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Effects of Early-Life Stress on 5-HT$_{1A}$ Receptors in Juvenile Rhesus Monkeys Measured by PET

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Abstract

Background—Traumatic experiences in early childhood are associated with increased risk for developing mood and anxiety disorders later in life. Low serotonin$_{1A}$ receptor (5-HT$_{1A}$R) density during development has been proposed as a trait-like characteristic leading to increased vulnerability of stress-related neuropsychiatric disorders.

Methods—To assess the relationship between early-life stress and alterations in the serotonin system during development, we used positron emission tomography (PET) to measure *in vivo* 5-HT$_{1A}$R density and apparent dissociation constant (K_D^{app}) in the brain of juvenile rhesus monkeys exposed to the early-life stress of peer-rearing.

Results—In general, 5-HT$_{1A}$R density and K_D^{app} were decreased in peer-reared compared with control mother-reared animals. However, increase in receptor density was found in the dorsomedial prefrontal cortex of peer-reared females.

Conclusions—These findings suggest that exposure to an adverse early-life environment during infancy is associated with long-term alterations in the serotonin system and, support previous studies suggesting that reduced 5-HT$_{1A}$R density during development may be a factor increasing vulnerability to stress-related neuropsychiatric disorders. Further, alterations in the serotonin system

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Conflict of Interest
The authors report no biomedical financial interests or potential conflicts of interest.

appeared to be gender- and region-specific, providing a biological basis for the higher prevalence of affective disorders in women.

Keywords

serotonin$_{1A}$ receptor; PET; early-life stress; nonhuman primate; development

Low 5-HT$_{1A}$R binding, measured by PET and *in vitro* binding assays of postmortem brain tissue, is found in individuals with several anxiety-related disorders, including depression, social anxiety and panic disorders (1–8). Moreover, a spontaneous variation in the *HTR1A* gene promoter that results in lower levels of *HTR1A* expression (9) is also associated with anxiety-related personality traits (10).

Rodent studies have demonstrated that 5-HT$_{1A}$R knockout −/− and −/+ heterozygous mice demonstrate high levels of anxiety-like behaviors, while mice over expressing this receptor show reduced anxiety levels (11). Importantly, Gross and colleagues reported that the expression of 5-HT$_{1A}$Rs early during development, but not in adulthood, is critical to rescue the anxiety-related phenotype in knockout −/− mice (12). The above evidence from human and animal studies has lead to the proposal that low 5-HT$_{1A}$R density early in life may increase vulnerability for anxiety-related disorders (11,13).

Epidemiological data indicate a higher prevalence of mood and anxiety disorders in women compared to men, with these gender differences appearing first during puberty (14,15), suggesting that, in light of the above evidence, lower 5-HT$_{1A}$R density might be expected in women. However, previous studies reported either increased 5-HT$_{1A}$R availability in women (16,17), or no gender differences (18). In addition, higher 5-HT$_{1A}$R availability measured by PET (19,20) and increased 5-HT$_{1A}$Rs *in vitro* binding (1) has also been reported in depressed patients.

These results seem in contrast with the hypothesis that low 5-HT$_{1A}$R density increases vulnerability for affective disorders. However, at least three factors need to be considered when interpreting these data. First, the reported studies were all performed in adults, while low 5-HT$_{1A}$R expression during development, not in the adulthood, is hypothesized to be a critical risk factor for anxiety-related disorders (12). Second, the primary outcome of PET studies is receptor availability, measured by binding potential. Although receptor availability is directly proportional to receptor density, it also reflects competition at the binding sites between the endogenous neurotransmitter and PET radioligand. This competition can be influenced by several factors, including variations in endogenous synaptic 5-HT levels, receptor internalization and/or changes in receptor affinity for 5-HT, all of which would affect receptor occupancy, and consequently, receptor availability for the exogenous PET radioligand. Finally, *in vitro* binding studies in humans are often conducted in post mortem tissue from suicide victims, which may not be representative of generalized affective disorders in the population or the *in vivo* characteristics of the receptor.

