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Early life adversity in primates: Behavioral, endocrine, and neural effects

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ABSTRACT

Background: Evidence suggests that early life adversity is associated with maladaptive behaviors and is commonly an antecedent of stress-related psychopathology. This is particularly relevant to rearing in primate species as infant primates depend on prolonged, nurturant rearing by caregivers for normal development. To further understand the consequences of early life rearing adversity, and the relation among alterations in behavior, physiology and brain function, we assessed young monkeys that had experienced maternal separation followed by peer rearing with behavioral, endocrine and multimodal neuroimaging measures.

Methods: 50 young rhesus monkeys were studied, half of which were rejected by their mothers and peer reared, and the other half were reared by their mothers. Assessments were performed at approximately 1.8 years of age and included: threat related behavioral and cortisol responses, cerebrospinal fluid (CSF) measurements of oxytocin and corticotropin releasing hormone (CRH), and multimodal neuroimaging measures (anatomical scans, resting functional connectivity, diffusion tensor imaging, and threat-related regional glucose metabolism).

Results: The results demonstrated alterations across behavioral, endocrine, and neuroimaging measures in young monkeys that were reared without their mothers. At a behavioral level in response to a potential threat, peer reared animals engaged in significantly less freezing behavior ($p = 0.022$) along with increased self-directed behaviors ($p < 0.012$). Levels of oxytocin in the CSF, but not plasma, were significantly reduced in the peer reared animals ($p = 0.019$). No differences in plasma cortisol or CSF CRH were observed. Diffusion tensor imaging revealed significantly decreased white matter density across the brain. Exploratory correlational and permutation analyses suggest that the impact of peer rearing on behavior, endocrine and brain structural alterations are mediated by separate parallel mechanisms.

Conclusions: Taken together, these results demonstrate in NHPs the importance of maternal rearing on the development of brain, behavior and hormonal systems that are linked to social functioning and adaptive responses. The findings suggest that the effects of maternal deprivation are mediated via multiple independent pathways which may account for the heterogeneity in behavioral and biological alterations observed in individuals that have experienced this early life adversity.

1. Introduction

In humans prolonged parental nurturing of offspring and a secure supportive environment is critical for the development of adaptive social, emotional and cognitive functioning (Rutter, 1979). It is thought that a secure attachment is critical for typical psychological and socio-emotional development, and is the mechanism by which caretakers exert their positive influence on children's development (Bowlby, 1984). When parents are unable to fulfill this role, the consequences for the child can be long-term and increase the risk to develop psychopathology (King et al., 2023; Slopen et al., 2012; Wade et al., 2022; Zeanah et al., 2017). Follow-up studies in children exposed to parental neglect

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and those in which children have been placed in orphanages report increased levels of stereotypical, internalizing, externalizing and self-injurious behaviors, as well as deficits in cognitive and social functioning and physical health (Bowlby, 1951; Casler, 1961; Fisher et al., 1997; Kaler and Freeman, 1994). Furthermore, stress related psychopathology, including anxiety disorders, depression and substance abuse, are reported to be associated with parental neglect and early adversity, and individuals with these disorders that have a history of early adversity may be less likely to respond to treatment (Anda et al., 2007; Dube et al., 2003; Gilbert et al., 2009; Kaufman et al., 2000; Kessler et al., 2010; Kessler and Magee, 1993; Nanni et al., 2012).

A nonhuman primate model of maternal deprivation and early adversity was developed to create a more in depth understanding of the mechanisms underlying the deleterious effects of parental neglect and early life adversity in humans. The nonhuman primate model is particularly valuable due to the recent evolutionary divergence between monkeys and humans. Both human and nonhuman primates require prolonged parental nurturing to facilitate adaptive social and cognitive development. In addition to similarities in brain structure, function, and development, both species display similar affiliative and threat related social behaviors, further enhancing the translational relevance of the nonhuman primate model of early adversity.

Early studies by Harry Harlow demonstrated the importance of early life attachment in primates, and the potentially devastating consequences of neglect and social isolation (Harlow, 1965, 1958; Harlow and Harlow, 1962). Initial studies demonstrated that maternal deprivation followed by prolonged social isolation markedly increased stereotypical and self-directed behaviors, anxiety related behaviors and aggression (Harlow, 1965; Harlow and Harlow, 1962). In severe cases animals had lifelong social and cognitive deficits (Harlow and Harlow, 1962), which in part were mitigated when infant monkeys were reared with peers. However, maternally separated infants reared with peers were observed to still have significant alterations in behavior, such as dysregulated social and emotional responses (Chamove, 2016; Chamove et al., 1973; Champoux and Metz, 1991). Many of these behavioral effects are similar to those in humans that have experienced neglect early in life, even though they were later raised in more supportive environments (Fisher et al., 1997; Kaler and Freeman, 1994).

Early adversity is one of the strongest predictors of the later development of psychopathology. It is noteworthy that early life adversity is a non-specific risk factor associated with numerous disorders, and across disorders is associated with poor treatment outcomes. To further understand early adversity related alterations in behavior, underlying neural systems, and hormonal systems involved in stress and attachment, we used a multimodal approach studying nonhuman primates. Here we define early adversity in the nonhuman primate model as the abnormal experience of living solely with peers during very early development, and maternal deprivation as the permanent absence of a mother early in life. We hypothesized that the risk to develop psychopathology that is associated with early adversity is due to the widespread effects of adversity across neural, endocrine and behavioral systems. Studying these multiple measures within an individual allows us to better understand their interrelatedness as well as the extent to which common or differing mechanisms may underlie alterations in behavior, neural and endocrine systems. Specifically, we compared maternally reared offspring (controls) with those that were rejected by their mothers at birth, then placed in a nursery, and subsequently housed with peers (peer reared; PR). Behaviors that were assessed during exposure to a potential threat included anxiety related behaviors, hostile behaviors, vocalizations, locomotion and abnormal stereotypies. We elected to use the human intruder paradigm as it provides the opportunity to expose individuals to different types of threat that allow for the assessment of contextually appropriate adaptive responses. This paradigm has been well validated and is particularly useful in assessing trait-like anxiety related behaviors (Fox et al., 2008; Kalin and Shelton, 1989). At an endocrine level we assessed plasma levels of cortisol at baseline and in response to threat, plasma and cerebrospinal fluid (CSF) levels of oxytocin and CSF levels of corticotropin releasing hormone (CRH). From a neuroimaging perspective we assessed: regional brain metabolism in response to potential threat, resting functional connectivity with fMRI, volumetric brain measures, measures of brain tissue density, and white matter microstructure. Based on the findings reviewed above, we initially hypothesized that PR animals would show increased anxiety, as indexed by freezing in the human intruder paradigm, and that these changes would be associated with changes in endocrine and neuroimaging measures.

The correspondence between behavioral, endocrine, and neuroimaging measures is often implied by the existing literature (de Lima et al., 2023; Ochi and Dwivedi, 2022; Short and Baram, 2019), in which findings above are interpreted as either 1) maternal deprivation causing a single change that manifests in corresponding changes across modalities, or 2) maternal deprivation causes a sequence of events within each individual, such that PR causes endocrine changes early-in-life which induce lasting changes in the brain function that manifests as life-long anxiety and psychopathology. However, in the few studies that have taken multiple measurements, the modest correlations between measures begin to suggest these interpretations could be incomplete (Dannlowski et al., 2012; Gee et al., 2013). An alternative hypothesis, which is consistent with the literature, is that maternal deprivation can exert multiple independent "parallel" effects, with little to no relationship between measures, and that each effect may or may not result from maternal deprivation in any individual. Here, we leveraged multiple measures across behavioral, endocrine, and neuroimaging measures alongside correlational and multivariate approaches to assess the likelihood of these alternative models. Together, the results begin to paint a more complete picture of the many distinct ways that not having a mother can influence the emergence of maladaptive emotional behavior.

2. Materials and methods

2.1. Subjects

Behavioral, endocrine and neuroimaging assessments were performed in 50 young rhesus monkeys (Macaca mulatta). As part of the standard animal care procedures, after birth all newborns were housed with their mothers.

Because of our interest in early adversity, we selected infants that were rejected or abused by their mother shortly after birth. Of the 25 infants that experienced maternal rejection, 13 infants were rejected by their mothers post C-section, which was medically indicated or because the dam was past due. For the remaining 12 infants, eleven were rejected by a first- or second-time dam, and one infant was physically abused by its mother. Placement with a foster mom was attempted where possible but was ultimately unsuccessful in all these animals. Twenty-five (7 females) age and sex matched controls were also included, who as a part of their standard upbringing were mother-reared. All animals were pair housed at the time of testing. Procedures were performed using protocols approved by the University of Wisconsin Institutional Animal Care and Use Committee (IACUC).

2.2. Rearing conditions

Maternally rejected infants that were initially placed in the nursery were raised in accordance with Wisconsin National Primate Research Center (WNPRC) standard nursery protocols. When reintroduction to mom failed and no suitable foster mom was found, each infant was moved to the incubator where it was kept warm and bottle fed, until capable of being cage housed. After approximately the first month, infants were moved to standard caging within the nursery and socially housed with peers, either in pairs or in a group. At approximately 3 months of age, after the animals were weaned from formula, they were moved to the general colony and socially housed in pairs or in groups with peers, and later adults. Across the first year of life from 0–8 months none of the PR animals were exposed to adults. At around 8 months 11 out of 25 animals were exposed to groups that included adults. At 11 months an additional 8 animals were exposed to groups that included adults. The remaining 6 animals were not exposed to adults for the full first year of life.

Control animals were selected from the same age cohort as the peer reared monkeys. All animals were housed with their mother for the first year of life. Animals were weaned from mom at approximately 1 year of age, after which they were pair or group housed.

2.3. Early life environment

PR animals were initially reared in the incubator and nursery for an average of 104.56 days (22.34 standard deviation [SD]). PR animals were placed into pairs or groups for an average of 253.31 days (29.93 SD) across the first year. Maternally reared animals remained with their mothers for the full first year (376.34 days +–15.34 SD). After weaning from their mothers, maternally reared animals were socially housed in pairs or groups similar to PR animals.

In accordance with the study design, there were significant group differences in the early life environments between the PR and control animals. The number of days the PR animals were with their mothers was significantly fewer compared to the control group ($p < 0.001$; Supplementary Table S1). After maternal rejection, separated animals were initially housed in an incubator, within a nursery and subsequently pair and/or group housed. Therefore, the housing environments of the control and peer reared animals were different beyond the amount of time spent with their mother. The number of days the maternally separated animals were housed alone, predominantly while they were in the incubator, was significantly greater compared to the control group ($p < 0.001$; Supplementary Table S1). Furthermore, the number of unique cagemates was significantly higher for the PR group ($p < 0.001$; Supplementary Table S1). However, the amount of time in a pair or group setting was not significantly different between groups when combining settings with or without mom (pair: $p = 0.658$, group: $p = 0.213$; Supplementary Table S1**).** However, time in a pair with just a peer was significantly higher in the PR group ($p < 0.001$; Supplementary Table S1). Similarly, time in a group with others but no mom was significantly greater ($p < 0.001$; Supplementary Table S1).

2.4. Behavioral assessment

Rhesus monkeys were exposed to multiple paradigms to assess behavioral, endocrine and metabolic brain changes in response to a mild threat (Supplementary Fig. S1). Methods have previously been described (Fox et al., 2008; Oler et al., 2010), and are detailed in the Supplement. Briefly, animals were exposed to the no eye contact (NEC) condition of the human intruder paradigm (HIP) (Kalin and Shelton, 1989). After the exposure the animals were anesthetized in order to image brain metabolism of the radiolabeled glucose that was injected right before the exposure, using a positron emission tomography (PET) scanner. Blood samples were collected to measure plasma cortisol and oxytocin levels post exposure. Approximately 3 months later the animals were exposed to the full 50-minute HIP. Blood was collected after the full exposure for cortisol assessment.

During both tests an array of behaviors were observed and assessed by trained raters using a closed circuit television system (Supplementary Table S2)(Kalin and Shelton, 1989). Behaviors were assessed according to the definitions found in the supplementary methods (Supplementary Table S2). All behaviors were log-transformed when the duration of the behavior was quantified, and square root transformed when the frequency was quantified, as previously described (Fox et al., 2008; Oler et al., 2010). To create the composite measure of AT, an average of the z-scores of freezing, inverse cooing and cortisol was computed for each subject. Because self-directed behaviors were prominent, we also examined the extent to which they co-occurred with "freezing". Therefore, a class of behaviors was added, freezing with self-directed behaviors, which included freezing with saluting and/or digit sucking.

2.5. Endocrine assessments

2.5.1. Plasma & CSF collection

Hormone levels were assessed in all 50 animals from both plasma and cerebrospinal fluid (CSF). To assess group differences in endocrine responsiveness we measured both baseline hormone levels, as well as reactive hormone levels. Baseline hormone level assessments were scheduled on days without other tests taking place, while the reactive hormone level assessments were measured after exposure to a mild stressor (NEC & HIP). All sampling occurred while the animal was under sedation with ketamine (15 mg/kg, IM). For baseline hormone levels, both blood (plasma; 7 ml EDTA tube) and CSF (3 ml) were collected. Average time between capture and blood draw was 7 min (2 min SD), and average time between capture and CSF draw was 13 min (3 min SD). For reactive hormone levels, blood (plasma; 7 ml EDTA tube) was collected at the conclusion of the mild stressor. Plasma was prepared from blood by immediately spinning down the EDTA tubes for 10 min at 1900 x g at 4 °C and the supernatant collected and stored at –80 °C until assayed.

2.5.2. Cortisol analysis

Cortisol was measured in plasma by radioimmunoassay (RIA) using the DPC Coat-a-count assay following the manufacturer's instructions (Siemens, Los Angeles, CA). Samples were diluted 8-fold prior to being measured in duplicate, and samples that had a coefficient of variance (CV) % >20 were repeated. The limit of detection, defined as the lowest cortisol standard used in the assay, was 0.71 μg/dL. The inter-assay and intra-assay coefficients of variation were both 7.0 %.

2.5.3. Corticotropin-releasing hormone analysis

CRH levels were measured in CSF using an RIA established in our laboratory with an antibody (rC68 – 5/31/83 bleed) generously provided by Dr. Wylie Vale (Salk Institute for Biological Studies, La Jolla, CA). All samples were run in triplicate following a previously described protocol (Raper et al., 2014), and the assay had a limit of detection of 0.4 pg.

2.5.4. Oxytocin analysis

Plasma and CSF oxytocin levels were measured in plasma and unextracted CSF in duplicate using commercially available enzyme-linked immunoabsorbent assays (ELISA) (Catalog #ADI-900–153, Enzo Life Sciences, Farmingdale, NY). The limit of detection of this assay was 1.2 pg. See supplement for additional details.

2.6. Neuroimaging assessment

Methods have previously been described (Fox et al., 2008; Oler et al., 2010, 2017; Tromp et al., 2019), and are detailed in the Supplement. Briefly, in order to assess neural structure and function, magnetic resonance imaging (MRI) scans and positron emission tomography (PET) scans were collected for both peer and mother reared animals. MRI scans included: T1w-anatomical, diffusion-weighted imaging (DWI) with a corresponding field map, "resting" state functional MRI (rs-fMRI) with its own corresponding field map. A [^{18}F]-fluoro-2-deoxyglucose (FDG) PET scan was obtained immediately after exposure to the 30-minute NEC paradigm.

2.7. Statistical analyses

Our overall analytic strategy was to first identify between group differences in the measures collected, and then to understand how the relations among these multi-modal variables differed between groups. Next, we tested multiple models that could provide insight into the pathway by which early adversity, characterized by maternal separation followed by peer rearing, affected the observed relations among these variables.

2.7.1. Main effects of group

Tests of between group differences were run with robust linear regression models in order to mitigate the effect of outliers on the results. To account for potential confounds, analyses covaried for age and sex when appropriate. Behavioral, endocrine and tract-based DTI analyses were run using the *statsmodels* package in Python (Seabold and Perktold, 2010). For the tract-based DTI analyses we applied a Šidák familywise error correction ($\alpha_{SID} = 1 - [1 - \alpha]^{1/m}$), where m is the number of tracts for each diffusion measure; 6 total. Leading to a value of $\alpha \leq 0.0085$. For all neuroimaging modalities a voxel-wise statistical analysis was performed using nonparametric permutation inferences with FSL's randomise tool (Winkler et al., 2014). This method makes fewer assumptions about the underlying distribution of the data, which can be more robust to abnormally distributed noise. Multiple comparison corrections were applied with FSL's threshold-free cluster enhancement (TFCE) (Smith and Nichols, 2009).

2.7.2. Group differences in the correlations between behavior, endocrine and neuroimaging measures

To explore if the relationships *between* dependent measures were altered in the PR group compared to the control group, we selected all measures with significant between group differences and correlated them with each other. This created two large correlation matrixes, one for all the correlations within the PR group and one for all the correlations within the control group. Because the *r*-scores within the PR group cannot directly be compared to *r*-scores for the control group, we calculated a *z*-transformation that enabled us to run a formal *t*-test for each correlation that was run. To transform *r*-values to *z*-values we used the Fisher's r-to-z transformation method, which was computed as the inverse hyperbolic tangent of r, i.e. $z = arctanh(r)$, implemented by the *NumPy* package in Python (Van Der Walt et al., 2011). This allowed us to test which correlations were significantly different between the PR and control groups.

