding in paired females.

AVPR1a binding was quantified in paired females reared under biparental care (BPC), monoparental care (MON), and maternal-plus-alloparental care (MPA) conditions in 8 regions of interest: (**a**) BNST, (**b**) CeA, (**c**) LDTh, (**d**) LS, (**e**) MDTh, (**f**) MeA, (**g**) VMH, and (**h**) VP. Bar graphs depict mean and standard error of the mean. Significant differences were identified according to early life experience in the CeA and VMH, as illustrated by representative film autoradiographs in (**i**) and (**j**), respectively. ROI Abbreviations: bed nucleus of the stria terminalis (BNST); central amygdala (CeA); lateral dorsal thalamus (LDTh); lateral septum (LS); mediodorsal thalamus (MDTh); medial amygdala (MeA); ventromedial nucleus of the hypothalamus (VMH); and ventral pallidum (VP).

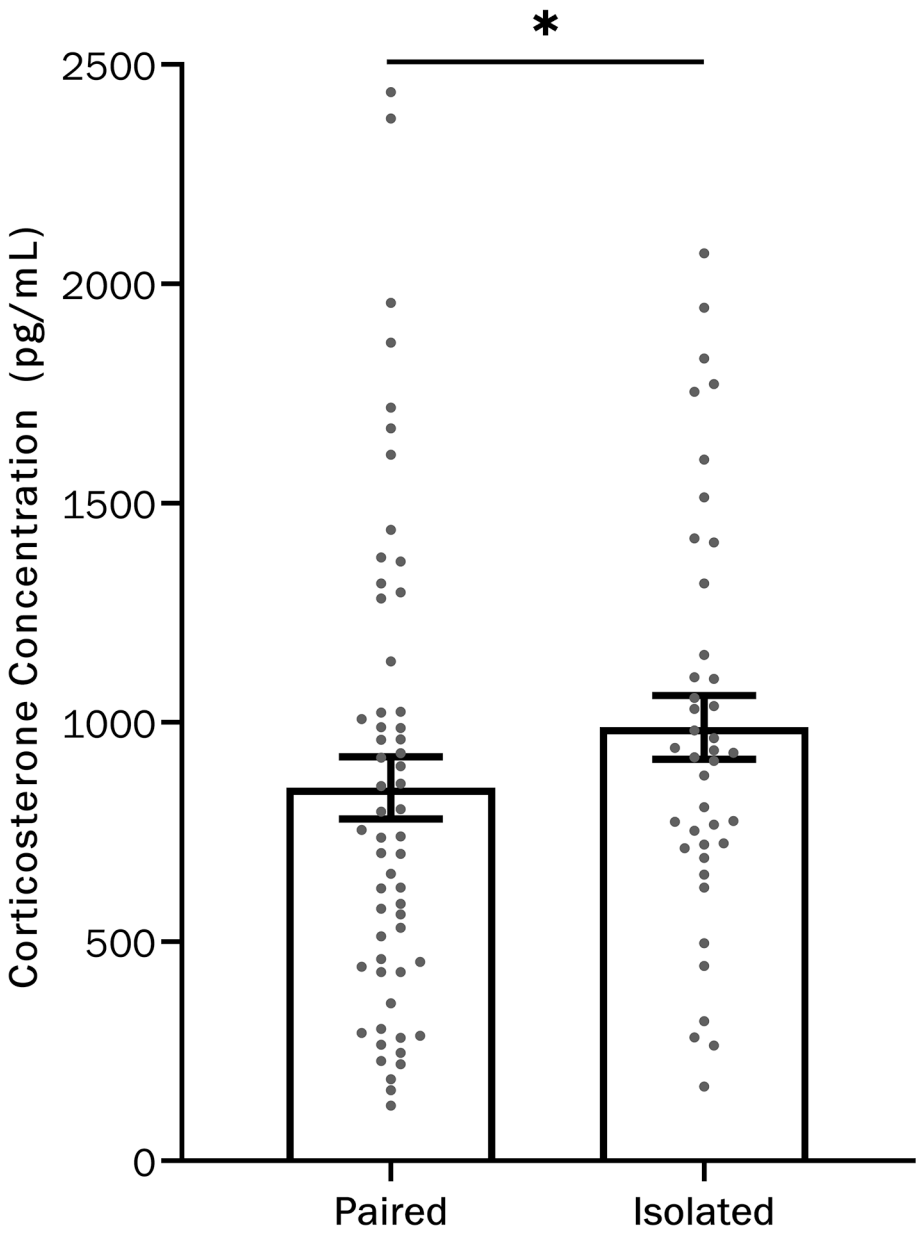

Figure 6: Corticosterone at sacrifice following a 48-hour acute pre-sacrifice experience.
Basal corticosterone concentrations were measured in samples taken from individuals after 48-hours of pairing with an opposite-sex partner, or alternatively after 48-hours of social isolation. Paired individuals, regardless of sex or early life condition, had significantly lower concentrations of circulating corticosterone than socially isolated individuals ($p = .034$). Bar graphs depict mean and standard error of the mean.

Table 1:

Test Statistics from OXTR Autoradiography in Paired Males.

For each region of interest (ROI), marginal means (M) and 95% confidence interval (CI.95) for optical density (dpg/mg) are presented for groups of paired individuals reared under biparental (BPC), monoparental (MON), or maternal-plus-alloparental (MPA). One-way ANOVA test statistics are reported for all tests with partial eta-squared (η_P^2). Post-hoc comparisons between groups (FDR adjusted p-values and Cohen's D) are presented only for those with significant effects in the omnibus test. ROI Abbreviations: bed nucleus of the stria terminalis (BNST); central amygdala (CeA); caudate putamen (CP); insular cortex (IC); lateral septum (LS); medial preoptic area (MPOA); nucleus accumbens (NAc); prefrontal cortex (PFC); septohippocampal nucleus (SH); and ventromedial nucleus of the hypothalamus (VMH).

