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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.

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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.

Keywords

Early Life Adversity; Rhesus Monkeys; Behavioral Assessment; Endocrine Measures; Neuroimaging Measures; Parallel Biological Pathways

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 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 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 Figure S1). Methods have previously been described (Andrew S 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 (Andrew S 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 minutes (2 minutes standard deviation [SD]), and average time between capture and CSF draw was 13 minutes (3 minute 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 1,900 × 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 (Andrew S 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 Figure 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 (Figure 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 (Figure 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) (Figure 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 (Figure 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 minutes 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 Figure 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 Figure S4B, Table 2, Supplementary Table S4). Self-directed behaviors include: self-mouth, self-groom, self-clasp, self-sex, and self-aggression (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 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 & Figure S3). No significant group differences were observed in plasma oxytocin levels, either at baseline or after NEC-PET (Table 3).

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 Figure S5, for overview Supplementary Figure 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 Figure 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 Figure S7A, Supplementary Figure S6, Supplementary Table S5, Supplementary Table S6). These clusters overlap in many regions with fibers from the internal capsule (Supplementary Figure 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 Figure 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 Figure S7C, Supplementary Figure 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 Figure 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 Figure 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 Figure S9 for the Control group and Supplementary Figure 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 Figure 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.

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 (Figure 2A, Figure 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; Figure 2). Importantly, numerous principal components differed between groups ($p<0.05$; Figure 2). These results are in accordance with a parallel model as outlined in Figure 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., 2013), 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 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; Brittany R Howell et al., 2013). 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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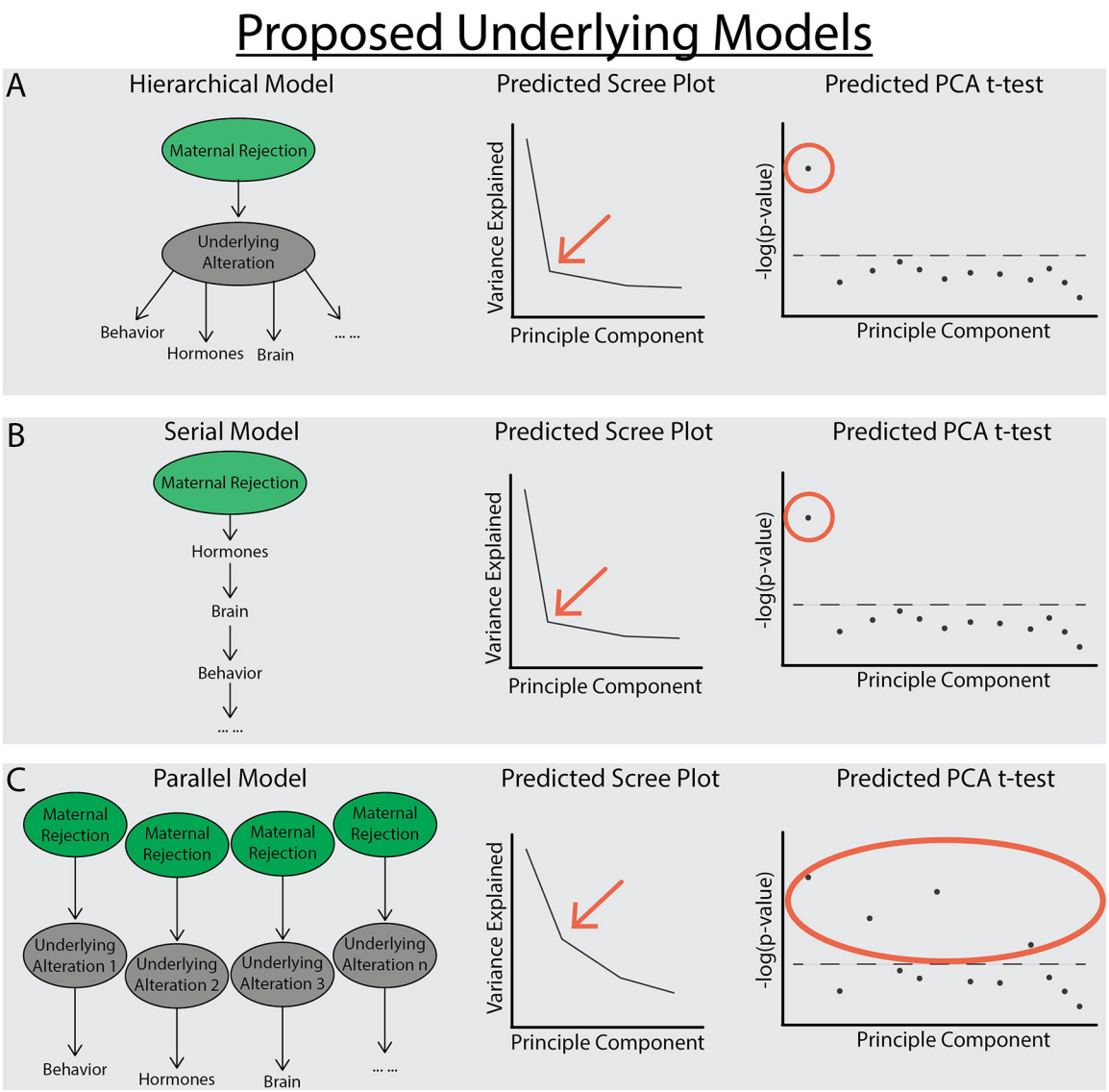