Next, we wanted to know if the frequency with which a significant z-score difference occurred was non-randomly distributed across the full matrix. The matrix consisted of 5 sections; correlations between *behavior* measures, correlations between *behavior & endocrine* measures, correlations between *behavior & neuroimaging* measures, correlations between *endocrine & neuroimaging* measures, and correlations between *neuroimaging* measures (Supplementary Fig. S2). We set out to understand if there were group differences in the average magnitude of the correlations when running a pairwise comparison between each of these 5 sections (e.g. do correlations between *behavior & neuroimaging* measures alter more after peer rearing than correlations between *neuroimaging & endocrine* measures). In order to determine whether the pairwise comparisons between these 5 sections significantly differed between groups, we ran 10 permutation tests to identify the percent times the observed group difference in the z-scored correlations was greater than the simulated difference. The permutation tests were implemented using the NumPy package in Python (Van Der Walt et al., 2011) (Link to github code: https://github.com/dotromp/Rearing-Adversity). Each test ran 10,000,000 permutations, to allow for *p*-values as small as 0.0000001.

2.7.3. Testing underlying biological models

As a final but important step we wanted to better understand the potential mechanisms by which maternal separation followed by peer rearing altered the various behavioral, hormonal and brain measures. Most previous studies have focused on either brain measures, behaviors or hormones. This makes it difficult to draw conclusions about how each of those systems interact with each other. Due to the multimodal nature of the data collected in the current study we were able to investigate the inter-relations of these maternal deprivation-related alterations in behavior, hormones and brain structure/function, as well as examine the extent to which alterations in these measures are related to early adversity in a hierarchical, serial or parallel manner. As such we tested these three models by which early adversity could influence the observed behavioral, hormonal, and brain alterations observed (Fig. 1).

Using principal component analyses (PCA) as implemented by the *Scikit-learn* package in Python (Pedregosa et al., 2011), we tested each of these models against the predicted outcome given the characteristics of the model. With a hierarchical model, the early adversity is thought to influence one general underlying biological alteration which in turn alters all other phenotypes. In this case one can expect a single principal component to explain the majority of the variance, resulting in a steep Scree plot and a single significant component in the PCA *t*-test (Fig. 1A). We would expect a similar result if the underlying biological mechanism is serial in nature, such that the early adversity alters hormones, which in turn alter the brain, which then alters behavior (or in any other order) (Fig. 1B). Since both the hierarchical and serial model predict that a single component would dominate the PCA analysis, a mediation analysis may be used in order to distinguish between the two. However, if early adversity influences the phenotypes through a parallel model, we would expect the PCA to identify more than 1 principal component, and thus produce a shallow Scree Plot, as well as reaching significance for multiple components in a PCA *t*-test (Fig. 1C).

3. Results

3.1. Demographics

There were no significant differences between control and PR animals with respect to sex, weight at birth, weight at tests, and age at tests (*ps* > 0.7, Table 1). However, there was a significant group difference with regard to the number of pregnancies the mothers had experienced, where mothers of PR animals had significantly fewer previous pregnancies (Table 1). The average age of the mothers of PR animals was also significantly lower than that of the mothers of control animals (Table 1). Furthermore, there was a significant group difference in the delivery method, where PR animals were delivered by caesarian section more frequently than control animals (Table 1).

3.2. Behavioral outcomes

To test if peer rearing altered responses to a mild threat, both PR and control monkeys were exposed to 30 min of the no eye contact condition with a subsequent PET scan (NEC-PET), as well as a 50-minute HIP with the alone, no eye contact and stare conditions.

Freezing is the predominant response of monkeys when exposed to the NEC condition, which is an adaptive reaction to a potential threat. Our results indicated that contrary to some expectations, peer reared monkeys froze significantly less than control animals during the 30-minute NEC-PET paradigm ($z = -2.284$, df = 46, $p = 0.022$; Table 2 **&** Supplementary Fig. S4A). Also, a significant reduction in freezing was observed in the alone condition of the HIP ($z = -4.182$, df = 46, $p < 0.001$; Supplementary Table S4).

No significant differences between groups were found for threat-induced cooing in either the NEC-PET or the HIP paradigms ($p > 0.356$; Table 2, Supplementary Table S4). Furthermore, AT, the composite measure of threat-induced freezing, cooing reductions and threat-induced cortisol, did not significantly differ between groups ($p = 0.140$; Table 2).

While freezing behavior was reduced in peer reared animals, self-directed behaviors, as observed across both paradigms and all conditions, dramatically increased (*ps* < 0.012; Supplementary Fig. S4B, Table 2, Supplementary Table S4). Self-directed behaviors include: self-mouth, self-groom, self-clasp, self-sex, and self-aggression

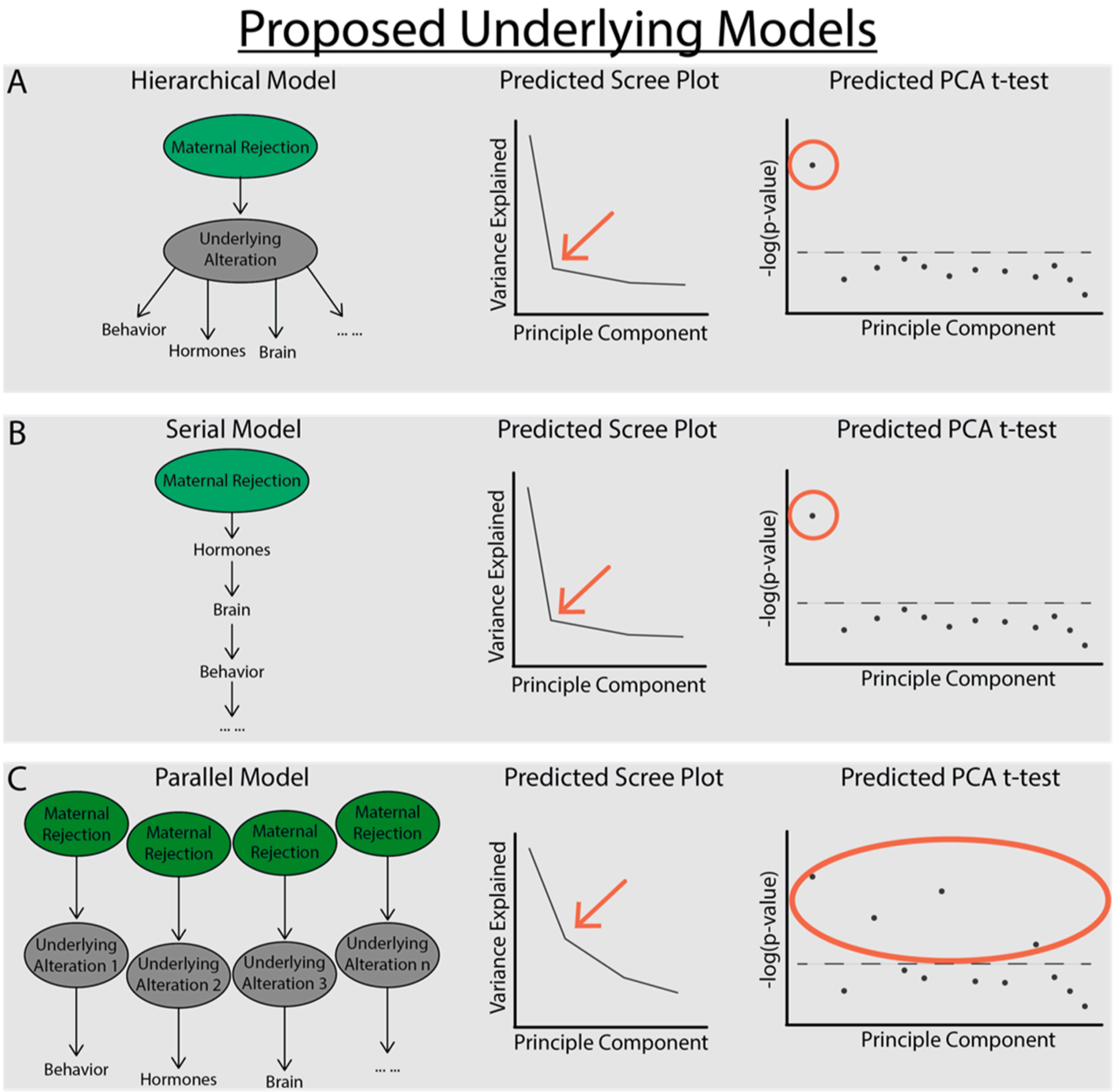

Fig. 1. Proposed underlying models. A. In the hierarchical model early adversity is thought to influence one underlying alteration which in turn influences all behavior, hormones, brain and other unknown effects. The predicted Scree Plot is steep due to only one principal component explaining variance, and the predicted PCA group t-test will have only a single significant principal component. B. In the serial model early adversity is thought to directly influence hormones, then the brain, then behavior and other unknown effects (or in any other order). The predicted Scree Plot is steep due to the variance being explained by only one principal component, and the predicted PCA group t-test will have only a single significant principal component. C. In the parallel model early adversity is thought to separately influence underlying alterations that in turn influence behavior, hormones, brain, and other unknown effects. The predicted Scree Plot is shallow due to more than one principal component explaining variance, and the predicted PCA group t-test will have more than one significant principal component.

Table 1
Demographic information. Demographics of control and peer reared (PR) animals. Mean, standard deviations and group significance are noted.

Demographics	Control (n = 25)	PR (n = 25)	*p*-values
Sex (female); number (%)	7 (28 %)	7 (28 %)	1
Weight at birth (kg); mean (SD)	0.56 (0.10)	0.57 (0.10)	0.835
Weight at tests (kg); mean (SD)	3.08 (0.47)	3.01 (0.44)	0.724
Age at test (years); mean (SD)	1.76 (0.32)	1.82 (0.40)	0.831
Age mom at birth infant (years); mean (SD)	10.46 (3.62)	8.42 (3.36)	**0.037***
Maternal parity (number); mean (SD)	3.80 (2.35)	2.48 (1.85)	**0.036***
Delivery method (c-section); number (%)	1 (4 %)	13 (52 %)	**<0.001***

[*] indicates significance at $p < 0.05$. Abbreviations: kilogram (kg), standard deviation (SD), peer reared (PR)

(Supplementary Table S2). Other stereotypies such as those involved with gross locomotor movements (stereo-locomotion) were not found to be significantly different between peer reared and control animals (ps > 0.16; Table 2, Supplementary Table S4).

Because freezing is defined as the absence of active behaviors, when self-directed behaviors were scored by definition freezing could not occur. It is possible that the animals' inability to inhibit their self-directed behaviors could account for the observed reductions in freezing in the PR animals. To examine this, we used the data from the HIP paradigm and broadened the definition of freezing to allow for concomitant self-directed behaviors. Results did not change using this composite score of freezing + self-directed behaviors (Supplementary Table S4). Indicating that freezing, with or without self-directed behaviors, was significantly reduced in the PR animals during the alone condition of the HIP (Supplementary Table S4).

Finally, we observed significantly increased locomotion and environmental exploration, as well as decreased experimenter orient in the

Table 2
Behaviors during NEC-PET. Behavioral observations during the positron emission tomography-no eye contact (NEC-PET) condition for control and peer reared (PR) animals. Mean log-transformed values and standard deviations are noted by group, significance of group difference is reported with the p-value.

NEC-PET Behaviors	Control	PR	*p*-values
AT; mean (SD)	0.13 (0.58)	–0.13 (0.80)	0.14
Coo Vocalizations; mean frequency (SD)	1.84 (2.78)	2.76 (4.04)	0.374
Environmental Explore; mean duration (SD)	1.23 (0.81)	2.04 (1.01)	**0.002***
Experimenter Hostility; mean duration (SD)	0.28 (0.43)	0.27 (0.40)	0.903
Experimenter Orient; mean duration (SD)	3.54 (0.65)	3.14 (0.64)	**0.028***
Freezing; mean duration (SD)	2.60 (1.67)	1.60 (1.64)	**0.022***
Locomotion; mean duration (SD)	3.32 (1.23)	3.81 (1.08)	0.065
Stereo-locomotion; mean duration (SD)	1.18 (1.63)	1.10 (1.37)	0.931
Self-directed; mean duration (SD)	0.70 (0.86)	2.57 (1.63)	**<0.001***

[*] indicates significance at $p < 0.05$. Abbreviations: anxious temperament (AT), peer reared (PR), no eye contact - positron emission tomography (NEC-PET), standard deviation (SD)

peer reared group compared to the control group (*ps* < 0.03, see Table 2, Supplementary Table S4).

3.3. Endocrine outcomes

To assess differences in HPA-axis activation, cortisol was measured at baseline and after the mildly stressful behavioral paradigms, NEC-PET and HIP. Furthermore, we measured baseline levels of CRH in the CSF. Results did not indicate significant differences between groups for baseline or stress induced cortisol or for CSF CRH (*ps* > 0.6; Table 3).

To investigate differences in oxytocin levels, we measured oxytocin in plasma and CSF at baseline and we also measured plasma levels of oxytocin after NEC-PET. Results indicated significantly reduced CSF oxytocin levels in peer reared monkeys compared to controls ($p = 0.019$; Table 3, See also Supplementary Table S3 & Fig. S3). No significant group differences were observed in plasma oxytocin levels, either at baseline or after NEC-PET (Table 3).

Table 3
Endocrine measures. Endocrine measures for control and peer reared (PR) animals, with standard deviations in parentheses. Significance of the regression statistics for the main effect of group are noted. All endocrine measures are corrected for time of day, and tests include age and sex as covariates.

Endocrine Measures		Control	PR	p-values
Baseline:	**Plasma Cortisol (µg/dl)**; mean (SD)	32.84 (9.26)	33.65 (9.84)	0.901
	Plasma Oxytocin (pg/ml); mean (SD)	305.22 (289.05)	367.40 (421.13)	0.697
	CSF CRH (pg/ml); mean (SD)	37.35 (17.73)	38.78 (15.06)	0.615
	CSF Oxytocin (pg/ml); mean (SD)	21.79 (10.07)	15.62 (9.57)	**0.019***
NEC-PET:	**Plasma Cortisol (µg/dl)**; mean (SD)	67.13 (9.75)	68.05 (14.62)	0.928
	Plasma Oxytocin (pg/ml); mean (SD)	376.90 (414.71)	321.29 (309.83)	0.796
HIP:	**Plasma Cortisol (µg/dl)**; mean (SD)	62.20 (10.55)	62.41 (11.52)	0.839

[*] indicates significance at $p < 0.05$. Abbreviations: corticotropin releasing hormone (CRH), cerebrospinal fluid (CSF), peer reared (PR), standard deviation (SD)

3.4. Neuroimaging outcomes

3.4.1. T1w-anatomical results

Here, we performed a voxel-wise analysis on T1w-anatomical scans, to assess differences in regional brain volume between peer reared and control monkeys, using log Jacobian determinants of the deformation fields. While none of the findings passed multiple comparison correction, PR animals had decreased volume in bilateral regions of dorsal amygdala that overlapped with the lateral division of the central nucleus (CeL), the amygdalostriatal transition zone and the white matter of the ventral amygdalofugal pathway/anterior commissure ($p < 0.005$, uncorrected; Supplementary Fig. S5, for overview Supplementary Fig. S6). A number of other regions were found to differ between groups, such that PR animals had volume decreases in the dorsolateral prefrontal cortex (dlPFC) and the posterior hippocampus ($p < 0.005$, uncorrected, for overview Supplementary Fig. S6).

3.4.2. DTI results

We measured white matter microstructure using diffusion tensor imaging (DTI) with both whole brain voxel-wise and tract-based methods. Voxel-wise results demonstrated that peer reared animals displayed significantly higher MD, AD and RD in a large bilateral cluster extending frontally from the lateral OFC/area 47, orbital proisocortex, anterior insula to the parietal-temporal-occipital association area in the posterior temporal lobe and to the brainstem ($p < 0.05$, TFCE corrected; Supplementary Fig. S7A, Supplementary Fig. S6, Supplementary Table S5, Supplementary Table S6). These clusters overlap in many regions with fibers from the internal capsule (Supplementary Fig. S7B). While reductions in FA were observed in PR animals these FA changes did not survive multiple comparison corrections. At the uncorrected level findings include reductions in FA in PR animals that were located in the bilateral anterior amygdala and the posterior cingulate ($p < 0.005$, uncorrected; Supplementary Fig. S6). Results from the tract-based analyses demonstrated higher average internal capsule AD levels in peer reared animals compared to controls ($z = -2.452$, $p = 0.014$, uncorrected; Supplementary Fig. S7C, Supplementary Fig. S6, Supplementary Table S6). Tract based analyses for FA, MD and RD for all tracts did not significantly differ between groups (Supplementary Table S6).

3.4.3. rs-fMRI results

To explore if peer rearing was associated with alterations in functional connectivity between the amygdala and other regions of the brain we tested group differences in rs-fMRI between bilateral CeA and the rest of the brain. Findings at the $p < 0.005$ uncorrected level demonstrated higher connectivity between the CeA and posterior cingulate cortex in the PR group compared to the control group ($p < 0.005$, uncorrected; Supplementary Fig. S6 & S8).

3.4.4. FDG-PET results

To test the effect of peer rearing on brain metabolism induced by a potential threat, we assessed glucose metabolism by performing FDG-PET scans after 30-minutes of NEC exposure. While no group differences passed multiple-comparison correction, less metabolism was observed in the insular cortex of the peer reared monkeys ($p < 0.005$, uncorrected; Supplementary Fig. S6).

3.5. Associations between measures across modalities

The results from the group t-tests indicate that peer rearing affected various behavioral, endocrine and brain measures. However, the analyses so far provided no indication of the extent to which the relationships among these measures differed between groups and to what extent these altered measures related to each other. Due to the unique multimodal nature of the data, we further interrogated these relations with both correlational and principal component analyses across the measures identifying, with multiple comparison corrections, those that were significantly different between groups. Of note, although we present many individual correlations, we are not trying to interpret each correlation, instead our aim is to characterize the pattern of correlations across groups and modalities.