	BPC	MON	MPA	One-Way ANOVA Test Statistics				BPC-MON		BPC-MPA		MON-MPA	
ROI	**M [CI.95]**	**M [CI.95]**	**M [CI.95]**	**F**	**DF**	**p**	η_P^2	**adj. p**	**|d|**	**adj. p**	**|d|**	**adj. p**	**|d|**
BNST	1020 [835, 1206]	969 [694, 1244]	970 [775, 1165]	0.09	2, 23	.913	.01	--	--	--	--	--	--
CeA	7300 [6278, 8321]	4798 [3282, 6313]	5671 [4649, 6692]	4.86	2, 24	.017	.29	**.028**	**1.52**	**.043**	**0.99**	.334	0.53
CP	1276 [850, 1703]	788 [237, 1338]	454 [28, 880]	4.01	2, 23	.032	.26	.241	0.75	**.029**	**1.26**	.331	0.51
IC	3123 [2627, 3620]	3533 [2861, 4206]	2856 [2359, 3353]	1.40	2, 25	.266	.10	--	--	--	--	--	--
LS	2598 [2217, 2979]	2088 [1549, 2628]	1998 [1596, 2400]	2.84	2, 21	.081	.21	--	--	--	--	--	--
MPOA	709 [382, 1035]	834 [392, 1276]	686 [359, 1012]	0.16	2, 25	.849	.01	--	--	--	--	--	--
NAc	4939 [3916, 5962]	3605 [2219, 4990]	2531 [1399, 3662]	5.39	2, 23	.012	.32	.184	0.81	**.010**	**1.47**	.227	0.65
PFC	5414 [4473, 6355]	5823 [4549, 7097]	4167 [3227, 5108]	2.96	2, 25	.070	.19	--	--	--	--	--	--
SH	7333 [5191, 9475]	5856 [3232, 8479]	5687 [3750, 7625]	0.77	2, 23	.473	.06	--	--	--	--	--	--
VMH	2358 [1698, 3018]	2046 [1153, 2940]	2875 [2215, 3536]	1.33	2, 25	.282	.10	--	--	--	--	--	--

Table 2:

Test Statistics from OXTR Autoradiography in Paired Females.

For each region of interest (ROI), marginal means (M) and 95% confidence interval (CI.95) for optical density (dpg/mg) are presented for groups of paired individuals reared under biparental (BPC), monoparental (MON), or maternal-plus-alloparental (MPA). One-way ANOVA test statistics are reported for all tests with partial eta-squared (η_P^2). Post-hoc comparisons between groups (FDR adjusted p-values and Cohen's D) are presented only for those with significant effects in the omnibus test. ROI Abbreviations: bed nucleus of the stria terminalis (BNST); central amygdala (CeA); caudate putamen (CP); insular cortex (IC); lateral septum (LS); medial preoptic area (MPOA); nucleus accumbens (NAc); prefrontal cortex (PFC); septohippocampal nucleus (SH); and ventromedial nucleus of the hypothalamus (VMH).