Among environmental factors, traumatic experiences during childhood are consistently linked to an increased risk for developing anxiety-related neuropsychiatric diseases (21). For example, early loss of a parent leads to increased risk for anxiety, depression, and abnormalities of the hypothalamic-pituitary-adrenal (HPA) axis (22,23). In this respect, rhesus macaques deprived of their parents during infancy provide a particularly germane animal model to investigate the long-term consequences of early-life adversity on 5-HT$_{1A}$R expression. Previous studies show that peer-reared (PR) monkeys exhibit dysfunctions of the 5-HT system, HPA axis, and high levels of anxiety-like behaviors compared to animals reared with their mothers (MR) (24–26). We employed this model to study the long-term effects of early-life stress on 5-HT$_{1A}$R

availability using PET and [^{18}F]FPWAY, an analogue of WAY100636 and a selective 5-HT_{1A}R antagonist (27,28).

By definition, receptor availability is proportional to total receptor density (B_{max}) and radioligand affinity for the receptor ($1/K_D^{app}$, where K_D^{app} is the apparent dissociation constant). We employed an experimental design that allowed us to calculate B_{max} and K_D^{app} of [^{18}F]FPWAY to determine the contribution of each parameter to potential receptor availability alterations. Importantly, K_D^{app} represents the dissociation constant of radioligand (K_D) adjusted for occupancy by the endogenous neurotransmitter (29). We hypothesized that juvenile PR monkeys would show decreased 5-HT_{1A}R density and availability compared to MR monkeys and that the effect would be more pronounced in females.

Methods and Materials

Subjects

Protocols for the care and use of experimental animals were approved by the Institutional Animal Care and Use Committee of the National Institute on Alcohol Abuse and Alcoholism, National Institute on Drug Abuse (NIDA) and National Institute of Child Health and Human Development, National Institutes of Health (NIH).

Twenty-one rhesus macaques (*Macaca mulatta*), representing 2 birth cohorts, were born and housed at the NIH Animal Center in Poolesville, MD. At birth, subjects were randomly assigned into two groups with different social and rearing experiences. MR monkeys (n=11; 6 males) were reared for the first 6 months of life with their biological or adopted mothers and fathers in social groups comprised of 8 to 12 adult females (about half of whom had same-aged infants) and 2 adult males.

PR monkeys (n=10; 5 males) were separated from their mothers and housed in an incubator for the first 14 days of life. From day 14 to 37, they were placed alone in a nursery cage and provided a blanket and a terry cloth–covered, rocking surrogate. At day 37, they were placed in a cage with 3 other age-mates with whom they thereafter had continuous access. At 6 months of age, MR subjects were removed from their mother and social group and housed together with the PR monkeys. Thereafter, both groups received identical treatment (26).

The study was conducted when the monkeys were about 24 months old (Table 1), corresponding to childhood in terms of human brain development (6–8 years old) (30). The animals were transported in groups of four to the NIDA-IRP in Baltimore, where they were housed in pairs for about one month, during which time the study was performed. One week before their transport, cerebrospinal fluid (CSF) was collected and assayed for baseline levels of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) [(26) and Supplemental Methods and Materials in Supplement 1].

PET Imaging

Radiochemistry—[^{18}F]FPWAY was produced as described (31). There were no differences in the specific activity across groups [males: 1521.2 ± 341.4, females: 2014.8 ± 331.9 ($p>0.3$); MR: 1454.5 ± 206.9, PR: 2081.5 ± 419.0 ($p>0.2$)].

Data acquisition—PET data were acquired on a Siemens Exact ECAT HR+ whole body tomograph (63 slices, center to center spacing of 2.4mm, with an in-plane resolution, full width at half maximum (FWHM), of 4.7mm at the center of the field of view and axial spatial resolution (or slice thickness) of 4.2mm in 3D mode. Before each radioligand administration, transmission scans were acquired with three rotating ^{68}Ge-^{68}Ga sources. Transmission scans were used to correct the emission scans for the attenuation of 511keV photons by body tissue

and face mask. PET images were reconstructed from the raw data with a standard filtered back-projection algorithm and a RAMP filter.