3.5.1. Group differences in the correlations among the variables

Here we tested the hypothesis that maternal deprivation not only altered individual measures, but also disrupted the relationship *between* measures. First, in both the PR and control animals we separately performed correlations between all variables that were significantly different between the PR and control groups. The magnitude of the correlations is shown in Supplementary Fig. S9 for the Control group and Supplementary Fig. S10 for the PR group. Next, we tested the extent to which the correlations within each group differed between the two groups using a Fischer's r-to-z transform. Supplementary Fig. S11 displays the *p*-values for the differences in the correlation between the groups. We then examined if significant *z*-score differences were non-randomly distributed across the matrix containing the five correlation sections. We performed 10 pairwise permutation tests to identify group differences in mean correlations between each section pair. Table 4 shows that for 6 out of 10 pairs the relation between measures was altered by peer rearing. Specifically, results indicate that the group difference in average correlation (regardless of positive or negative direction) between *behavior & endocrine* measures was significantly greater than the group difference in average correlation between *behavior* measures, *behavior & neuroimaging* measures, and *endocrine & neuroimaging* measures. We also found that the group difference in average correlations between *neuroimaging* measures was significantly greater than the group difference in average correlations between *behavioral* measures, *behavior & neuroimaging* measures, and *endocrine & neuroimaging* measures. Indicating that peer rearing specifically altered the relation within *behavior & endocrine* measures as well as within *neuroimaging* measures.

Table 4

Group difference in average correlation by cluster; Our results indicate that the group difference in average correlation (irrespective of positive or negative direction) between behavior & endocrine measures was significantly greater than group difference in the average correlation between behavior measures, behavior & neuroimaging measurements, and endocrine & neuroimaging measures. We also found that the group difference in average correlations between neuroimaging measures was significantly greater than the group difference in average correlations between behavioral measures, behavior & neuroimaging measures, and endocrine & neuroimaging measures. Significant *p*-values indicate that the observed group differences in the z-scored correlations for that cluster pair are greater than the simulated group difference.

Measurement cluster 1	Direction	Measurement cluster 2	*p*-value
Correlations between *behavior & endocrine* measures	>	Correlations between *behavior* measures	**0.015** *
Correlations between *behavior & endocrine* measures	>	Correlations between *behavior & neuroimaging* measures	**0.002** *
Correlations between *behavior & endocrine* measures	>	Correlations between *endocrine & neuroimaging* measures	**0.004** *
Correlations between *behavior & endocrine* measures	=	Correlations between *neuroimaging* measures	0.947
Correlations between *neuroimaging* measures	>	Correlations between *behavior* measures	**<0.001** *
Correlations between *neuroimaging* measures	>	Correlations between *behavior & neuroimaging* measures	**<0.001** *
Correlations between *neuroimaging* measures	>	Correlations between *endocrine & neuroimaging* measures	**<0.001** *
Correlations between *behavior* measures	=	Correlations between *behavior & neuroimaging* measures	0.166
Correlations between *behavior* measures	=	Correlations between *endocrine & neuroimaging* measures	0.332
Correlations between *endocrine & neuroimaging* measures	=	Correlations between *behavior & neuroimaging* measures	0.843

3.5.2. Underlying biological models

Finally, to test which of three proposed underlying models (hierarchical, serial, or parallel) were supported by the current data, we ran principal component analyses on the measures for which there were significant group differences after peer rearing. Principle components were estimated in each group separately (Fig. 2A, Fig. 2B respectively) and subsequently projected to the full sample. This revealed a similar outcome, in which numerous principal components explained substantive variance (shallow Scree plots; Fig. 2). Importantly, numerous principal components differed between groups ($p < 0.05$; Fig. 2). These results are in accordance with a parallel model as outlined in Fig. 1C.

4. Discussion

Parents provide the foundation for who we are. These early life experiences are carried with us and passed on to our children. Here we have explored what it is to be without these core features early in life. Building on studies of maternally deprived animals that have been performed over decades, we examined hormones, behavior and brain imaging measures in peer reared rhesus monkeys. Counter to our expectations we did not find increases in AT (including freezing behaviors or cortisol levels) or FDG-PET metabolism. However, our results indicate that peer rearing in the absence of maternal nurturance does not specifically alter fear circuits within an individual, but rather the effects appear less specific, influencing various other behaviors and biology. The most reliable findings appear to be a preponderance of self-directed behaviors, decreased threat-related freezing behaviors, decreased CSF oxytocin, and alterations in cell packing throughout white matter as indexed by higher diffusivity. Beyond group differences we also investigated the relationship between these altered neural systems, behavioral responses, and endocrine functions. We acknowledge that the current study was a naturalistic observational study, not a prospective experiment in which peer reared infants would be determined by random assignment. Thus, the possibility exists that some of the alterations detected in the maternally deprived animals could be due to a genetic predisposition associated with their mothers difficulty related to nurturing her infant. Furthermore, because our study is not longitudinal we can not comment on what might be observed at later ages. Future work will need to extend these findings to include longitudinal data with additional age groups, as well as other forms of early-life stress, including maltreatment (e.g. Howell et al., 2013a), with large sample sizes and randomly assigned animals to obtain definitive results.

4.1. Effects on behavior

Contrary to what might be expected, we did not find an increase in anxious behaviors in peer reared animals when tested in the human intruder paradigm. Instead, levels of freezing behaviors both in response to a human intruder, as well as when alone were *decreased* in the peer reared animals. This finding remained when we also assessed "freezing" when it included self-directed behaviors that occurred in the absence of locomotion. Although review papers focused on early life adversity in nonhuman primates often describe an association with increased anxiety (Heim and Nemeroff, 1999; Kaufman et al., 2000; Latham and Mason, 2008; Sánchez et al., 2001), after closer inspection of the original research paper in which this was reported there is little empirical evidence for this assertion (Chamove et al., 1973).

While we did not see an increase in anxious behaviors, and actually saw a decrease, other abnormal behaviors, specifically self-directed

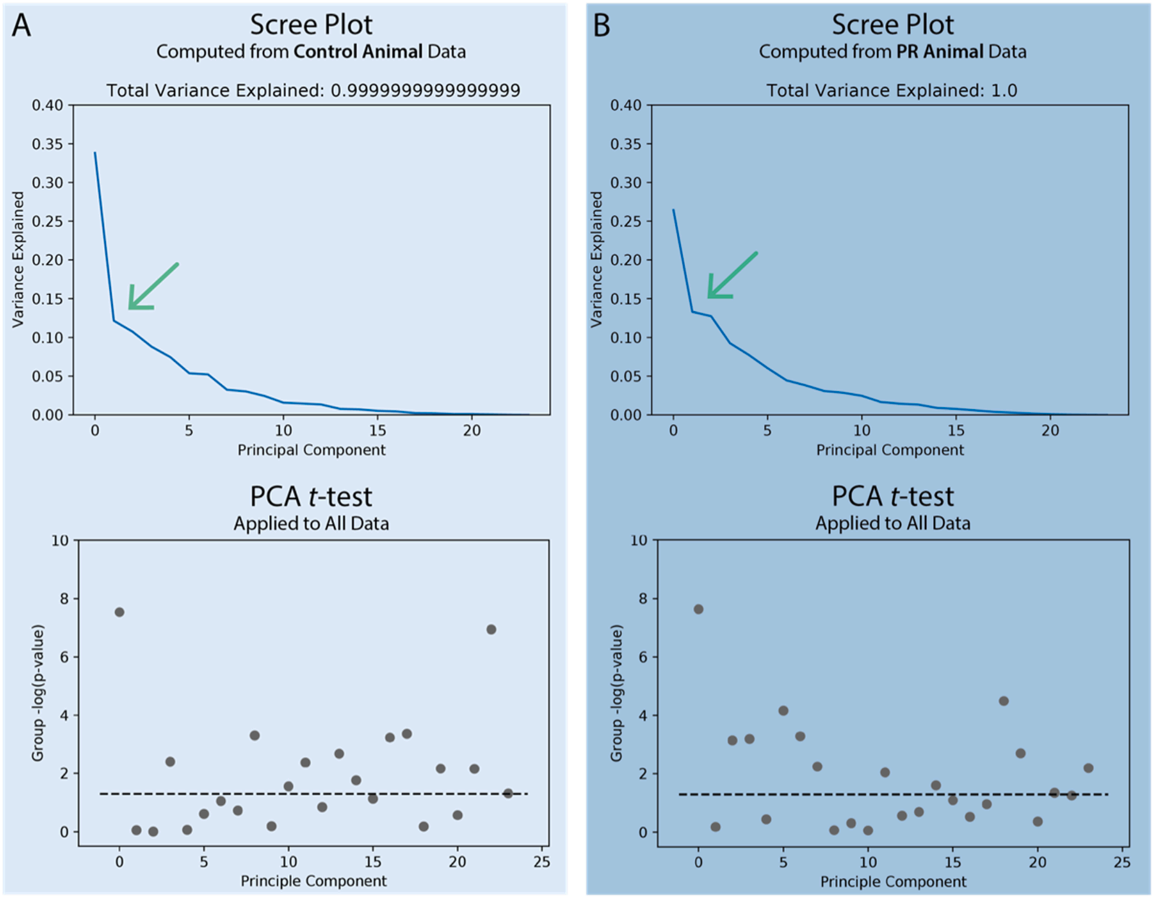

Fig. 2. Overview of PCA results. A. Results of PCA on significant variables as fit on the control group and subsequently projected to the full sample. Top: Scree Plot indicates multiple principal components explaining variance. Bottom: PCA group t-tests indicates 12 principal components for which the between group difference is $p < 0.05$ (striped line). B. Results of PCA on significant variables as fit on the PR group and subsequently projected to the full sample. Top: Scree Plot indicates multiple principal components explaining variance. Bottom: PCA group t-tests indicates 11 principal components for which the significance is $p < 0.05$ (striped line).

behaviors were prominent. Behaviors are considered self-directed when the animal displays self-mouth, self-groom, self-clasp, self-sex, or self-aggression. Self-directed behaviors are sometimes grouped with stereotypical behaviors (Berkson, 1968; Latham and Mason, 2008; Novak et al., 2006). Our observation of increased self-directed behavior is consistent with the many previous papers which have observed stereotypical behavioral responses across species after neglect or maternal deprivation (Berkson, 1968; Latham and Mason, 2008). Furthermore, researchers observed that human children who lived their first years of life in Romanian orphanages, displayed more frequent stereotypical behaviors compared to Canadian and Romanian children that were not orphaned (Fisher et al., 1997).

4.2. Effects on endocrine systems

Oxytocin is thought to play a central role in mediating affiliative behaviors, and is an important substrate thought to facilitate attachment between infant and caregiver (Eapen et al., 2014; Nelson and Panksepp, 1998). Numerous studies in humans and primates support an association between oxytocin and attachment quality (Eapen et al., 2014; Meyer-Lindenberg et al., 2011). Levels of Oxytocin can be assessed in the blood as well as in the CSF (Carson et al., 2015), and some studies demonstrate a correlation between plasma and CSF levels (Carson et al., 2015), whereas others do not (Amico et al., 1990; Kagerbauer et al., 2013). Oxytocin is thought to only minimally penetrate the blood-brain-barrier (Leng and Ludwig, 2016) and there is considerable support for independent but coordinated functions of peripheral and brain oxytocin systems (Meyer-Lindenberg et al., 2011). Human studies indicate altered plasma oxytocin levels after early maternal deprivation, for example children who were reared in an orphanage for the first year of life displayed significantly lower plasma oxytocin responses after exposure to their (adoptive) mothers compared to controls (Fries et al., 2005). Regarding CSF levels of oxytocin, a study in children demonstrated a negative relation between oxytocin levels and individual differences in anxiety (Carson et al., 2015). A prior study in rhesus monkeys demonstrated reduced CSF oxytocin in peer reared animals (Winslow et al., 2003). In the current study we replicate this finding, as we observe reduced CSF oxytocin in PR animals compared to controls. We did not find an association between peer rearing and blood plasma levels of oxytocin.

Research across humans, monkeys and rodents indicate mixed results when assessing the effects of early life stress on HPA-axis functioning. Similar to previous work (Winslow et al., 2003) examining the effects of peer rearing on rhesus monkeys, our results did not indicate any significant influences of peer rearing on stress related hormones (plasma cortisol or CSF CRH). While others did find HPA related effects (Bunea et al., 2017; Coplan et al., 1996).

4.3. Effects on brain structure and function

Analysis of the neuroimaging data from the different modalities revealed several interesting effects related to peer rearing. These data point to a number of potential mechanisms by which early adversity may have effects on brain development.

In relation to volumetric changes, we found reductions at the $p < 0.005$ uncorrected level in volume that were associated with peer rearing in a number of regions, including dlPFC, posterior hippocampus and dorsal amygdala. The effects observed in the dorsal amygdala are of particular interest in relation to the role that the central nucleus plays in emotion, anxiety, and fear-related processing (Fox et al., 2015). This finding is in agreement with a meta-analyses of early life stress associated with PTSD in which there was a significant but small reduction in amygdala volume (Woon and Hedges, 2008). However other literature in humans on the effects of early adversity on amygdala volume have been inconsistent, with reports of both volumetric increases and decreases after early life stress (Hanson et al., 2014; Hart and Rubia, 2012; Tottenham et al., 2010).

More prominent effects of peer rearing were observed in relation to white matter parameters. We found white matter microstructure to significantly differ between groups such that peer reared animals had increases in AD, MD and RD, extending frontally from the lateral OFC to the brainstem. The increase in these parameters likely reflects a lower density of neurons, axons and myelin across these regions. These results agree with research in children and monkeys that experienced early life stress, where changes in white matter microstructure are reported in regions including the corpus callosum, cingulum, inferior fronto-occipital fasciculus, and internal capsule (Bick et al., 2015; Coplan et al., 2010; Eluvathingal et al., 2006; Hanson et al., 2013, 2015; Howell et al., 2013b). Research in rodents provides insights into our findings in nonhuman primates. Early weaning in rat pups has been associated with reduced levels of myelin basic protein, a key constituent of myelin (Kodama et al., 2008). Additionally, experimentally induced reductions in social interactions in juvenile mice was found to alter prefrontal cortex myelination, through reduced oligodendrocyte processes and branching, decreased myelin basic protein and myelin associated glycoprotein (Makinodan et al., 2012). Electron microscopy investigations of affected regions in these juvenile mice indicated reductions in myelin thickness, while axon diameter was unaffected (Makinodan et al., 2012). These findings would be consistent with our observations of increases in AD, MD and RD in the peer reared rhesus monkeys.

Results from our functional connectivity analyses did not find group differences that passed multiple comparison corrections. However, at the $p < 0.005$ uncorrected level the PR group indicated higher connectivity between the CeA and posterior cingulate cortex in comparison to the control group. CeA is a critical component of the limbic system and posterior cingulate cortex is a hub for the default mode network, the interaction between these regions is likely important for adaptive behavioral and emotional responding (Pearson et al., 2011). We found no previous reports of alterations in functional connectivity between these regions after maternal deprivation in children or monkeys. However, some previous studies did identify negative functional connectivity between the amygdala and the posterior cingulate cortex in human adolescents after abuse and non-human primates after maternal maltreatment (Cheng et al., 2021; Morin et al., 2020).

Brain metabolism during threat as measured with FDG-PET did not find group differences that passed multiple comparison corrections. However, at an uncorrected $p < 0.005$ level peer reared monkeys indicated lower glucose metabolism in the insular cortex. The insular cortex is generally involved with interoceptive processes and is part of the circuitry involved in the expression of emotion and anxiety (Nieuwenhuys, 2012). Alterations in insular cortex function have been reported in individuals with anxiety disorders and depression. Most analogous to our current study, a previous study in a small number of children adopted from Romanian orphanages did not report alterations in insula glucose metabolism with FDG PET while awake resting, but did report reductions in various brain regions including the orbital frontal cortex, the amygdala and hippocampus (Chugani et al., 2001).

4.4. Group differences in the associations between the different measurement modalities

We found that maternal deprivation altered the relationships between measurement modalities and found multiple variance components that differed between groups. These data, derived from the unique multimodal nature of this study, allowed us to examine the likelihood of whether the effects of maternal deprivation are mediated by one or many underlying mechanisms. The multimodal nature of our study contrasts with many previous studies of maternal deprivation that have been more limited in the number of dependent measures examined. In summarizing these studies, one might conclude that a serial model could account for the diverse alterations reported across studies, e.g. where maternal deprivation may lead to oxytocin reductions, which would change white matter microstructure, leading to functional alterations in the insula, leading to changes in self grooming and decreased freezing. However, our data instead point to a parallel model, in which it is likely that multiple mechanisms underlie the effects of maternal deprivation that we detected in behavioral, hormonal and neural systems. Here, we used a variety of approaches to examine the extent to which different models may account for the various deficits we observed in relation to maternal deprivation. Had we observed an unchanged correlation structure between groups, and/or a single principal component that reflected group differences, these results would have supported a serial model. However, the complex nature of maternal deprivation was reflected in altered correlations between modalities, and group differences across multiple variance components. Together, these data support a more complex parallel model, in which maternal deprivation can differentially impact brain, endocrine, and behavioral measures to a different extent in different subjects.