	BPC	MON	MPA	One-Way ANOVA Test Statistics				BPC-MON		BPC-MPA		MON-MPA	
ROI	**M [CI.95]**	**M [CI.95]**	**M [CI.95]**	**F**	**DF**	**p**	η_P^2	**adj. p**	**|d|**	**adj. p**	**|d|**	**adj. p**	**|d|**
BNST	1056 [841, 1270]	1074 [746, 1401]	936 [713, 1158]	0.41	2, 30	.671	.03	--	--	--	--	--	--
CeA	7241 [6329, 8152]	6184 [4850, 7518]	6264 [5245, 7282]	1.42	2, 31	.257	.08	--	--	--	--	--	--
CP	1546 [1014, 2079]	603 [−177, 1382]	523 [−129, 1176]	3.86	2, 29	.033	.21	.075	0.95	.057	1.06	.874	0.11
IC	3489 [3043, 3934]	3247 [2595, 3899]	2993 [2514, 3471]	1.19	2, 32	.316	.07	--	--	--	--	--	--
LS	3043 [2667, 3419]	3378 [2885, 3870]	2361 [2000, 2723]	6.78	2, 29	.004	.32	.279	0.53	**.018**	**1.07**	**.006**	**1.6**
MPOA	945 [714, 1175]	1008 [671, 1345]	449 [192, 707]	5.45	2, 31	.009	.26	.754	0.14	**.017**	**1.13**	**.017**	**1.28**
NAc	5150 [4205, 6095]	3409 [1915, 4903]	3075 [1918, 4233]	4.68	2, 28	.018	.25	.080	0.97	**.025**	**1.16**	.720	0.19
PFC	5284 [4689, 5879]	5836 [4965, 6708]	5194 [4554, 3833]	0.79	2, 32	.461	.05	--	--	--	--	--	--
SH	6407 [4978, 7836]	6673 [4581, 8764]	4934 [3399, 6469]	1.37	2, 32	.270	.08	--	--	--	--	--	--
VMH	2599 [2093, 3106]	3181 [2439, 3922]	2289 [1745, 2833]	1.95	2, 32	.159	.11	--	--	--	--	--	--

Table 3:

Test Statistics from AVPR1a Autoradiography in Paired Males.

For each region of interest (ROI), marginal means (M) and 95% confidence interval (CI.95) for optical density (dpg/mg) are presented for groups of paired individuals reared under biparental (BPC), monoparental (MON), or maternal-plus-alloparental (MPA). One-way ANOVA test statistics are reported for all tests with partial eta-squared (η_P^2). Post-hoc comparisons between groups (FDR adjusted p-values and Cohen's D) are presented only for those with significant effects in the omnibus test. ROI Abbreviations: bed nucleus of the stria terminalis (BNST); central amygdala (CeA); lateral dorsal thalamus (LDTh); lateral septum (LS); mediodorsal thalamus (MDTh); medial amygdala (MeA); ventromedial nucleus of the hypothalamus (VMH); and ventral pallidum (VP).

	BPC	MON	MPA	One-Way ANOVA Test Statistics				BPC-MON		BPC-MPA		MON-MPA	
ROI	M [CI.95]	M [CI.95]	M [CI.95]	F	DF	p	η_P^2	adj. p	|d|	adj. p	|d|	adj. p	|d|
BNST	4421 [3840, 5003]	4265 [3478, 5052]	4676 [4066, 5286]	0.40	2, 24	.674	.03	--	--	--	--	--	--
CeA	6187 [5450, 6923]	5664 [4667, 6661]	6092 [5319, 6864]	0.40	2, 24	.677	.03	--	--	--	--	--	--
LDTh	14010 [12197, 15823]	12452 [9997, 14906]	13144 [11243, 15046]	0.59	2, 24	.561	.05	--	--	--	--	--	--
LS	6038 [5159, 6917]	5477 [4286, 6668]	5330 [4407, 6252]	0.72	2, 24	.499	.06	--	--	--	--	--	--
MDTh	9924 [8809, 11039]	7548 [5894, 9202]	9089 [7781, 10396]	3.08	2, 21	.067	.23	--	--	--	--	--	--
MeA	3692 [3091, 4293]	4086 [3310, 4862]	3866 [3265, 4467]	0.35	2, 23	.711	.03	--	--	--	--	--	--
VMH	4837 [4064, 5611]	4257 [3259, 5256]	3780 [3007, 4553]	2.00	2, 23	.158	.15	--	--	--	--	--	--
VP	4506 [3945, 5066]	4285 [3638, 4932]	3972 [3471, 4473]	1.11	2, 21	.348	.10	--	--	--	--	--	--

Table 4:

Test Statistics from AVPR1a Autoradiography in Paired Females.