Each monkey was anesthetized with 1.5mg/kg alfadolone and alfaxolone acetate (Saffan®, Arnolds Veterinary Products, Shropshire, U.K.), given intramuscularly. The monkey was intubated, an arterial line inserted for subsequent blood sampling and an intravenous line was set for the continuous intravenous infusion of 9–14mg/kg/h Saffan® to allow transport of the animal from the Animal Facility to the PET Center. Anesthesia was subsequently maintained throughout the PET study by 1.5–2.0% isoflurane.

An individually molded thermoplastic face mask was secured to a custom-made monkey head-holder attached to a backboard. The monkey's head was positioned in the gantry with the aid of orthogonal laser lines. [^{18}F]FPWAY, 6–18mCi, was administered intravenously as a bolus followed by a constant infusion (B/I) to reach an equilibrium state of radioactivity distribution. The bolus component delivered a volume of radiotracer equivalent to the volume that would be administered in the subsequent 60min infusion period (K_{bol}=60min). Acquisition of dynamic PET scans started with the beginning of the bolus component administration and continued for 180–210min. At 120min after the bolus infusion, an unlabelled FPWAY injection started as B/I (K_{bol}=40min) corresponding to the equilibrium infusion rate of 0.15nmol/kg/min. Specific activity in the second period was calculated by the ratio of the radioactivity infusion rate to the mass infusion rate. Blood samples were drawn at predetermined times from the arterial catheter with the first sample before dosing and subsequent samples following attainment of equilibrium (70 to 210min); plasma radioactivity and concentration of non-metabolized [^{18}F]FPWAY not bound to plasma proteins were measured (32). Vital signs, including heart rate, EKG, respiration rate, end tidal CO_2 and blood oxygen saturation (maintained above 95%) were continuously monitored during the study.

Magnetic resonance imaging (MRI)—Structural brain images were acquired on a 3.0 Tesla Siemens Magnetom Allegra MRI (Siemens Medical Solutions, Inc., Malvern, PA, U.S.A.). MRI and PET acquisitions were separated by at least 2 days to allow full recovery between studies [(34) and Supplement 1].

Co-registration and region-of-interest (ROI) placement—A ROI analysis was performed on brain regions previously implicated in emotional regulation and stress reactivity (21,33) and known to be vulnerable to stress exposure (21,34): amygdala (AMY), hippocampus (HC), dorsomedial prefrontal cortex (dmPFC) and anterior and medial cingulate cortex (ACC and MCC). We also included the midbrain raphe nuclei (RN), an area where 5-HT_{1A}Rs are present in high density and are organized as somatodendritic autoreceptors that help regulate 5-HT neurotransmission in other brain regions (35). ROIs were drawn on the T1-weighted MRI images of each monkey with reference to a stereotaxic atlas (36) (in Supplement 1, see Methods and Materials and Figure S1), and were co-registered to the PET images. For co-registration, MRI images of each monkey were re-sliced to the PET voxel size (1.14mm × 1.14mm × 2.45mm) and averaged between 90 and 120min after the start of [^{18}F]FPWAY administration. PET images were manually aligned to their corresponding T1 MRI images (Fig. 1) using Fusion mode in PMOD v.2.7, (PMOD Technologies Ltd., Zurich, Switzerland). The obtained transformation parameters were applied to the respective re-sliced dynamic PET images. Time–activity curves were calculated based on the mean radioactivity (kBq/ml) for each ROI (Fig. 2a).