Work by Zelikowsky *at al.* characterizing the role of Tac2/NkB in mediating the various behavioral effects of social isolation in mice revealed dissociable, region-specific requirements for both the peptide and its receptor (Zelikowsky et al., 2018). This provides a useful framework for demonstrating that the diverse effects on behavior in mice induced by social isolation are mediated in parallel by different brain regions. Along these lines, our results in maternally deprived primates support a similar parallel model to explain the diverse consequences of maternal deprivation across behavioral, endocrine and neural systems. Future studies in primates will be needed to more precisely tease out the molecular mechanisms that underlie these distributed changes across behavioral, endocrine and neural systems (Gee, 2021). Together our data provide compelling evidence for maternal deprivation impacting multiple parallel mechanisms to impact primate behavior and biology.

4.5. Considerations on the overall statistical framework used to understand the structure of the multimodal data and its implications

Throughout this manuscript, we presented many statistical tests, and took a number of novel approaches to data analysis. The majority of these analyses were focused on understanding the different biological models that could account for observed differences as a function of maternal deprivation. We identified significant effects of maternal deprivation within each modality, and explored the relationship between individual differences across measures using differences in within-group correlations, and data reduction through PCA. Although we identified some individual relationships between measures across modality to be nominally significant, we remain cautious in our interpretation of individual correlations with respect to the likelihood of replication. Although we had power to detect large effects, even this unusually large NHP study provides insufficient power to detect more subtle relationships with small effect sizes. Had the relationship between measures been “strong and localized” we would have found them, suggesting a more “weak and diffuse” relationship between measures (Cremers et al., 2017). Nevertheless, the overall pattern was consistent –

there were few to no robust relationships that would support a serial or hierarchical model. When taken together, these findings support a parallel model. Perhaps this should not be surprising, as parental care is titrated for each individual and context. Parents provide much more than a singular presence, guiding the development of their children through many distinct actions and influences in the hopes of supporting their child through the many things that could go awry.

4.6. Conclusions

Healthy and secure attachment with parents and/or caregivers facilitate healthy and strong attachment bonds, which are critical for normal social, emotional and physical development. Individual differences in genetic predisposition, temperament, and social support, interacting with the severity of deprivation, determines each individuals' unique response to maternal deprivation. As our results demonstrate, the effects of maternal deprivation can manifest in alterations in multiple domains, including behavior, hormones, brain structure and function. Furthermore, by assessing the impact of maternal deprivation on these multiple domains our findings support a parallel model by which the effects of maternal deprivation are mediated and manifested. Further research aimed at characterizing the different mechanisms that result in altered behavior, physiology and brain function will provide a foundation for developing personalized interventions focused on promoting resilience in affected individuals.

CRediT authorship contribution statement

Tromp Do: Conceptualization, Data curation, Formal analysis, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. **Fox Andrew S.:** Conceptualization, Funding acquisition, Methodology, Software, Visualization, Writing – review & editing, Writing – original draft. **Zhou Xiaojue:** Formal analysis, Writing – review & editing. **Roseboom Patrick H.:** Investigation, Resources, Validation, Writing – review & editing. **Riedel Marissa K.:** Investigation, Project administration, Resources, Writing – review & editing. **Oler Jonathan A.:** Funding acquisition, Project administration, Supervision. **Alexander Andrew L.:** Methodology, Writing – review & editing. **Kalin Ned H.:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

No conflict.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2023.106953.

References

Amico, J.A., Challinor, S.M., Cameron, J.L., 1990. Pattern of oxytocin concentrations in the plasma and cerebrospinal fluid of lactating rhesus monkeys (Macaca mulatto,): evidence for functionally independent oxytocinergic pathways in primates. J. Clin. Endocrinol. Metab. 71, 1531–1535. https://doi.org/10.1210/JCEM-71-6-1531.

Anda, R.F., Brown, D.W., Felitti, V.J., Bremner, J.D., Dube, S.R., Giles, W.H., 2007. Adverse Childhood Experiences and Prescribed Psychotropic Medications in Adults 32, 389–394. https://doi.org/10.1016/j.amepre.2007.01.005.

Berkson, G., 1968. Development of abnormal stereotyped behaviors. Dev. Psychobiol. 1, 118–132. https://doi.org/10.1002/dev.420010210.

Bick, J., Zhu, T., Stamoulis, C., Fox, N.A., Zeanah, C., Nelson, C.A., 2015. Effect of early institutionalization and foster care on long-term white matter development a randomized clinical trial. JAMA Pedia 169, 211–219. https://doi.org/10.1001/jamapediatrics.2014.3212.

Bowlby, J., 1951. Maternal care and mental health. Bull. World Health Organ 3, 179.

Bowlby, J., 1984. Attachment and loss, vol. 1. attachment. J. Am. Psychoanal. Assoc. 32, 216–218. https://doi.org/10.1177/000306518403200125.

Bunea, I.M., Szentágotai-Tătar, A., Miu, A.C., 2017. Early-life adversity and cortisol response to social stress: a meta-analysis. Transl. Psychiatry 2017 712 7, 1–8. https://doi.org/10.1038/S41398–017-0032–3.

Carson, D.S., Berquist, S.W., Trujillo, T.H., Garner, J.P., Hannah, S.L., Hyde, S.A., Sumiyoshi, R.D., Jackson, L.P., Moss, J.K., Strehlow, M.C., Cheshier, S.H., Partap, S., Hardan, A.Y., Parker, K.J., 2015. Cerebrospinal fluid and plasma oxytocin concentrations are positively correlated and negatively predict anxiety in children. Mol. Psychiatry 20, 1085–1090. https://doi.org/10.1038/mp.2014.132.

Casler, L., 1961. Maternal deprivation: a critical review of the literature. Monogr. Soc. Res. Child Dev. 26, 1. https://doi.org/10.2307/1165564.

Chamove, A.A.S., 2016. Rearing Infant Rhesus Together Published by: Brill Stable URL: http://www.jstor.org/stable/4533541 REFERENCES Linked references are available on JSTOR for this article: You may need to log in to JSTOR to access the linked references. 47, 48–66.

Chamove, A.S., Rosenblum, L.A., Harlow, H.F., 1973. Monkeys (Macaca mulatta) raised only with peers. A pilot study. Anim. Behav. 21 https://doi.org/10.1016/S0003-3472(73)80073-9.

Champoux, M., Metz, B., 1991. Behavior of nursery / peer-reared and mother-reared rhesus monkeys from birth through 2 years of age, 32, 509–514.

Cheng, T.W., Mills, K.L., Miranda Dominguez, O., Zeithamova, D., Perrone, A., Sturgeon, D., Feldstein Ewing, S.W., Fisher, P.A., Pfeifer, J.H., Fair, D.A., Mackiewicz Seghete, K.L., 2021. Characterizing the impact of adversity, abuse, and neglect on adolescent amygdala resting-state functional connectivity. Dev. Cogn. Neurosci. 47, 100894 https://doi.org/10.1016/J.DCN.2020.100894.

Chugani, H.T., Behen, M.E., Muzik, O., Juhász, C., Nagy, F., Chugani, D.C., 2001. Local brain functional activity following early deprivation: a study of postinstitutionalized Romanian orphans. Neuroimage 14, 1290–1301. https://doi.org/10.1006/nimg.2001.0917.

Coplan, J.D., Andrews, M.W., Rosenblum, L.A., Owens, M.J., Friedman, S., Gorman, J. M., Nemeroff, C.B., 1996. Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult nonhuman primates exposed to early-life stressors: implications for the pathophysiology of mood and anxiety disorders. Proc. Natl. Acad. Sci. 93, 1619–1623. https://doi.org/10.1073/PNAS.93.4.1619.

Coplan, J.D., Abdallah, C.G., Tang, C.Y., Mathew, S.J., Martinez, J., Hof, P.R., Smith, E.L. P., Dwork, A.J., Perera, T.D., Pantol, G., Carpenter, D., Rosenblum, L. a, Shungu, D. C., Gelernter, J., Kaffman, A., Jackowski, A., Kaufman, J., Gorman, J.M., 2010. The role of early life stress in development of the anterior limb of the internal capsule in nonhuman primates. Neurosci. Lett. 480, 93–96. https://doi.org/10.1016/j.neulet.2010.06.012.

Cremers, H.R., Wager, T.D., Yarkoni, T., 2017. The relation between statistical power and inference in fMRI. PLoS One 12, e0184923. https://doi.org/10.1371/JOURNAL.PONE.0184923.

Dannlowski, U., Stuhrmann, A., Beutelmann, V., Zwanzger, P., Lenzen, T., Grotegerd, D., Domschke, K., Hohoff, C., Ohrmann, P., Bauer, J., Lindner, C., Postert, C., Konrad, C., Arolt, V., Heindel, W., Suslow, T., Kugel, H., 2012. Limbic scars: long-term consequences of childhood maltreatment revealed by functional and structural magnetic resonance imaging. Biol. Psychiatry 71, 286–293. https://doi.org/10.1016/j.biopsych.2011.10.021.

de Lima, R.M.S., Couto Pereira, N., de, S., Dalmaz, C., Mar Arcego, D., 2023. Editorial: early life events: shedding light on neurobiological mechanisms. Front. Behav. Neurosci. 17, 1209494 https://doi.org/10.3389/FNBEH.2023.1209494/BIBTEX.

Dube, S.R., Felitti, V.J., Dong, M., Ph, D., Giles, W.H., Anda, R.F., S, M., 2003. The impact of adverse childhood experiences on health problems: evidence from four birth cohorts dating back to 1900, 37, 268–277. https://doi.org/10.1016/S0091-7435(03)00123-3.

Eapen, V., Dadds, M., Barnett, B., Kohlhoff, J., Khan, F., Radom, N., Silove, D.M., 2014. Separation anxiety, attachment and inter-personal representations: disentangling the role of oxytocin in the perinatal period. PLoS One 9. https://doi.org/10.1371/journal.pone.0107745.

Eluvathingal, T.J., Chugani, H.T., Behen, M.E., Juhász, C., Muzik, O., Maqbool, M., Chugani, D.C., Makki, M., 2006. Abnormal brain connectivity in children after early severe socioemotional deprivation: a diffusion tensor imaging study. Pediatrics 117, 2093–2100. https://doi.org/10.1542/peds.2005-1727.

Fisher, L., Ames, E.W., Chisholm, K., Savoie, L., Columbia, B., 1997. Problems Reported by Parents of Romanian Orphans Adopted to British Columbia, 20, pp. 67–82.

Fox, A.S., Oler, J. a, Tromp, D.P.M., Fudge, J.L., Kalin, N.H., 2015. Extending the amygdala in theories of threat processing. Trends Neurosci. 38, 319–329. https://doi.org/10.1016/j.tins.2015.03.002.

Fox, Andrew S., Shelton, S.E., Oakes, T.R., Davidson, R.J., Kalin, N.H., 2008. Trait-like brain activity during adolescence predicts anxious temperament in primates. PLoS One 3, e2570. https://doi.org/10.1371/journal.pone.0002570.

Fries, A.B.W., Ziegler, T.E., Kurian, J.R., Jacoris, S., Pollak, S.D., 2005. Early experience in humans is associated with changes in neuropeptides critical for regulating social behavior, 102.
Gee, D.G., 2021. Early adversity and development: parsing heterogeneity and identifying pathways of risk and resilience. Am. J. Psychiatry 178, 985–1069. https://doi.org/10.1176/APPI.AJP.2021.21090944.
Gee, D.G., Gabard-durnam, L.J., Flannery, J., Goff, B., Humphreys, K.L., Telzer, E.H., 2013. Early developmental emergence of human amygdala – prefrontal connectivity after maternal deprivation. https://doi.org/10.1073/pnas.1307893110/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1307893110.
Gilbert, R., Widom, C.S., Browne, K., Fergusson, D., Webb, E., 2009. Series Child Maltreatment 1 Burden and consequences of child maltreatment in high-income countries 373. https://doi.org/10.1016/S0140–6736(08)61706–7.
Hanson, J.L., Adluru, N., Chung, M.K., Alexander, A.L., Davidson, R.J., Pollak, S.D., 2013. Early neglect is associated with alterations in white matter integrity and cognitive functioning, 00, n/a-n/a Child Dev.. https://doi.org/10.1111/cdev.12069.
Hanson, J.L., Nacewicz, B.M., Sutterer, M.J., Cayo, A. a, Schaefer, S.M., Rudolph, K.D., Shirtcliff, E. a, Pollak, S.D., Davidson, R.J., 2014. Behavioral problems after early life stress: contributions of the hippocampus and amygdala. Biol. Psychiatry. https://doi.org/10.1016/j.biopsych.2014.04.020.
Hanson, J.L., Knodt, A.R., Brigidi, B.D., Hariri, A.R., 2015. Lower structural integrity of the uncinate fasciculus is associated with a history of child maltreatment and future psychological vulnerability to stress 27, 1611–1619. https://doi.org/10.1017/S0954579415000978.
Harlow, H.F., 1958. The nature of love. Am. Psychol. 13, 673–685.
Harlow, H.F., 1965. Total social isolation: effects on macaque monkey behavior. Science 148 (80), 666. https://doi.org/10.1126/science.148.3670.666-a.
Harlow, H.F., Harlow, M., 1962. Social deprivation in monkeys. Sci. Am. 207, 136–146. https://doi.org/10.1038/scientificamerican1162-136.
Hart, H., Rubia, K., 2012. Neuroimaging of child abuse: a critical review. Front. Hum. Neurosci. 6, 1–24. https://doi.org/10.3389/fnhum.2012.00052.
Heim, C., Nemeroff, C.B., 1999. The impact of early adverse experiences on brain systems involved in the pathophysiology of anxiety and affective disorders. Biol. Psychiatry 46, 1509–1522. https://doi.org/10.1016/S0006-3223(99)00224-3.
Howell, Brittany R., McCormack, K.M., Grand, A.P., Sawyer, N.T., Zhang, X., Maestripieri, D., Hu, X., Sanchez, M.M., 2013. Brain white matter microstructure alterations in adolescent rhesus monkeys exposed to early life stress: associations with high cortisol during infancy. Biol. Mood Anxiety Disord. 3 (1), 14. https://doi.org/10.1186/2045-5380-3-21.
Howell, Brittany R., Godfrey, J., Gutman, D. a, Michopoulos, V., Zhang, X., Nair, G., Hu, X., Wilson, M.E., Sanchez, M.M., 2013. Social subordination stress and serotonin transporter polymorphisms: associations with brain white matter tract integrity and behavior in juvenile female macaques. Cereb. Cortex 1–16. https://doi.org/10.1093/cercor/bht187.
Kagerbauer, S.M., Martin, J., Schuster, T., Blobner, M., Kochs, E.F., Landgraf, R., 2013. Plasma oxytocin and vasopressin do not predict neuropeptide concentrations in human cerebrospinal fluid. J. Neuroendocrinol. 25, 668–673. https://doi.org/10.1111/jne.12038.
Kaler, S.R., Freeman, B.J., 1994. Analysis of environmental deprivation: cognitive and social development in Romanian orphans. J. Child Psychol. Psychiatry 35, 769–781. https://doi.org/10.1111/j.1469-7610.1994.tb01220.x.
Kalin, N., Shelton, S., 1989. Defensive behaviors in infant rhesus monkeys: environmental cues and neurochemical regulation. Science 243 (80), 1718–1721. https://doi.org/10.1126/science.2564702.
Kaufman, J., Plotsky, P.M., Nemeroff, C.B., Charney, D.S., 2000. Effects of early adverse experiences on brain structure and function: clinical implications. Biol. Psychiatry 48, 778–790. https://doi.org/10.1016/S0006-3223(00)00998-7.
Kessler, R.C., Magee, W.J., 1993. Childhood adversities and adult depression: basic patterns of association in a US national survey. Psychol. Med. 23, 679–690 https://doi.org/https://doi.org.ez.statsbiblioteket.dk:2048/10.1017/S0033291700025460.
Kessler, R.C., Mclaughlin, K.A., Green, J.G., Gruber, M.J., Sampson, N.A., Zaslavsky, A. M., Aguilar-gaxiola, S., Alhamzawi, A.O., Alonso, J., Angermeyer, M., Benjet, C., Bromet, E., Chatterji, S., Girolamo, G. De, Demyttenaere, K., Fayyad, J., Florescu, S., Gal, G., Gureje, O., Haro, J.M., Hu, C., Karam, E.G., Ormel, J., Sagar, R., Vassilev, S., Viana, M.C., Williams, D.R., 2010. Childhood adversities and adult psychopathology in the WHO World Mental Health Surveys 378–385. https://doi.org/10.1192/bjp.bp.110.080499.
King, L.S., Guyon-Harris, K.L., Valadez, E.A., Radulescu, A., Fox, N.A., Nelson, C.A., Zeanah, C.H., Humphreys, K.L., 2023. A comprehensive multilevel analysis of the bucharest early intervention project: causal effects on recovery from early severe deprivation. Am. J. Psychiatry 180, 573–583. https://doi.org/10.1176/APPI.AJP.20220672/ASSET/IMAGES/LARGE/APPI.AJP.20220672F4.JPEG.
Kodama, Y., Kikusui, T., Takeuchi, Y., Mori, Y., 2008. Effects of early weaning on anxiety and prefrontal cortical and hippocampal myelination in male and female Wistar rats. Dev. Psychobiol. 50, 332–342. https://doi.org/10.1002/dev.20289.
Latham, N.R., Mason, G.J., 2008. Maternal deprivation and the development of stereotypic behaviour 110, 84–108. https://doi.org/10.1016/j.applanim.2007.03.026.
Leng, G., Ludwig, M., 2016. Intranasal oxytocin: myths and delusions. Biol. Psychiatry 79, 243–250. https://doi.org/10.1016/J.BIOPSYCH.2015.05.003.
Makinodan, M., Rosen, K.M., Ito, S., Corfas, G., 2012. A critical period for social experience-dependent oligodendrocyte maturation and myelination. Science 337 (80), 1357–1360. https://doi.org/10.1126/science.1220845.
Meyer-Lindenberg, A., Domes, G., Kirsch, P., Heinrichs, M., 2011. Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine. Nat. Rev. Neurosci. 12, 524–538. https://doi.org/10.1038/nrn3044.
Morin, E.L., Howell, B.R., Feczko, E., Earl, E., Pincus, M., Reding, K., Kovacs-Balint, Z.A., Meyer, J.S., Styner, M., Fair, D., Sanchez, M.M., 2020. Developmental outcomes of early adverse care on amygdala functional connectivity in nonhuman primates. Dev. Psychopathol. 32, 1579–1596. https://doi.org/10.1017/S0954579420001133.
Nanni, V., Uher, R., Danese, A., 2012. Childhood maltreatment predicts unfavorable course of illness and treatment outcome in depression: a meta-analysis. Am. J. Psychiatry 169, 141–151. https://doi.org/10.1176/appi.ajp.2011.11020335.
Nelson, E.E., Panksepp, J., 1998. Brain substrates of infant-mother attachment: contributions of opioids, oxytocin, and norepinephrine. Neurosci. Biobehav. Rev. 22, 437–452. https://doi.org/10.1016/S0149-7634(97)00052-3.
Nieuwenhuys, R., 2012. The insular cortex: a review. Prog. Brain Res. 195, 123–163. https://doi.org/10.1016/B978-0-444-53860-4.00007-6.
Novak, M., Meyer, J., Lutz, C., Tiefenbacher, S., 2006. Deprived environments: developmental insights from primatology. In: Mason, G., Rushen, J. (Eds.), Stereotypic Animal Behaviour. Fundamentals and Applications to Welfare, pp. 153–189.
Ochi, S., Dwivedi, Y., 2022. Dissecting early life stress-induced adolescent depression through epigenomic approach. Mol. Psychiatry 28, 141–153. https://doi.org/10.1038/s41380-022-01907-x, 2022 281.
Oler, J.A., Fox, A.S., Shelton, S.E., Rogers, J., Dyer, T.D., Davidson, R.J., Shelledy, W., Oakes, T.R., Blangero, J., Kalin, N.H., 2010. Amygdalar and hippocampal substrates of anxious temperament differ in their heritability. Nature 466, 864–868. https://doi.org/10.1038/nature09282.
Oler, J.A., Tromp, D.P.M., Fox, A.S., Kovner, R., Davidson, R.J., Alexander, A.L., McFarlin, D.R., Birn, R.M., E. Berg, B., deCampo, D.M., Kalin, N.H., Fudge, J.L., 2017. Connectivity between the central nucleus of the amygdala and the bed nucleus of the stria terminalis in the non-human primate: neuronal tract tracing and developmental neuroimaging studies. Brain Struct. Funct. 222 https://doi.org/10.1007/s00429-016-1198-9.
Pearson, J.M., Heilbronner, S.R., Barack, D.L., Hayden, B.Y., Platt, M.L., 2011. Posterior cingulate cortex: adapting behavior to a changing world. Trends Cogn. Sci. 15, 143–151. https://doi.org/10.1016/J.TICS.2011.02.002.
Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel, M., Prettenhofer, P., Weiss, R., Dubourg, V., Vanderplas, J., Passos, A., Cournapeau, D., Brucher, M., Perrot, M., Duchesnay, E., 2011. Scikit-learn: machine learning in python. J. Mach. Learn. Res. 12, 2825–2830.
Raper, J., Stephens, S.B.Z., Henry, A., Villarreal, T., Bachevalier, J., Wallen, K., Sanchez, M.M., 2014. Neonatal amygdala lesions lead to increased activity of brain CRF systems and hypothalamic-pituitary-adrenal axis of juvenile rhesus monkeys. J. Neurosci. 34, 11452–11460. https://doi.org/10.1523/JNEUROSCI.0269-14.2014.
Rutter, M., 1979. Maternal deprivation, 1972-1978: new findings, new concepts, new approaches. Child Dev. 50, 283–305.
Sánchez, M.M., Ladd, C.O., Plotsky, P.M., 2001. Early adverse experience as a developmental risk factor for later psychopathology: evidence from rodent and primate models. Dev. Psychopathol. 13, 419–449. https://doi.org/10.1017/S0954579401003029.
Seabold, S., Perktold, J., 2010. Statsmodels: econometric and statistical modeling with Python. Proc. 9th Python Sci. Conf. 57–61.
Short, A.K., Baram, T.Z., 2019. Early-life adversity and neurological disease: age-old questions and novel answers, 2019 1511 Nat. Rev. Neurol. 15, 657–669. https://doi.org/10.1038/s41582-019-0246-5.
Slopen, N., McLaughlin, K. a, Fox, N. a, Zeanah, C.H., Nelson, C. a, 2012. Alterations in neural processing and psychopathology in children raised in institutions. Arch. Gen. Psychiatry 1–9. https://doi.org/10.1001/archgenpsychiatry.2012.444.
Smith, S.M., Nichols, T.E., 2009. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. Neuroimage 44, 83–98. https://doi.org/10.1016/j.neuroimage.2008.03.061.
Tottenham, N., Hare, T. a, Quinn, B.T., McCarry, T.W., Nurse, M., Gilhooly, T., Millner, A., Galvan, A., Davidson, M.C., Eigsti, I.M., Thomas, K.M., Freed, P.J., Booma, E.S., Gunnar, M.R., Altemus, M., Aronson, J., Casey, B.J., 2010. Prolonged institutional rearing is associated with atypically large amygdala volume and difficulties in emotion regulation. Dev. Sci. 13, 46–61. https://doi.org/10.1111/j.1467-7687.2009.00852.x.
Tromp, D.P.M., Fox, A.S., Oler, J.A., Alexander, A.L., Kalin, N.H., 2019. The relationship between the uncinate fasciculus and anxious temperament is evolutionarily conserved and sexually dimorphic. Biol. Psychiatry 86, 890–898. https://doi.org/10.1016/j.biopsych.2019.07.022.
Van Der Walt, S., Colbert, S.C., Varoquaux, G., 2011. The NumPy array: a structure for efficient numerical computation. Comput. Sci. Eng. https://doi.org/10.1109/MCSE.2011.37.
Wade, M., Parsons, J., Humphreys, K.L., McLaughlin, K.A., Sheridan, M.A., Zeanah, C.H., Nelson, C.A., Fox, N.A., 2022. The Bucharest early intervention project: adolescent mental health and adaptation following early deprivation. Child Dev. Perspect. 16, 157–164. https://doi.org/10.1111/CDEP.12462.
Winkler, A.M., Ridgway, G.R., Webster, M.A., Smith, S.M., Nichols, T.E., 2014. Permutation inference for the general linear model. Neuroimage 92, 381–397. https://doi.org/10.1016/j.neuroimage.2014.01.060.
Winslow, J.T., Noble, P.L., Lyons, C.K., Sterk, S.M., Insel, T.R., 2003. Rearing effects on cerebrospinal fluid oxytocin concentration and social buffering in rhesus monkeys. Neuropsychopharmacology 28, 910–918. https://doi.org/10.1038/sj.npp.1300128.