For each region of interest (ROI), marginal means (M) and 95% confidence interval (CI.95) for optical density (dpg/mg) are presented for groups of paired individuals reared under biparental (BPC), monoparental (MON), or maternal-plus-alloparental (MPA). One-way ANOVA test statistics are reported for all tests with partial eta-squared (η_P^2). Post-hoc comparisons between groups (FDR adjusted p-values and Cohen's D) are presented only for those with significant effects in the omnibus test. ROI Abbreviations: bed nucleus of the stria terminalis (BNST); central amygdala (CeA); lateral dorsal thalamus (LDTh); lateral septum (LS); mediodorsal thalamus (MDTh); medial amygdala (MeA); ventromedial nucleus of the hypothalamus (VMH); and ventral pallidum (VP).

	BPC	MON	MPA	One-Way ANOVA Test Statistics				BPC-MON		BPC-MPA		MON-MPA	
ROI	**M [CI.95]**	**M [CI.95]**	**M [CI.95]**	**F**	**DF**	**p**	η_P^2	**adj. p**	**|d|**	**adj. p**	**|d|**	**adj. p**	**|d|**
BNST	3862 [3383, 4342]	4455 [3753, 5157]	4057 [3542, 4573]	1.01	2, 32	.376	.06	--	--	--	--	--	--
CeA	4767 [4260, 5274]	6126 [5409, 6843]	5594 [5068, 6120]	5.63	2, 31	.008	.27	**.011**	**1.46**	**.042**	**0.89**	.232	0.57
LDTh	12714 [11280, 14149]	12011 [9911, 14111]	13670 [12129, 15211]	0.92	2, 32	.408	.06	--	--	--	--	--	--
LS	5781 [5138, 6423]	6213 [5273, 7154]	6152 [5461, 6842]	0.45	2, 32	.643	.03	--	--	--	--	--	--
MDTh	9535 [8430, 10640]	10352 [8734, 11970]	10740 [9553, 11927]	1.19	2, 32	.318	.07	--	--	--	--	--	--
MeA	3863 [3441, 4285]	3923 [3255, 4590]	4132 [3679, 4586]	0.41	2, 31	.886	.03	--	--	--	--	--	--
VMH	3964 [3450, 4478]	3968 [3215, 4720]	4916 [4363, 5468]	3.86	2, 32	.032	.19	.994	0.00	**.045**	**0.97**	.070	0.97
VP	3508 [3043, 3974]	3484 [2850, 4118]	4134 [3669, 4599]	2.35	2, 30	.113	.14	--	--	--	--	--	--

Table 5:

Post-hoc Behavioral Correlations.

For regions of interest with significant group differences in OXTR and AVPR1a autoradiography, post-hoc behavioral correlations were run. Behavioral measures were selected from partner preference testing and observations of parental care, of which methods and major findings are summarized briefly in the preceding introduction and more thoroughly in Rogers and Bales[20]. Within each applicable receptor-sex-ROI nesting, Pearson correlation (r) and p-values are presented for behavioral outcomes of parental care (total, from the mother, and from the secondary caregiver) and partner preference (contact time with a partner and preference for partners cage). ^{125}I-LVA binding did not significantly differ amongst males from different early life experiences (BPC, MON, and MPA) in any of the selected ROIs; accordingly, no post-hoc behavioral correlations were run for AVPR1a in males. Significant correlations are in bold. ROI Abbreviations: bed nucleus of the stria terminalis (BNST); central amygdala (CeA); lateral dorsal thalamus (LDTh); lateral septum (LS); mediodorsal thalamus (MDTh); medial amygdala (MeA); ventromedial nucleus of the hypothalamus (VMH); and ventral pallidum (VP).