PET Data Analyses

Binding potential (BP) was calculated as BP_{ND} and BP_F defined by consensus nomenclature for *in vivo* imaging of reversible radioligand binding (37). Here we report BP_{ND} calculated as

the ratio of specifically bound radioligand concentration to that of non-displaceable radioligand concentration in tissue (($C_{ROI} - C_{ND}$)/C_{ND}) at equilibrium ($BP_{ND} = (C_{ROI} - C_{ND})/C_{ND} = f_{ND}B_{max}/K_D^{app}$) [results for BP_F were similar and given in Supplement 1].

The 5-HT_{1A}R B_{max} and the K_D^{app} of [^{18}F]FPWAY in each brain region were calculated using Scatchard analysis (29). K_D^{app} is defined as the *in vivo* K_D adjusted for occupancy by 5-HT and by [^{18}F]FPWAY non-displaceable fraction in tissue. After equilibrium was reached (first point: 90 to 120min after the start of [^{18}F]FPWAY injection; second point 50 to 90min after the beginning of unlabelled [^{18}F]FPWAY administration (Fig. 2b), specifically bound radioactivity was calculated as the difference between the total radioactivity measured in a brain ROI (C_{ROI}) and that in the cerebellum (C_{CB}). ($C_{ROI} - C_{CB}$) was plotted against ($C_{ROI} - C_{CB}$)/ C_{CB}. Due to technical problems, data acquisition for one male MR monkey was stopped after 120min and thus B_{max} and K_D^{app} could not be calculated for this animal.

Statistical Analyses

StatView 5.0.1 (SAS Institute, Inc., Cary, NC) was used for all statistical analyses. When ROIs were considered as a group, a multivariate analysis of variance (MANOVA) was conducted to assess the overall effects of rearing condition on B_{max}, K_D^{app} and BP_{ND}. Analyses were performed on the natural logarithm of the data to achieve homogeneity of variances, after adding 2.5 to all measures to ensure positivity. However, Tables and Figures report actual, not log-transformed values. A two-way analysis of variance (ANOVA) was conducted with rearing condition (MR or PR) and sex (male or female) as independent variables for BP_{ND} data in each ROI, as well as physiological measures. Significance was set at $p<0.05$ two-tailed alternatives, not adjusted for multiple comparisons.

Results

Effects of Rearing Conditions and Sex on Physiological Measures

ANOVA showed no difference in age across rearing group ($F(1,17)=1.70$, $p>0.20$) or sex ($F(1,17)=0.34$, $p>0.33$), as well as no difference in body weight across rearing conditions ($F(1,17)=2.63$, $p>0.74$). However, as expected, females had lower body weight than males ($F(1,17)=12.62$, $p<0.003$). CSF 5-HIAA concentrations were lower in PR animals ($F(1,12)=5.23$, $p<0.05$), but there was no difference between sexes ($F(1,12)=1.42$, $p>0.26$; Table 1).

Pattern of 5-HT_{1A}R Distribution in the Brain and K_D^{app}

Generally, the pattern of BP_{ND} values (Table 4) across studied brain areas within each of the four groups was similar to that previously reported in human PET experiments (18) and followed the pattern of 5-HT_{1A}R brain distribution observed using *in vitro* binding assay of postmortem human brain tissue (38). BP_{ND} and B_{max} values varied across studied brain areas. The HC and MCC showed the highest 5-HT_{1A}R density and BP_{ND} values under both rearing conditions followed by MCC, ACC, AMY and DmPFC, respectively. The lowest BP_{ND} and B_{max} values were determined in RN (Fig. 3, Table 2 and 4).

The AMY showed the highest K_D^{app} values for both rearing conditions followed by HC (Table 3, but see PR males). Compared to the AMY, K_D^{app} values were 30–50% lower in the other regions (MCC, ACC, dmPFC, RN), which also showed limited variability (2–12%) across brain areas within each experimental group.