Woon, F.L., Hedges, D.W., 2008. Hippocampal and amygdala volumes in children and adults with childhood maltreatment-related posttraumatic stress disorder: a meta-analysis. Hippocampus. https://doi.org/10.1002/hipo.20437.
Zeanah, C.H., Humphreys, K.L., Fox, N.A., Nelson, C.A., 2017. Alternatives for abandoned children: insights from the Bucharest early intervention project. Curr. Opin. Psychol. 15, 182–188. https://doi.org/10.1016/J.COPSYC.2017.02.024.
Zelikowsky, M., Hui, M., Karigo, T., Choe, A., Yang, B., Blanco, M.R., Beadle, K., Gradinaru, V., Deverman, B.E., Anderson, D.J., 2018. The neuropeptide Tac2 controls a distributed brain state induced by chronic social isolation stress. Cell 173, 1265–1279.e19. https://doi.org/10.1016/j.cell.2018.03.037.

__Table S1. Early life environment.__ Early life environment of control and peer reared (PR) animals. Mean, standard deviations and group significance are noted.

Early life environment	Control (n = 25)	PR (n = 25)	p-values
Time with mom (days; year 1); mean (SD)	358.44 (4.19)	2.11 (9.79)	**<0.001***
Time alone (days; year 1); mean (SD)	0.07 (0.29)	22.63 (12.47)	**<0.001***
Time in pair - with a peer or with mom (days; year 1); mean (SD)	102.90 (130.70)	106.88 (93.45)	0.658
Time in pair - with a peer (days; year 1); mean (SD)	1.49 (4.20)	108.70 (93.21)	**<0.001***
Time in group - with or without mom (days; year 1); mean (SD)	257.03 (130.73)	228.65 (90.30)	0.213
Time in group - without mom (days; year 1); mean (SD)	0.00 (0.00)	212.03 (85.29)	**<0.001***
Number of unique cagemates (birth till testing); mean (SD)	5.12 (3.13)	17.32 (6.81)	**<0.001***

* indicates significance at $p < 0.05$. Abbreviations: standard deviation (SD), peer reared (PR)

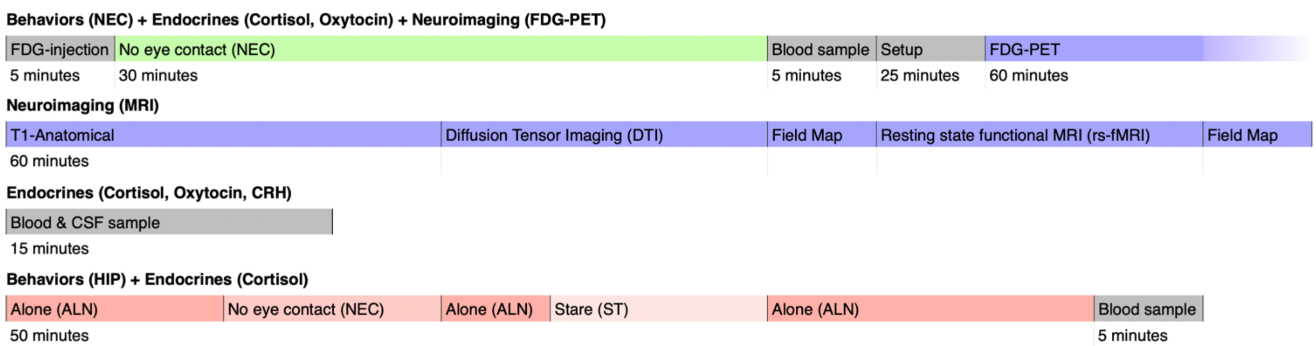


__Figure S1. Assessments.__ Overview of behavioral, endocrine and neuroimaging assessments. From top to bottom; animals were exposed to the NEC condition with concurrent FDG-PET imaging, blood was collected for cortisol and oxytocin measurements. Next, animals were scanned in the MRI scanner to collect T1-anatomical, DTI and rs-fMRI scans. Later, blood and CSF were collected to measure baseline levels of cortisol, oxytocin and CRH. Finally, the animals were exposed to the full human intruder paradigm, with NEC, alone and stare conditions. Blood was collected for cortisol measures.

__Table S2. Behavioral assessment.__ Description of behavioral coding methods.

Behavior	Description
Bark	Vocalization made by forcing air through vocal cords from the abdomen producing a short, rasping low frequency sound. *(frequency)*
Coo	Vocalization made by rounding and pursing the lips with an increase then decrease in frequency and intensity. *(frequency)*

Environment Explore	Any manual or oral exploration or manipulation of the physical surroundings such as cage, pan, droppings, hair, urine, or chow. Includes non-locomotive cage shaking. *(duration)*
Experimenter Hostility	Any hostile behavior directed at or stimulated by the presence of the intruder (e.g., head bobbing, ear flapping, locomotive cage shaking, open mouth threat, etc.). Only scored when intruder is present. *(duration)*
Experimenter Orient	Any non-hostile orienting behavior towards the intruder. Only scored when intruder is present. *(duration)*
Freezing	A period of at least three seconds characterized by tense body posture, no vocalizations, and no movement other than slow movements of the head. *(duration)*
Girn	A soft intermittent complex nasal warbling sound. It is a quiet sound of variable duration of approximately 500 ms and a peak frequency of about 800 Hz. *(frequency)*
Huddle	Self-enclosed, fetal like position with the head at or lower than the shoulders. *(duration)*
Lip Smack	Repeatedly pressing the lips together and pulling them apart quickly. *(duration)*
Locomotion	Ambulation of one or more full steps at any speed. Includes such behaviors as dropping from ceiling to floor or swinging from cage. *(duration)*
Lying Down	Animal is lying down ventrally, dorsally, or on its side. *(duration)*
Other Vocals	Vocalizations other than coo, bark, girn, and shriek. *(frequency)*
Resting	Animal is inactive with eyes closed and appears to be sleeping. *(duration)*
Self-Directed	Any self-directed behaviors which includes self-mouth, self-groom, self-clasp, self-sex, and self-aggression. *(duration)*
Freezing + Self-Directed	Any freezing behavior that is accompanied with a selection of self-directed behaviors. The self-directed behavior should only include those behaviors with no movement. i.e.: The animal is freezing but is also saluting or finger sucking. Examples that should not be counted are: Freezing with scratching or freezing with grooming which are self-directed behaviors that have movement components. *(duration)*
Shriek	Harsh shrill sound made by pulling back the lips. This vocalization is high pitched with little change in frequency. *(frequency)*
Stereo Locomotion	Any repetitive, patterned, and rhythmic movements, alone or in combination. The first occurrence during a test session is scored after three cycles. Thereafter, it is scored whenever it occurs unless a new pattern is developed. *(duration)*
Teeth Grinding	Any visual and/or audible gnashing of the teeth. *(duration)*

2 Supplementary Methods

2.4 Behavioral Assessment

Animals were first exposed to the no eye contact (NEC) condition of the human intruder paradigm (Kalin and Shelton, 1989). During this exposure the animals are placed in a test-cage after which a human "intruder" enters the room and displays the profile of his/her face to the animal for 30 minutes at 2.5 meters distance, while making no eye contact. After the exposure the animals were anesthetized in order to image brain metabolism of the radiolabeled glucose that was injected right before the exposure, using a positron emission tomography (PET) scanner. Blood samples were collected to measure plasma cortisol and oxytocin levels post exposure.

Approximately 3 months later the animals were exposed to the full 50-minute human intruder paradigm (HIP). During this test the monkeys are put in a test cage alone and observed there for 10 minutes. Then a human "intruder" enters as described above for the NEC exposure for 10 minutes. This is followed by 5 minutes alone, and 10 minutes of stare condition, where the human faces the animal at 2.5 meters distance, while maintaining eye contact with the animal. Finally, the animal is left alone for 15 minutes. Blood was collected after the full exposure for cortisol assessment.

During both tests an array of behaviors were observed and assessed by trained raters using a closed circuit television system (Supplementary **Table S2**)(Kalin and Shelton, 1989). Behaviors were assessed according to the definitions found in the supplementary methods (Supplementary **Table S2**). However, only behaviors for which the median value across the sample was above zero were included in subsequent analyses. This led to inclusion of coo vocalizations, environmental explore, experimenter hostility, experimenter orient, freezing, locomotion, stereo-locomotion and self-directed behaviors. All behaviors were log-transformed when the duration of the behavior was quantified, and square root transformed when the frequency was quantified, as previously described (Fox et al., 2008; Oler et al., 2010). Because self-directed behaviors were prominent, we also examined the extent to which they co-occurred with "freezing". Therefore, a class of behaviors was added, freezing with self-directed behaviors, which included freezing with saluting and/or thumb sucking. To create the composite measure of AT, an average of the z-scores of freezing, inverse cooing and cortisol was computed for each subject.

2.5 Endocrine Assessments

2.5.4 Oxytocin analysis

Oxytocin levels were measured in unextracted CSF and plasma samples in duplicate using a commercially available enzyme-linked immunosorbent assay (ELISA; Catalog #ADI-900-153, Enzo Life Sciences, Farmingdale, NY). By the time the plasma samples were assayed, the ELISA kit had been modified to use a different oxytocin antibody due to exhaustion of the original antibody supply. The average CV% for the CSF assay was 33.8 and for the plasma assay was 10.5. The higher CV% for the CSF assay was likely due to the ~16-fold lower concentrations of CSF oxytocin compared to plasma oxytocin. Because of the higher CV% for the CSF assay and because of the value of having CSF oxytocin levels, the group statistics were run a number of different ways to better understand the extent to which the individuals with high CV% influenced the outcomes of the data analysis. Specifically, we compared 4 approaches: each of the within subject duplicates, the average of the duplicates, covarying for the CV%, as well as the average values excluding subjects with CV%s greater than 30%. In general, the different analyses yielded very similar results. In the body of the paper, we report the analysis based on the average of the duplicates (See **Supplementary Table S3 & Figure S3** for the additional analyses described above).

2.6 Neuroimaging Assessment

2.6.1 Neuroimaging acquisition

Methods have previously been described (Fox et al., 2008; Oler et al., 2010), and are detailed in the supplement. Briefly, in order to assess neural structure and function, magnetic resonance imaging (MRI) scans were collected of both peer and mother reared animals. MRI images were acquired after the animals received ketamine (15 mg/kg, IM) and dexmedetomidine (0.015 mg/kg, IM). The animal was then placed in the sphinx position using a custom stereotactic frame while heart rate and oxygen saturation were monitored. Ketamine (up to 5 mg/kg, IM) was repeated as needed approximately every 20-40 minutes throughout the scan. At the end of the scan the dexmedetomidine was reversed with atipamezole (0.15 mg/kg, IM) and animals were removed from the scanner and monitored until they fully recovered from anesthesia.