			Parental Care			Partner Preference Test	
Receptor	**Sex**	**ROI**	**Total**	**Mother**	**Second Caregiver**	**Contact time**	**Cage Preference**
OXTR	Male	CeA	r = 0.15, p = .499	r = −0.14, p = .511	r = 0.21, p = .318	**r = 0.42, p = .037**	**r = 0.40, p = .045**
		CP	r = −0.14, p = .514	r = −0.12 , p = .562	r = −0.09, p = .681	r = 0.32, p = .115	r = 0.38, p = .060
		NAc	r = 0.21, p = .314	r = 0.20 , p = .350	r = 0.13, p = .559	r = 0.03, p = .873	r = 0.23, p = .278
	Female	CP	r = 0.16, p = .368	r = −0.05 , p = .758	r = 0.19, p = .291	r = 0.01, p = .938	r = −0.03, p = .879
		LS	r = −0.19, p = .289	r = 0.33, p = .057	**r = −0.35, p = .049**	r = −0.11, p = .526	r = −0.09, p = .614
		MPOA	r = −0.09, p = .605	r = 0.12 , p = .489	r = −0.15, p = .399	r = −0.31, p = .077	r = −0.16, p = .353
		NAc	r = −0.33, p = .956	r = −0.05 , p = .794	r = 0.03, p = .861	r = −0.04, p = .842	r = 0.05, p = .766
AVPR1a	Female	CeA	r = −0.26, p = .149	r = −0.13, p = .457	r = −0.20, p = .268	r = 0.11, p = .532	r = 0.23, p = .198
		VMH	r = 0.28, p = .110	r = 0.26, p = .136	r = 0.17, p = .357	r = −0.08, p = .636	r = 0.18, p = .300

Table 6:

Summary of Previous Adult Behavioral Findings under the Paternal Absence Paradigm.

References are compared to the preceding study by Rogers and Bales[20]. Results are organized by species and order of publication. Results are limited to observed adult behavior. Abbreviations: biparental care (BPC); maternal-only care (MON); maternal-plus-alloparental care (MPA).

Reference	Species	Conditions and Adult Behavioral Summary
Rogers & Bales[20]	Prairie vole	BPC, MON, and MPA conditions: Partner preferences formed in all BPC animals and MPA females but not MON animals and MPA males. MON males show significantly more alloparental care than MPA (but not BPC) males. No group differences in exploratory or anxiety-like behavior on the elevated plus maze.
Ahern & Young[18]	Prairie vole	BPC and MON conditions: Partner preferences were formed in BPC animals but delayed in MON animals. MON females showed less alloparental care. MON females and males show more movement in the first five minutes of an open field test. MON females show more exploratory behavior on the elevated plus maze.
Tabbaa et al.[35]	Prairie vole	BPC and MON conditions: MON males showed more social affiliation toward a same-sex conspecific. BPC animals entered the open arms of the elevated plus maze more frequently; paternal absence is indicated to alter locomotion but not anxiety-like behavior. Social recognition was not different between groups.
Kelly et al.[21]	Prairie vole	BPC and MON conditions with or without a tradeoff manipulation: Partner preferences were formed in both groups, but bonds were stronger in BPC pairs. MON animals showed less anxiety-like behavior in an open field test. No differences were found in dominance behavior or social approach.
Valera-Marín et al.[47]	Prairie vole	BPC and MON conditions: There were no significant differences in sexual behavior. BPC males demonstrate partner preference, whereas MON males do not. Olfactory capacity did not differ by group.
Jia et al.[46]	Mandarin vole	BPC, MON, and MON conditions with social deprivation: Less movement and more sniffing in MON vs BPC females in an open field. In social interactions MON females autogroom less while MON males are less active but retreat more.
Yu et al.[52]	Mandarin vole	BPC and MON conditions: Partner preferences were formed in all BPC animals but not MON females. MON males show partner preferences but severely reduced affiliative contact in general. Social interaction behavior of MON deviates from BPC animals in a sex dependent fashion.
Wang et al.[53]	Mandarin vole	BPC and MON conditions: MON animals show more contact with but less boxing/wrestling in a social play test.
Glasper et al.[17]	California mouse	BPC and MON conditions: MON animals show less exploratory behavior but not more anxiety-like behavior on the elevated plus maze. MON animals showed a greater percentage of time immobile in a forced swim task.
Agarwal et al.[44]	California mouse	BPC and MON conditions with or without chronic variable stress: MON animals traveled a greater distance during the acquisition phase of a novel object recognition task.