Effect of Rearing Condition and Sex on 5-HT_{1A}R B_{max} and K_D^{app}

For the 10 MR and 10 PR juvenile monkeys analyzed ([^{18}F]FPWAY specific activity was not available for one MR male monkey), a MANOVA for B_{max} values across all ROIs revealed a trend for lower 5-HT_{1A}R density ($F(6,11)=2.60$, $p>0.08$, Table 2) and significantly lower

K_D^{app} values ($F(6,11)=4.27$, $p<0.02$, Table 3) in PR animals. There was no effect of sex on B_{max} or K_D^{app} ($F(6,11)=0.99$, $p>0.47$ and $F(6,11)=0.93$, $p>0.51$, respectively) nor was there a rearing × sex interaction ($F(6,11)=0.55$, $p>0.76$ and $F(6,11)=0.91$, $p>0.52$ for B_{max} and K_D^{app} , respectively).

Similarly, MANOVA for K_D^{app} across all ROIs revealed lower values in PR animals ($F(6,11)=4.27$, $p<0.02$, Table 3). Again, there was no effect of sex ($F(6,11)=0.93$, $p>0.51$) or rearing × sex interaction ($F(6,11)=0.91$, $p>0.52$). Two-way ANOVAs on each ROI revealed a significant rearing effect in the HC ($F(1,16)=4.80$, $p<0.05$); no sex ($F(1,16)=1.20$, $p>0.28$) or rearing × sex interaction ($F(1,16)=0.32$, $p<0.57$) effects were found. There was no significant correlation between mean K_D^{app} (or B_{max} values in any brain ROI) and 5-HIAA concentrations.

Effect of Rearing Condition and Sex on 5-HT_{1A}R Availability

Peer-rearing affected BP_{ND} values in several brain regions as demonstrated by MANOVA ($F(6,12)=5.74$, $p<0.006$), in the absence of a main effect of sex ($F(6,12)=0.38$, $p>0.87$) or a rearing × sex interaction ($F(6,12)=1.63$, $p>0.22$). Two-way ANOVAs on each ROI revealed a significant rearing effect in the dmPFC ($F(1,17)=6.79$, $p<0.02$) but no sex effect ($F(1,17)=0.97$, $p>0.33$). There was a significant rearing × sex interaction in the following three brain regions: dmPFC ($F(1,17)=6.81$, $p<0.02$), MCC ($F(1,17)=4.79$, $p<0.05$) and ACC ($F(1,17)=5.88$, $p<0.03$), but no main effect of rearing or sex [MCC rearing ($F(1,17)=0.96$, $p>0.34$), sex ($F(1,17)=0.49$, $p>0.49$); ACC rearing ($F(1,17)=0.32$, $p>0.58$), sex ($F(1,17)=0.18$, $p>0.68$)]. Because of the rearing × sex interaction, we conducted an unpaired t-test for each sex separately.

In PR females, BP_{ND} values were greater in the dmPFC compared to control females ($t(1,8)=-3.73$, $p<0.006$; Table 4 and Fig. 3), while in males there was no significant effect of rearing condition in this area ($t(1,9)=0.00$, $p>0.99$). However in males, peer-rearing induced a significant reduction in BP_{ND} values in the MCC ($t(1,9)=2.66$, $p<0.03$) and the ACC ($t(1,9)=2.55$, $p<0.04$; Table 4 and Fig. 3), with no significant effect in female monkeys in either of these areas ($t(1,8)=-0.74$, $p>0.48$ and $t(1,8)=-1.12$, $p>0.29$, respectively). There was no significant correlation between 5-HIAA concentration and BP_{ND}.

Discussion

The present study shows that early-life stress as a result of parental deprivation affects 5-HT_{1A}Rs density and radioligand affinity. In both males and females, peer-rearing induced an overall decrease in B_{max} and in K_D^{app}. The exception was the dmPFC in females, where B_{max} values were elevated. Our results are consistent with the hypothesis that exposure to an adverse environment during infancy has long-term consequences on the 5-HT system. To our knowledge, these findings provide the first evidence in nonhuman primates for such developmental changes in an aspect of the 5-HT system that has been strongly linked to anxiety and depression.