First, a T1-weighted (T1w)-anatomical scan was acquired to assess gross anatomical variation, using an axial T1-weighted 3D inversion recovery prepared fast spoiled gradient recalled scan (IR-fSPGR; repetition time (TR) = 11.448 ms, echo time (TE) = 5.412 ms, inversion time (TI) = 600 ms, flip angle a = 10°, number of excitations (NEX) = 2, field of view (FOV) = 140 x 140 mm, matrix = 256 x 256 interpolated to 512 x 512, in-plane resolution = 0.27 mm, slice thickness/gap = 0.5/0 mm, 248 slices). Next, diffusion-weighted imaging (DWI) scans were collected with a corresponding field map. DWIs were collected using a two-dimensional, echo-planar, spin-echo sequence (TR/TE = 10000/85.3 ms, flip angle = 90°, NEX = 1, FOV = 144 mm, matrix = 128 x 128 interpolated to 256 x 256, in-plane resolution = 0.5625 mm, slice thickness/gap = 1.3/0 mm, 68 interleaved slices, echo-planar spacing = 816 μs. DWI at b = 1000 s/mm^2 was performed in 72 non-collinear directions with 6 non-diffusion-weighted images). Images were acquired in the coronal plane through the entire monkey brain. A co-planar field map was obtained for the diffusion-weighted images, using a gradient echo with images at two echo times: TE1 = 7 ms, TE2 = 10 ms. A "resting" state functional MRI (rs-fMRI) scan was collected to assess regional BOLD covariation, or functional connectivity, with its own corresponding field map. Rs-fMRI scans were acquired using a T2*-weighted echo planar imaging (EPI) sequence (TR/TE/Flip/FOV/Matrix: 2000 ms/25 ms/90°/140 mm/ 64x64; 26x3.1mm axial slices; gap: 0.5mm). A co-planar field map was obtained for the resting state images, using a gradient echo with images at two echo times: TE1 = 7 ms, TE2 = 10 ms. All MRI scans were obtained using a GE SIGNA 750 3.0T scanner (General Electrics, Milwaukee, WI, USA) with a HD T/R Quad extremity coil (Invivo Corp, Gainsville, FL), in which the stereotaxic frame fit in the center of the coil.

To measure group differences in brain glucose metabolism while the animal was behaving freely during a mild threat, the monkeys were injected with radiolabeled glucose before NEC exposure and then scanned using a positron emission tomography (PET) scanner. Specifically, animals received intravenous injections of up to 7 mCi [^{18}F]-fluoro-2-deoxyglucose (FDG) immediately before exposure to the 30-minute NEC paradigm, during which FDG-uptake occurred. After the behavioral paradigm animals were anesthetized with ketamine (15 mg/kg, IM) and atropine sulphate (0.04 mg/kg, IM). Blood cortisol samples were collected immediately after anesthesia. Next, subjects were fitted with an endotracheal tube and positioned in a stereotaxic head holder and given isoflurane gas anesthesia (1–2%) for the duration of the 60-minute scanning procedure, during which integrated FDG-uptake was measured that occurred during the behavioral paradigm. Scanning was performed using a Siemens microPET Focus 220 scanner (Siemens Healthcare, Knoxville, TN), which has an approximate resolution of ~2mm^3 FWHM.

2.6.2Neuroimaging processing

2.6.2.1 T1w-anatomical analysis

To allow for inter-individual comparisons the T1w-images of each animal were spatially normalized and registered to our previously published 592 Rhesus template (Fox et al., 2015). First, anatomical scans were manually skull stripped with SPAMALIZE (http://psyphz. Psych.wisc.edu/*oakes/spam/spam_frames.htm). Next, we corrected for low frequency intensity non-uniformity that often presents in MRI scans by applying a bias field correction using N3BiasFieldCorrection(Sled et al., 1998). The skull stripped and T1w-anatomical scans were iteratively registered to the Rhesus macaque template made from 592 pre-adolescent monkeys (http://www.pnas.org/content/112/29/9118; Supplementary Dataset S01) using Advanced Normalization Tools (ANTS; http://sourceforge.net/projects/advants (Avants et al., 2011, 2010)). The transformation parameters (both affine and nonlinear warp files) were saved to aid in calculating the Jacobian determinants, and for use in the PET normalization process.

Local volumetric variation was calculated using the Jacobian determinant of the nonlinear component of the transformation to template space. The proportion of volumetric change between each animal's native and template space was quantified as the absolute value of the Jacobian determinant of the non-linear transformation. To put volumetric expansions and reductions on the same scale, data were log transformed. Importantly, this procedure accounts for individual differences in total brain volume and produced a single map for each subject representing the relative volume at each voxel in the brain. All images were smoothed with a 4 mm full width at half maximum (FWHM) Gaussian smoothing kernel, to improve signal detection in cases of imperfect alignment. All images were visually inspected to ensure accurate pre-processing before statistical analyses were performed.

2.6.2.2 DTI analysis

DWI images were corrected for field inhomogeneities and eddy currents (Woolrich et al., 2009), and gradient directions were adjusted for this correction (Leemans and Jones, 2009). After which the tensors were calculated using a robust tensor estimation using Camino software (Cook et al., 2006). This method is especially effective for nonhuman primates as their smaller brain can lead to lower signal-to-noise ratios. Between-subject and cross-modality analyses were enabled by making a tensor-based template in 592 rhesus monkey T1w-space. Tensor images of all subjects were co-registered iteratively using non-linear tensor-based normalization tools (DTI-TK)(Zhang et al., 2006). The final population template was then aligned to the 592 rhesus monkey T1-template, and the warp was applied to all normalized images. In this template space, diffusion measures were extracted to quantify local white matter microstructure. Four diffusion measures were extracted that each characterize a different aspect of the local microstructure. Fractional anisotropy (FA) is a measure that weighs the longest vector of diffusion as a ratio of the average diffusion, generally this measure is highest in areas with organized white matter fiber pathways. Mean diffusivity (MD) is an average of all diffusion directions and is sensitive to the general density in the microstructure. Axial diffusivity (AD) and radial diffusivity (RD) reflect longitudinal and transverse diffusion respectively, and are selective to density in each respective direction specifically (for an overview (Tromp, 2016a)).

The population template created from all subjects was used for deterministic fiber tractography to delineate tracts of interest. Whole-brain fiber tractography was performed using Camino software (Cook et al., 2006), that implemented a tensor deflection (TEND) algorithm for optimal estimation of the fiber tracking directions (Basser et al., 2000; Lazar et al., 2003). Fiber tracking was terminated in voxels where FA was below 0.1, or where the angle between consecutive streamline steps was more than 90 degrees. Visualization software TrackVis (Wang et al., 2007) was used to iteratively delineate white matter pathways using anatomically defined waypoints (Mori et al., 2002; Tromp, 2016b). Tracts extracted included: corpus callosum, cingulum, internal capsule, inferior fronto-occipital fasciculus, stria terminalis/fornix and uncinate fasciculus (Bick et al., 2015; Eluvathingal et al., 2006; Hanson et al., 2015, 2013; Howell et al., 2013). Due to limited DTI resolution and close proximity of the STRIA and FX, the two pathways were combined. All tracts were homologues of human tracts explored in relation to anxiety disorders (Tromp et al., 2019). A weighted mean for each diffusion measure (FA, MD, AD, RD) was calculated for each tract. This approach assigns relatively more weight to values in voxels that have a higher fiber count, which is most frequently observed in areas more central to the white matter tract of interest. This method reduces the influence of voxels that are on tissue boundaries and often suffer from partial volume effects. To explore potential white matter group differences beyond the extracted regions in the tract-based analysis, voxel-based analyses of FA, MD, AD and RD were performed across the whole brain. All images were smoothed with a 4mm FWHM Gaussian kernel. All images were visually inspected to ensure accurate pre-processing before statistical analyses were performed.

2.6.2.3 rs-fMRI analysis

Functional connectivity was assessed with methods previously used in a large sample of preadolescent monkeys (Birn et al., 2014; Oler et al., 2012), and is briefly described here. All processing steps were carried out in AFNI (Cox, 1996), unless otherwise indicated. Specifically, resting state scans were slice time and motion corrected, had the first 3 volumes removed, and were adjusted for field inhomogeneities with a field map correction. The preprocessed resting state scans were warped into 592 rhesus monkey T1-space with the warps calculated from the anatomical scans, and up-sampled to anatomical resolution. In order to reduce the influence of non-neuronal fluctuations on functional connectivity estimates, average signal intensity time courses from the white matter and lateral ventricular CSF were regressed out of the EPI time series. The residualized resting state signal was further processed with a temporal bandpass filtering (low = 0.01 Hz, high = 0.1 Hz), and smoothed with a 4mm FWHM Gaussian kernel.

Functional connectivity between bilateral central nucleus of the amygdala (CeA) and the rest of the brain was investigated by seeding with this region. The CeA was chemoarchitectonically localized using a PET ligand that identified this serotonin transporter-rich area with [11-C] DASB (Christian et al., 2009; Oler et al., 2012). A temporal correlation analysis was run with the seed mean time-series using all available data, producing a z-map where each voxel represents the correlations with BOLD fluctuations in the seed region. All images were visually inspected to ensure accurate pre-processing before statistical analyses were performed.

2.6.2.4 FDG-PET analysis

FDG-PET images of each animal were transformed to our 592 Rhesus template space in order to allow for group comparisons. This was done through a multi-step process; first the FDG-PET image of each animal was aligned to its T1-anatomical image, using a rigid body mutual information warp. Next, the transformation from T1 to template-space was applied to the FDG-PET image.

Template-space FDG-PET images were scaled to correct for global intensity differences based on the mean FDG concentration across the whole brain in template space, and smoothed with 4mm FWHM Gaussian kernel. All images were visually inspected to ensure accurate pre-processing before statistical analyses were performed.

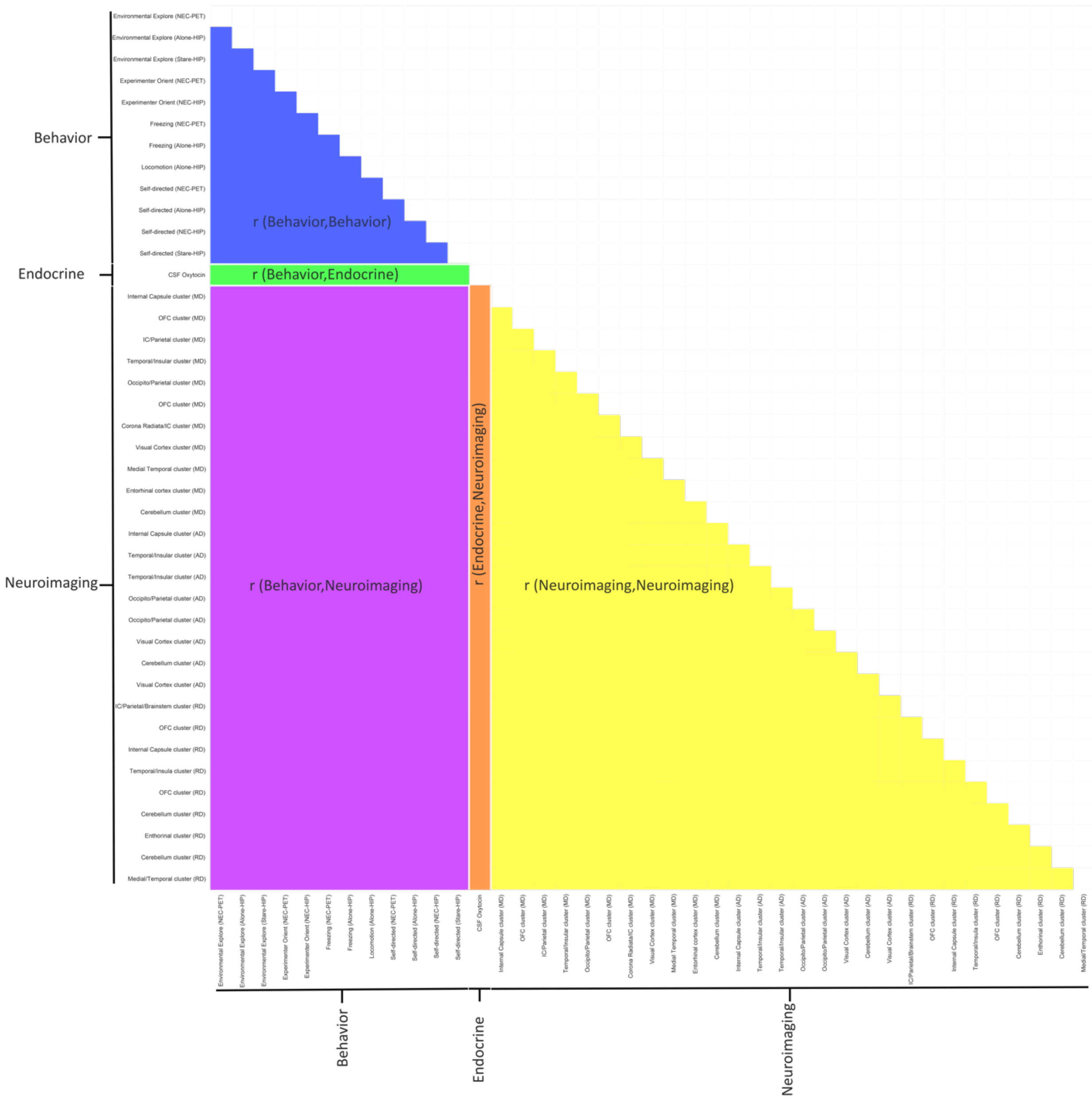


Figure S2. Here we visualize how the 3 modalities result in 5 correlation sections: r(Behavior,Behavior) in blue, r(Behavior,Endocrine) in green, r(Behavior,Neuroimaging) in purple, r(Endocrine,Neuroimaging) in orange, r(Neuroimaging,Neuroimaging) in yellow.

Table S3. Quality control of CSF Oxytocin. Group differences between control and peer reared (PR) animals for different analyses of CSF Oxytocin. Tests include group differences for the values of the first duplicate, for the values of the second duplicate, for the mean of the two duplicates, for the mean of the two duplicates when covarying for the coefficient of variance, and for the mean of the two duplicates when excluding all tests with a coefficient of variance above 30%.

Oxytocin CSF (pg/ml)	Control	PR	z	df	*p*-values
Duplicate 1	19.44 (7.68)	13.54 (9.68)	-2.743	42	**0.006***
Duplicate 2	23.33 (14.71)	16.69 (10.21)	-1.895	44	0.058
Mean of both assays	21.79 (10.07)	15.62 (9.57)	-2.349	44	**0.019***
Mean of both assays; covary for CV	21.79 (10.07)	15.62 (9.57)	-2.133	41	**0.033***
Mean of both assays; select only CV's < 30	20.44 (5.71)	16.35 (7.59)	-4.059	23	**<0.001***

* indicates significance at $p < 0.05$. Abbreviations: cerebrospinal fluid (CSF), coefficient of variance (CV), degrees of freedom (df), peer reared (PR).

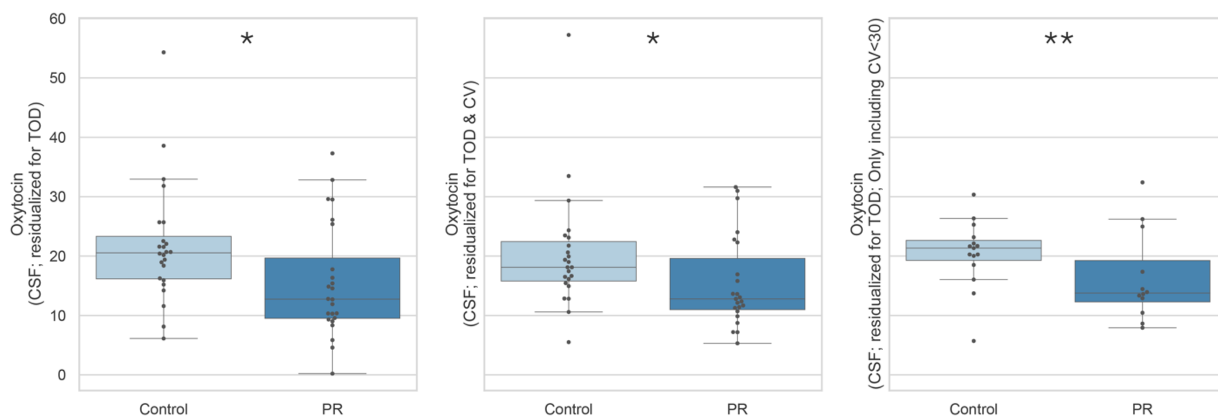


Figure S3. Quality control of CSF Oxytocin. Group differences between control and peer reared (PR) animals in three different oxytocin analyses; the mean of duplicates 1 and 2 (left graph), the mean when covarying for the CV (center graph), the mean when excluding all tests with a CV above 30% (right graph).