Figure 1. Proposed underlying models.

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.

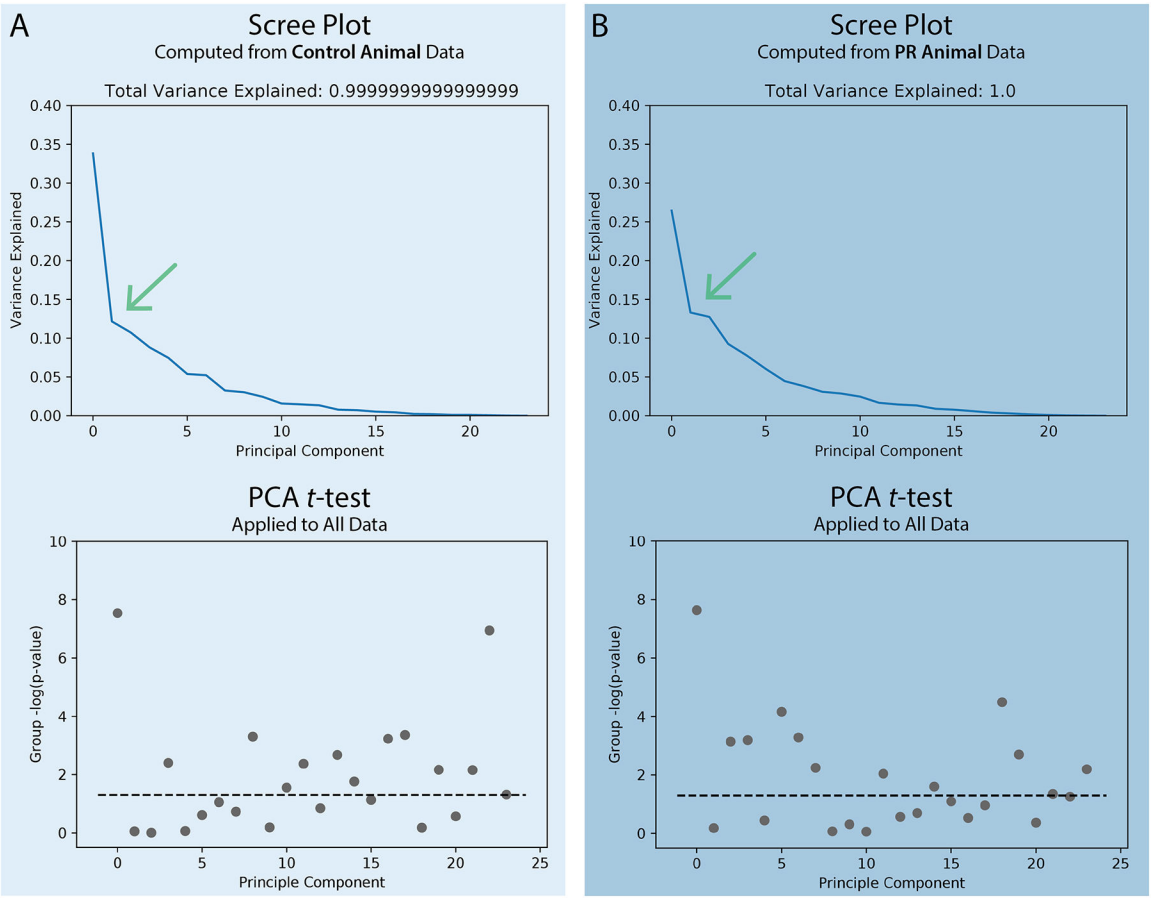

Figure 2. Overview of PCA results.

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).

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)

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)

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)

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