Overall our findings in nonhuman primates are consistent with and extend the data from human PET and *in vitro* binding studies of post mortem brain tissue suggesting a down-regulation of 5-HT_{1A}Rs associated with anxiety disorders (39,40). The present results are also consistent with previous evidence indicating decreased 5-HT_{1A}R availability using PET in adult females macaques with signs of behavioral “depression”(41), and decreased 5-HT_{1A}R binding and mRNA expression *in vitro* in the HC in marmoset monkeys exposed to early-life stress (42). Moreover, low 5-HT_{1A}R binding in the HC and ACC was found in male rats exposed to early-life stress (43).

Surprising, early-life stress was associated with increased 5-HT_{1A}R density the in the dmPFC in female monkeys. Using structural MRI in the same cohort of monkeys, we recently reported (34) increased volume of the dmPFC and cingulate cortex in male and female PR animals. As such, it is possible that partial volume effect contributed to the present result. However, lower B_{max} values in females PR monkeys were found in the ACC, a region that also showed increased gray matter volume in PR animals, suggesting that any structural changes in the dmPFC may not fully account for the present B_{max} differences. Gender differences in PFC 5-HT_{1A}R density have been observed in humans using *in vitro* binding assays from postmortem brain tissue (44). Moreover, specific changes in 5-HT_{1A}R protein levels were found in PFC of depressed female patients (45). As a part of the 'medial prefrontal network', the dmPFC, together with the cingulate cortex, is thought to modulate activity in limbic structures (e.g. AMY and HC) known to be important for the processing of emotional information (46,47). Taken together, these data suggest that gender differences in brain regions involved in the cognitive control of emotional regulation are present on a molecular level, and may contribute to the increased vulnerability to affective disorders in women.

In addition to the tendency for an overall decrease in B_{max} values, we also found an increased radioligand affinity for 5-HT_{1A}R in PR animals, as shown by the decreased K_D^{app} values, particularly in the HC. Reduced K_D^{app} values reflect less competition between radioligand and endogenous 5-HT at the receptor sites, which could be related to one or more of the following: lower baseline 5-HT levels, reduced 5-HT affinity for 5-HT_{1A}R as a result of conformational receptor alterations, or such other factors as receptor internalization (48). Unfortunately, PET analysis limitations do not allow us to define the exact reason for the observed changes in radioligand affinity. Nevertheless, the decreased CSF 5-HIAA concentrations found in PR animals in the present study, and throughout development by earlier findings (24,26), support the hypothesis of lower brain 5-HT levels in PR animals. However, since neither K_D^{app} nor CSF 5-HIAA concentrations represent direct measures of 5-HT levels in the brain, future investigations using other techniques, such as microdialysis, are warranted to confirm that early-life stress affects endogenous 5-HT in PR animals. In rodents, early-life stress has been shown to affect both basal 5-HT and 5-HIAA levels in several brain regions (49), and previous data obtained by *in vitro* assays in rodents exposed to chronic stress showed decreased 5-HT_{1A}R density and increased 5-HT_{1A}R affinity in the HC (50), indicating that stress might induce transcriptional or post-transcriptional effects on 5-HT_{1A}R in this region.

Our results on 5-HT_{1A}R availability calculated as BP_{ND} (and BP_F, see Supplement 1) indicate that the effect of peer-rearing on BP_{ND} was different in males and females. B_{max} values were decreased in most brain regions in male and female PR animals. However, while overall lower 5-HT_{1A}R availability was found in PR males, it was increased in PR females compared to control MR animals. These gender differences in receptor availability may be related to reported differences in SSRI efficacy between men and women (51). As mentioned in the Introduction, differences in 5-HT_{1A}R availability can be linked to changes in receptor density, radioligand apparent affinity ($1/K_D^{app}$) or both as demonstrated in the current study. Our results further emphasize the importance of considering changes in receptor availability along with concomitant changes in 5-HT_{1A}R density and *in vivo* affinity.