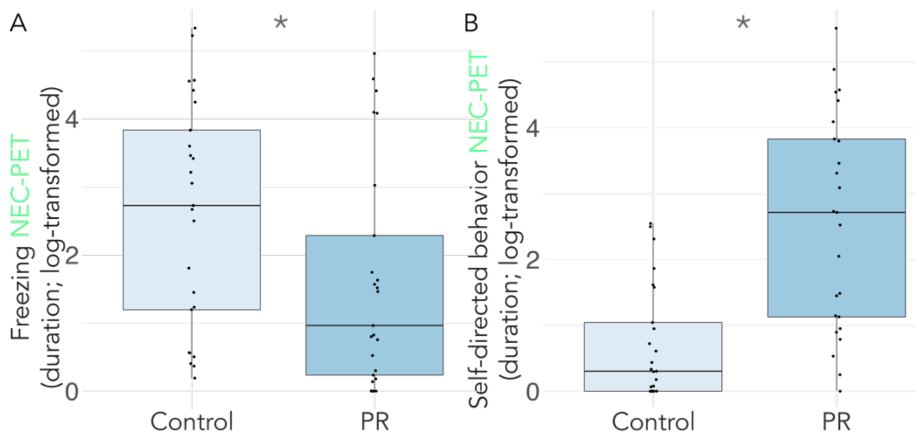


Figure S4. Overview of behavioral results. *A) The amount of freezing during NEC-PET is significantly less in PR animals compared to control animals. B) The amount of self-directed behaviors during NEC-PET is significantly greater in PR animals compared to control animals. * indicates p < 0.05.*

Table S1. Behaviors during HIP. *Behavioral observations during the human intruder paradigm (HIP) for control and peer reared (PR) animals. Values across alone, no eye contact (NEC) and stare conditions. Mean log-transformed values and standard deviations are noted by group, significance of group difference is reported with the p-value.*

	Control	**PR**	***p*-values**	**Control**	**PR**	***p*-values**	**Control**	**PR**	***p*-values**
HIP Behaviors		**Alone**			**NEC**			**Stare**	
Coo Vocalizations; mean frequency (SD)	1.22 (1.93)	1.80 (3.03)	0.677	1.46 (2.70)	1.91 (3.17)	0.657	2.07 (2.04)	2.98 (3.48)	0.356
Environmental Explore; mean duration (SD)	0.94 (0.65)	1.56 (1.03)	**0.021***	0.70 (1.08)	1.12 (1.24)	0.229	1.08 (0.76)	1.65 (0.99)	**0.018***
Experimenter Hostility; mean (SD)	N.A.	N.A.	N.A.	0.51 (1.00)	0.34 (0.75)	0.782	3.08 (1.47)	2.85 (1.44)	0.496
Experimenter Orient; mean duration (SD)	N.A.	N.A.	N.A.	4.03 (0.60)	3.64 (0.64)	**0.018***	3.81 (0.57)	3.66 (0.69)	0.077
Freezing; mean duration (SD)	2.83 (1.45)	1.45 (1.05)	**<0.001***	3.74 (1.89)	2.91 (1.87)	0.066	2.18 (1.52)	1.79 (1.62)	0.281
Freezing+Self-Directed; mean duration	2.83 (1.45)	1.83 (1.15)	**0.009***	3.74 (1.89)	3.15 (1.96)	0.231	2.18 (1.52)	2.06 (1.70)	0.571
Locomotion; mean duration (SD)	3.57 (0.98)	4.19 (0.55)	**0.007***	2.24 (1.58)	2.86 (1.70)	0.153	3.28 (1.22)	3.65 (1.22)	0.104
Stereo-locomotion; mean duration (SD)	1.94 (1.84)	2.73 (1.97)	0.162	0.95 (1.55)	1.49 (1.92)	0.378	0.80 (1.22)	1.09 (1.41)	0.492
Self-directed; mean duration (SD)	0.40 (0.57)	1.88 (1.60)	**<0.001***	0.26 (0.52)	1.60 (1.82)	**0.012***	0.74 (0.82)	1.95 (1.61)	**0.008***

* indicates significance at $p < 0.05$. Abbreviations: human intruder paradigm (HIP), no eye contact (NEC), peer reared (PR), standard deviation (SD)

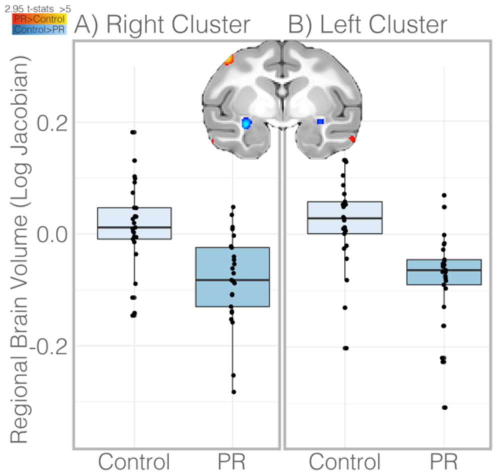


Figure S1. Overview of T1 results. *Group differences in T1 log jacobians were tested across the whole brain, significance is thresholded at p < 0.005, uncorrected. No clusters passed multiple comparison corrections. To visualize the direction of change we added boxplots of the volume difference in the A) right and B) left cluster between control and PR animals.*

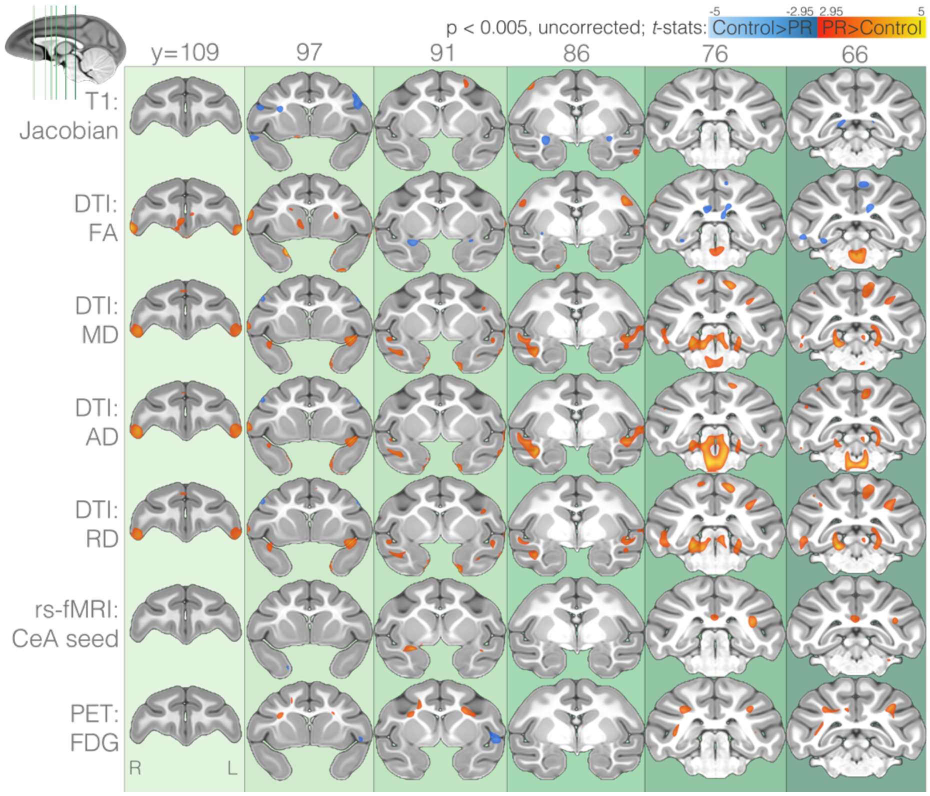


Figure S6. Overview of neuroimaging results. Group differences between control and peer reared (PR) animals in all tested neuroimaging modalities. Warm colors indicate PR greater than controls, and cool colors indicate controls greater than PR. All results are p < 0.005, uncorrected.

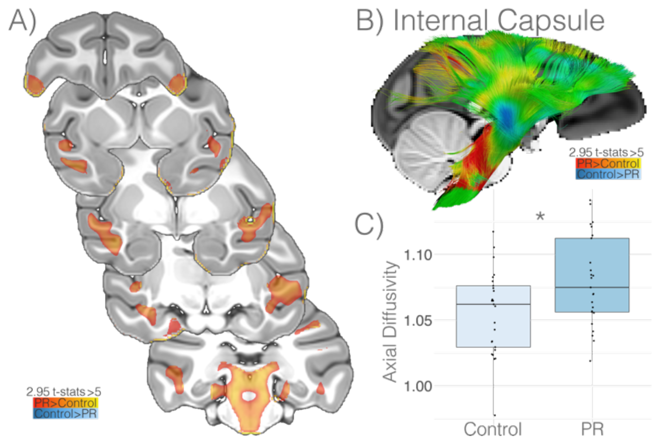


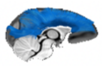
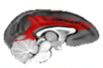
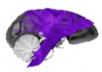
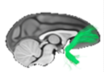
Figure S7. Overview of DTI results. *A) Whole brain analysis demonstrates significantly higher axial diffusivity in PR animals compared to control animals (p < 0.05; TFCE corrected). B) 3D view of internal capsule fibers colored by voxel-wise group differences in axial diffusivity. C) Tract based analysis demonstrates significantly higher axial diffusivity in internal capsule in the PR group compared to the controls (p = 0.014; uncorrected).*

Table S5. Overview of significant neuroimaging results after multiple comparison correction. Whole-brain voxel-based group differences in neuroimaging modalities after TFCE correction. Overview of size and locations of significant clusters after TFCE correction by modality.

Cluster Volume (mm^3)	Location	Side	*t*-value	x	y	z	*p*-value
DTI MD							
7661	Internal Capsule/Parietal Cortex/Brainstem	R/L	**3.04**	62	66	34	0.988
5878	Orbital Frontal Cortex (47L)	L	**3.09**	38	131	58	0.981
5670	Internal Capsule/Parietal Cortex	L/R	**3.05**	48	52	74	0.985
4183	Temporal/Insular Cortex	R	**2.99**	83	88	31	0.982
3012	Occipito-parietal area	R	**3**	74	44	69	0.978
785	Orbital Frontal Cortex (47L)	R	**3.14**	87	110	46	0.97
204	Corona Radiata/Internal Capsule	R	**3.06**	67	66	81	0.955

191	Visual Cortex (V3/4)	R	**2.97**	85	41	47	0.953
161	Medial Temporal (TH/TL)	R	**3.02**	69	78	28	0.955
130	Entorhinal Cortex	L	**3.42**	41	93	20	0.955
30	Cerebellum	R	**3.04**	79	56	34	0.951
DTI AD							
10469	Internal Capsule/Parietal Cortex/Brainstem	R/L	**3.24**	48	64	31	0.998
6038	Temporal/Insular Cortex	L	**3.15**	23	73	37	0.985
5831	Temporal/Insular Cortex	R	**3.11**	82	89	31	0.99
2945	Occipito-parietal area	L	**3.19**	32	45	70	0.98
1145	Occipito-parietal area	R	**3.22**	74	43	69	0.973
885	Visual Cortex (V3/4)	R	**2.88**	83	40	47	0.964
120	Cerebellum	R	**2.91**	79	54	30	0.953
115	Visual Cortex (V3/4)	R	**3.44**	89	54	67	0.958
DTI RD							
10086	Internal Capsule/Parietal Cortex/Brainstem	L/R	**3**	48	52	74	0.986
3808	Orbital Frontal Cortex (47L)	L	**3.22**	38	131	58	0.98
3739	Brainstem	R/L	**3.1**	67	59	37	0.987
3535	Temporal/Insular Cortex	R	**2.94**	83	88	31	0.973
633	Orbital Frontal Cortex (47L)	L	**3.08**	88	111	45	0.967
200	Cerebellum	R	**2.81**	78	56	34	0.953
101	Entorhinal Cortex	R	**3.14**	69	77	28	0.955
88	Cerebellum	R	**3.6**	62	47	17	0.953
84	Medial Temporal (TH/TL)	L	**3.56**	41	92	20	0.956

Table S6. Tract-based analyses. *Weighted mean FA, MD, AD and RD values for each white matter tract for control and peer reared (PR) animals, with standard deviations in parentheses. Tract-based analysis indicates differences in internal capsule white matter between control and PR animals. Age and sex were included as covariates.*

	FA			MD			AD			RD		
Bilateral WM Tract	**Control**	**PR**	***p*-values**	**Control**	**PR**	***p*-values**	**Control**	**PR**	***p*-values**	**Control**	**PR**	***p*-values**
CC 	0.427 (0.020)	0.426 (0.020)	0.347	0.780 (0.029)	0.784 (0.025)	0.626	1.191 (0.036)	1.199 (0.030)	0.464	0.574 (0.030)	0.577 (0.029)	0.726
CING 	0.300 (0.016)	0.293 (0.016)	0.079	0.734 (0.026)	0.735 (0.013)	0.204	0.974 (0.031)	0.969 (0.020)	0.748	0.614 (0.026)	0.618 (0.014)	0.062
IC 	0.414 (0.018)	0.423 (0.016)	0.051	0.703 (0.027)	0.715 (0.023)	0.085	1.056 (0.032)	1.080 (0.034)	**0.014***	0.527 (0.028)	0.532 (0.022)	0.339
IFO 	0.386 (0.019)	0.386 (0.013)	0.94	0.778 (0.019)	0.779 (0.013)	0.941	1.136 (0.030)	1.140 (0.026)	0.53	0.599 (0.018)	0.598 (0.012)	0.92
STRIA /FX 	0.247 (0.010)	0.241 (0.014)	0.064	0.900 (0.036)	0.887 (0.044)	0.377	1.149 (0.044)	1.126 (0.061)	0.256	0.775 (0.034)	0.767 (0.037)	0.553
UF 	0.275 (0.011)	0.278 (0.010)	0.365	0.773 (0.038)	0.764 (0.009)	0.997	1.006 (0.043)	0.998 (0.015)	0.644	0.656 (0.036)	0.647 (0.010)	0.879

**Sidák-corrected significance at p < 0.0085. * Uncorrected significance at p < 0.05. Abbreviations: axial diffusivity (AD), Corpus Callosum (CC), Cingulum bundle (CING), fractional anisotropy (FA), Internal Capsule (IC), Inferior Fronto-Occipital fasciculus (IFO), peer reared (PR), mean diffusivity (MD), radial diffusivity (RD), Stria Terminalis & Fornix (STRIA/FX), Uncinate Fasciculus (UF).

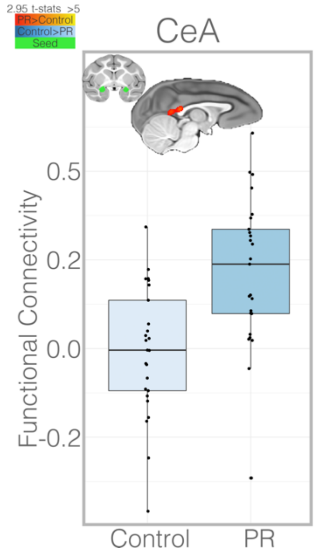


Figure S8. Overview of rs-fMRI results. *Boxplot of functional connectivity differences between control and peer reared (PR) animals. Using the central nucleus of the Amygdala (CeA) as a seed (in green) connectivity was increased between the amygdala and posterior cingulate in PR animals (p<0.005, uncorrected).*

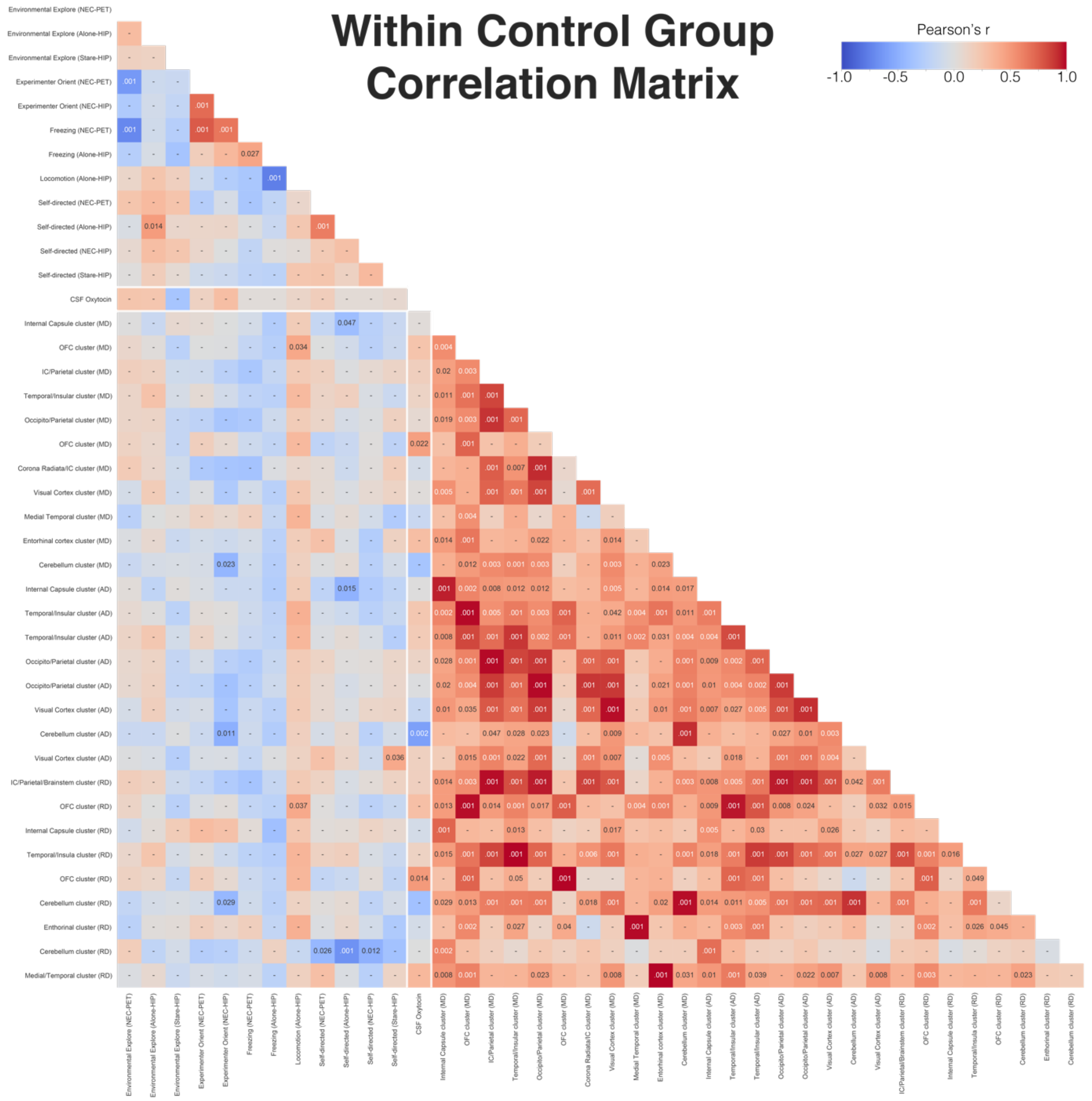


Figure S9. Within control group correlation matrix. Correlation matrix of select measures within the control group. Colors indicate the Pearson's r value, while numbers indicate the significance of the correlation (for p<0.05). Measures were selected when they displayed a significant group difference between the control and PR groups. White lines are drawn between different modalities; behavior, endocrine and neuroimaging.