Consequences of the findings

Overall these data suggest that early-life stress is associated with changes in the development of the 5-HT system in brain regions considered to be critically involved in major depression and to be modulated by antidepressant treatment (47). An impaired interaction between neural networks involved in emotional and cognitive processing is proposed as a key dysfunction in mood disorders (46,47). Specifically, it is suggested that the dmPFC, dorsal ACC and posterior cingulate cortex are part of a "dorsal network" involved in emotional and cognitive deficits,

while the HC and the AMY are part of a "ventral network" associated with vegetative and somatic symptoms of mood disorders; the (rostral) ACC, which is connected to both networks, is thought to have a more regulatory function (46). Moreover, antidepressant treatment, known to increase 5-HT levels, is known to modulate neural activity in these regions/networks (52).

In this context, our data indicate that peer-rearing stress affects 5-HT_{1A}R density and *in vivo* affinity in brain regions known to play a key role in affective disorders. Affinity changes, particularly in the HC, may be important considering that the SSRI efficacy seems to be mediated by 5-HT_{1A}R and by neurogenesis in the HC (53). It is also possible that region- and sex-specific changes in 5-HT_{1A}R function during development have important consequences on cognitive abilities (54). The 5-HT system is implicated in the behavioral and brain responsiveness to punishment (55). Modulation of the 5-HT system has been shown to affect neural activity in dmPFC during negative feedback processing in humans (56) and a disrupted top-down control by dmPFC over AMY has been proposed to underlie some of the negative feedback deficits reported in depression (57). Recently, increased responding to threat (and reward) has been reported specifically in two-year old PR males (58), supporting the hypothesis that 5-HT changes may affect the processing of negative information. However, it is unclear if and how 5-HT_{1A}R alterations during development may be related to cognitive differences in humans and potentially to increased vulnerability for affective disorders later in life.

Limitations of the study

We found no differences in B_{max} values between male and female monkeys in either the MR or PR group. Although the number of subjects (n=21) was relatively high for a nonhuman primate study, it may still not have been sufficient to demonstrate more subtle differences between males and females. Previous PET studies in humans showed either no changes or elevated 5-HT_{1A}R availability in healthy adult women (16–18). Conversely, there are as yet no data regarding the levels of brain 5-HT_{1A}R in children, which due to issues of radioactivity administration, are not likely to be easily obtained. Gender differences in 5-HT_{1A}R in the adult nonhuman primate brain is also not available at this point in time. However, since *in vitro* data from rodents show sex differences in 5-HT_{1A}R binding both in adults and throughout development (59,60), it is possible that sex specific differences are also present during development in nonhuman primates. Thus, future *in vitro* binding and microdialysis studies will be important to confirm our PET results and to investigate possible sex differences in 5-HT_{1A}R density and 5-HT levels occurring during development.