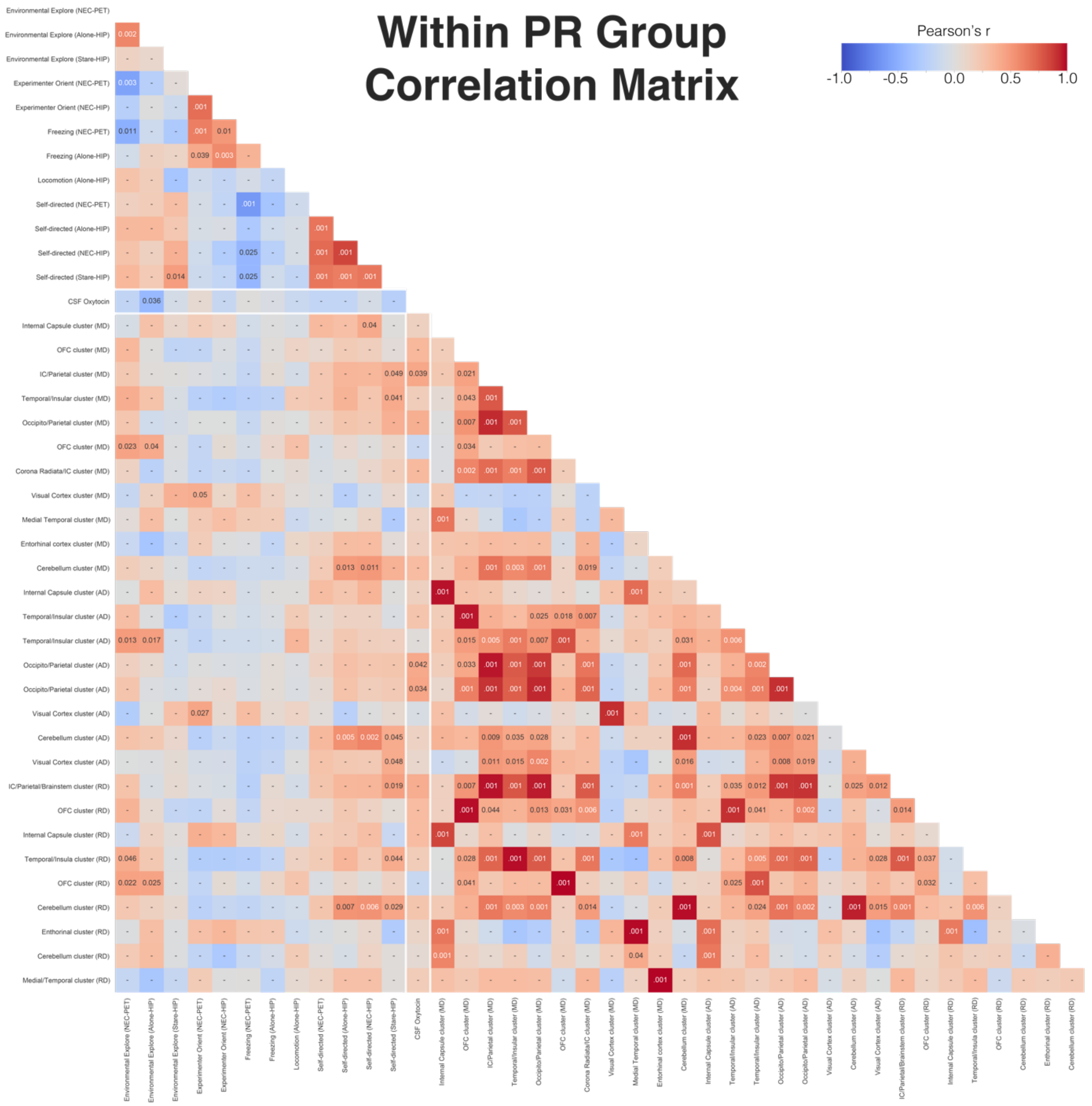


Figure S10. Within PR group correlation matrix. Correlation matrix of select measures within the PR group. Selected measures include those for which the difference between the control group and PR group was significant. Colors indicate the direction and strength of the correlation (darker red for stronger positive correlations, and darker blue for stronger negative correlations), while the numbers indicate the significance of the correlation (for p<0.05). White lines are drawn between different modalities; behavior, endocrine and neuroimaging.

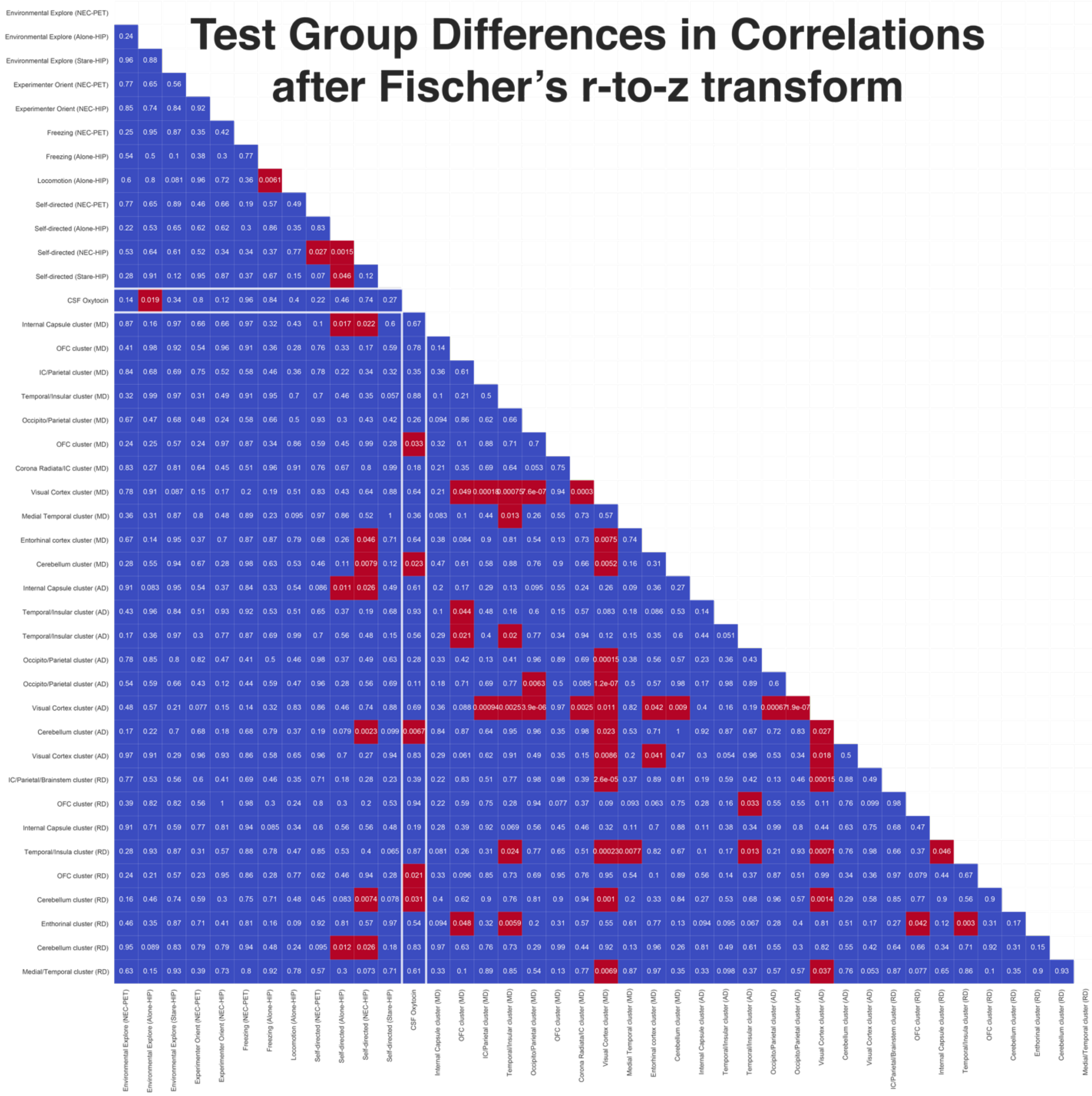


Figure S11. Group differences in correlations. To test if the correlations were significantly different between PR and control animals we ran a Fischer's r-to-z transformation so we could run t-tests on each measure comparison. Red indicates a significant difference ($p<0.05$), and the numbers indicate the actual p-value. White lines are drawn between different modalities; behavior, endocrine and neuroimaging.

6 References

Avants, B.B., Tustison, N.J., Song, G., Cook, P.A., Klein, A., Gee, J.C., 2011. A reproducible evaluation of ANTs similarity metric performance in brain image registration. Neuroimage 54, 2033–2044. https://doi.org/10.1016/j.neuroimage.2010.09.025

Avants, B.B., Yushkevich, P., Pluta, J., Minkoff, D., Korczykowski, M., Detre, J., Gee, J.C., 2010. The optimal template effect in hippocampus studies of diseased populations. Neuroimage 49, 2457–2466. https://doi.org/10.1016/j.neuroimage.2009.09.062

Basser, P.J., Pajevic, S., Pierpaoli, C., Duda, J., Aldroubi, A., 2000. In vivo fiber tractography using DT-MRI data. Magn. Reson. Med. 44, 625–632. https://doi.org/10.1002/1522-2594(200010)44:4<625::AID-MRM17>3.0.CO;2-O

Bick, J., Zhu, T., Stamoulis, C., Fox, N.A., Zeanah, C., Nelson, C.A., 2015. Effect of early institutionalization and foster care on long-term white matter development a randomized clinical trial. JAMA Pediatr. 169, 211–219. https://doi.org/10.1001/jamapediatrics.2014.3212

Birn, R.M., Shackman, A.J., Oler, J.A., Williams, L.E., McFarlin, D.R., Rogers, G.M., Shelton, S.E., Alexander, A.L., Pine, D.S., Slattery, M.J., Davidson, R.J., Fox, a S., Kalin, N.H., 2014. Evolutionarily conserved prefrontal-amygdalar dysfunction in early-life anxiety. Mol. Psychiatry 19, 915–922. https://doi.org/10.1038/mp.2014.46

Christian, B.T., Fox, a S., Oler, J. a, Vandehey, N.T., Murali, D., Rogers, J., Oakes, T.R., Shelton, S.E., Davidson, R.J., Kalin, N.H., 2009. Serotonin transporter binding and genotype in the nonhuman primate brain using [C-11]DASB PET. Neuroimage 47, 1230–6. https://doi.org/10.1016/j.neuroimage.2009.05.090

Cook, P.A., Bai, Y., Seunarine, K.K., Hall, M.G., Parker, G.J., Alexander, D.C., 2006. Camino : Open-Source Diffusion-MRI Reconstruction and Processing 14, 22858.

Cox, R.W., 1996. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. Comput. Biomed. Res. 29, 162–73. https://doi.org/10.1006/cbmr.1996.0014

Eluvathingal, T.J., Chugani, H.T., Behen, M.E., Juhász, C., Muzik, O., Maqbool, M., Chugani, D.C., Makki, M., 2006. Abnormal brain connectivity in children after early severe socioemotional deprivation: a diffusion tensor imaging study. Pediatrics 117, 2093–2100. https://doi.org/10.1542/peds.2005-1727

Fox, A.S., Oler, J. a., Shackman, A.J., Shelton, S.E., Raveendran, M., McKay, D.R., Converse, A.K., Alexander, A., Davidson, R.J., Blangero, J., Rogers, J., Kalin, N.H., 2015. Intergenerational neural mediators of early-life anxious temperament. Proc. Natl. Acad. Sci. 201508593. https://doi.org/10.1073/pnas.1508593112

Fox, A.S., Shelton, S.E., Oakes, T.R., Davidson, R.J., Kalin, N.H., 2008. Trait-like brain activity during adolescence predicts anxious temperament in primates. PLoS One 3, e2570. https://doi.org/10.1371/journal.pone.0002570

Hanson, J.L., Adluru, N., Chung, M.K., Alexander, A.L., Davidson, R.J., Pollak, S.D., 2013. Early Neglect Is Associated With Alterations in White Matter Integrity and Cognitive Functioning. Child Dev. 00, n/a-n/a. https://doi.org/10.1111/cdev.12069

Hanson, J.L., Knodt, A.R., Brigidi, B.D., Hariri, A.R., 2015. Lower structural integrity of the uncinate fasciculus is associated with a history of child maltreatment and future

psychological vulnerability to stress 27, 1611–1619. https://doi.org/10.1017/S0954579415000978
Howell, B.R., McCormack, K.M., Grand, A.P., Sawyer, N.T., Zhang, X., Maestripieri, D., Hu, X., Sanchez, M.M., 2013. Brain white matter microstructure alterations in adolescent rhesus monkeys exposed to early life stress: associations with high cortisol during infancy. Biol. Mood Anxiety Disord. 3, 21. https://doi.org/10.1186/2045-5380-3-21
Kalin, N., Shelton, S., 1989. Defensive behaviors in infant rhesus monkeys: environmental cues and neurochemical regulation. Science (80-.). 243, 1718–1721. https://doi.org/10.1126/science.2564702
Lazar, M., Weinstein, D.M., Tsuruda, J.S., Hasan, K.M., Arfanakis, K., Meyerand, M.E., Badie, B., Rowley, H. a, Haughton, V., Field, A., Alexander, A.L., 2003. White matter tractography using diffusion tensor deflection. Hum. Brain Mapp. 18, 306–21. https://doi.org/10.1002/hbm.10102
Leemans, A., Jones, D.K., 2009. The B-matrix must be rotated when correcting for subject motion in DTI data. Magn. Reson. Med. 61, 1336–1349. https://doi.org/10.1002/mrm.21890
Mori, S., Kaufmann, W.E., Davatzikos, C., Stieltjes, B., Amodei, L., Fredericksen, K., Pearlson, G.D., Melhem, E.R., Solaiyappan, M., Raymond, G. V, Moser, H.W., Zijl, P.C.M. Van, 2002. Imaging Cortical Association Tracts in the Human Brain Using Diffusion-Tensor-Based Axonal Tracking 223, 215–223. https://doi.org/10.1002/mrm.10074
Oler, J. a, Birn, R.M., Patriat, R., Fox, A.S., Shelton, S.E., Burghy, C. a, Stodola, D.E., Essex, M.J., Davidson, R.J., Kalin, N.H., 2012. Evidence for coordinated functional activity within the extended amygdala of non-human and human primates. Neuroimage 61, 1059–66. https://doi.org/10.1016/j.neuroimage.2012.03.045
Oler, J.A., Fox, A.S., Shelton, S.E., Rogers, J., Dyer, T.D., Davidson, R.J., Shelledy, W., Oakes, T.R., Blangero, J., Kalin, N.H., 2010. Amygdalar and hippocampal substrates of anxious temperament differ in their heritability. Nature 466, 864–868. https://doi.org/10.1038/nature09282
Sled, J.G., Zijdenbos, a P., Evans, a C., 1998. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. IEEE Trans. Med. Imaging 17, 87–97. https://doi.org/10.1109/42.668698
Tromp, D., 2016a. The diffusion tensor, and its relation to FA, MD, AD and RD. doi.org. https://doi.org/10.15200/winn.146119.94804
Tromp, D., 2016b. DTI Tutorial 3 - Fiber Tractography. doi.org. https://doi.org/10.15200/winn.146228.88526
Tromp, D.P.M., Fox, A.S., Oler, J.A., Alexander, A.L., Kalin, N.H., 2019. The Relationship Between the Uncinate Fasciculus and Anxious Temperament Is Evolutionarily Conserved and Sexually Dimorphic. Biol. Psychiatry 86. https://doi.org/10.1016/j.biopsych.2019.07.022
Wang, R., Benner, T., Sorensen, A.G., Wedeen, V.J., 2007. Diffusion Toolkit : A Software Package for Diffusion Imaging Data Processing and Tractography. Proc. Intl. Soc. Mag. Reson. Med. 15, 3720.
Woolrich, M.W., Jbabdi, S., Patenaude, B., Chappell, M., Makni, S., Behrens, T., Beckmann, C.,

Jenkinson, M., Smith, S.M., 2009. Bayesian analysis of neuroimaging data in FSL. Neuroimage 45, S173–S186. https://doi.org/10.1016/j.neuroimage.2008.10.055
Zhang, H., Yushkevich, P.A., Alexander, D.C., Gee, J.C., 2006. Deformable registration of diffusion tensor MR images with explicit orientation optimization. Med. Image Anal. 10, 764–85. https://doi.org/10.1016/j.media.2006.06.004