In conclusion, we provide the first evidence in nonhuman primates that a model of early-life stress affects 5-HT_{1A}R density and *in vivo* affinity in juvenile monkeys. Specifically, we found decreased 5-HT_{1A}R density and K_D^{app} in PR animals. However, in females, early-life stress induced an increase in 5-HT_{1A}R density in the dmPFC, a brain region involved in emotional regulation. Overall these findings support the hypothesis that low 5-HT_{1A}R density during development may be related to an increased risk for affective disorders. Moreover, sex difference may be present early in development and need to be considered in the context of the increased vulnerability for mood and anxiety disorders reported in women.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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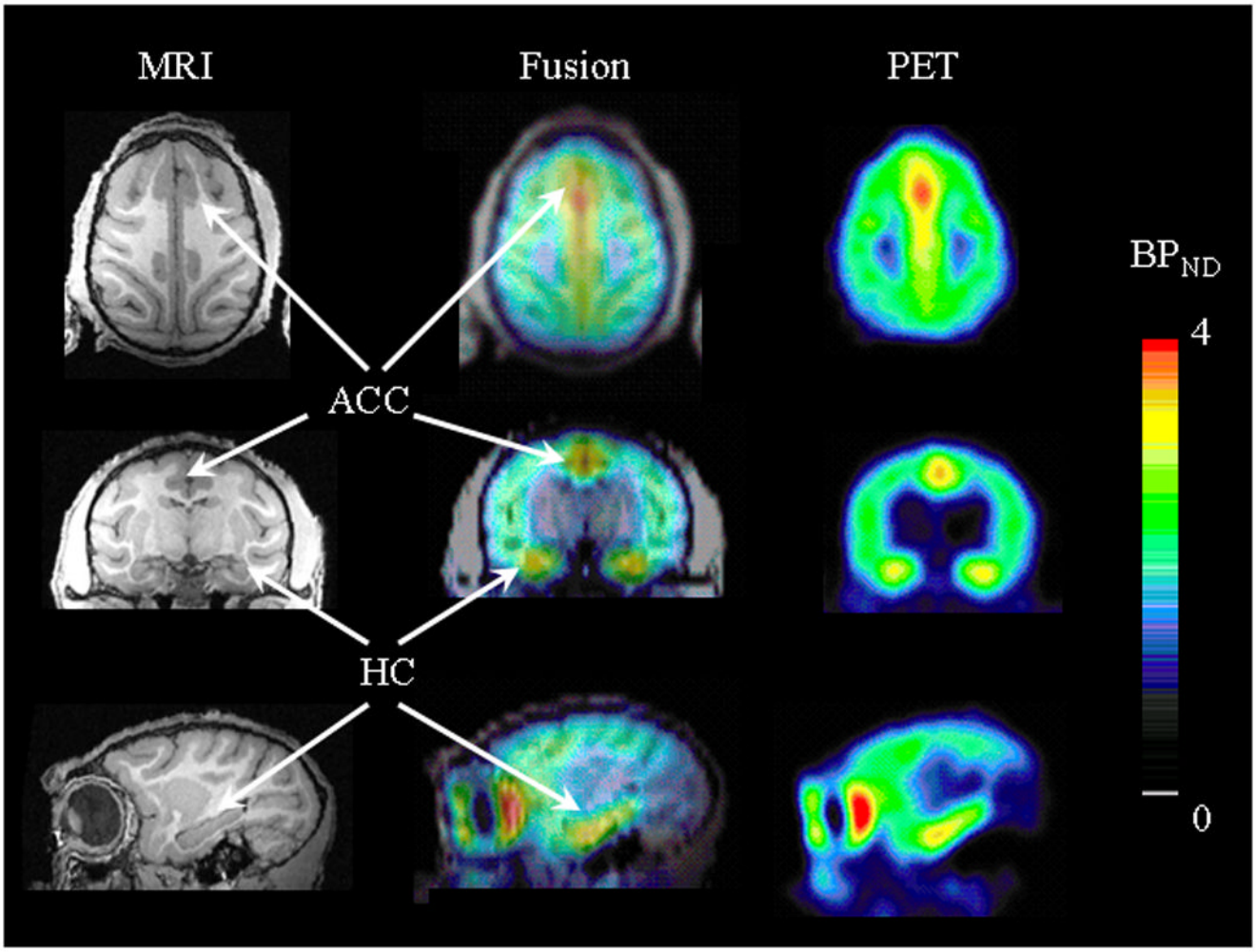

Figure 1.
Representative images of Rhesus monkey brain in transaxial (top panel), coronal (middle panel) and sagittal (bottom panel) view. On the left – T1 MRI scans used for co-registration with parametric BP_{ND} maps for ROI placement (middle panel). Parametric BP_{ND} maps were calculated from bolus plus infusion PET studies with [^{18}F]FPWAY and PMOD (right panel). Pseudocolor bar represents BP_{ND} values (arbitrary unit).

Figure 2a

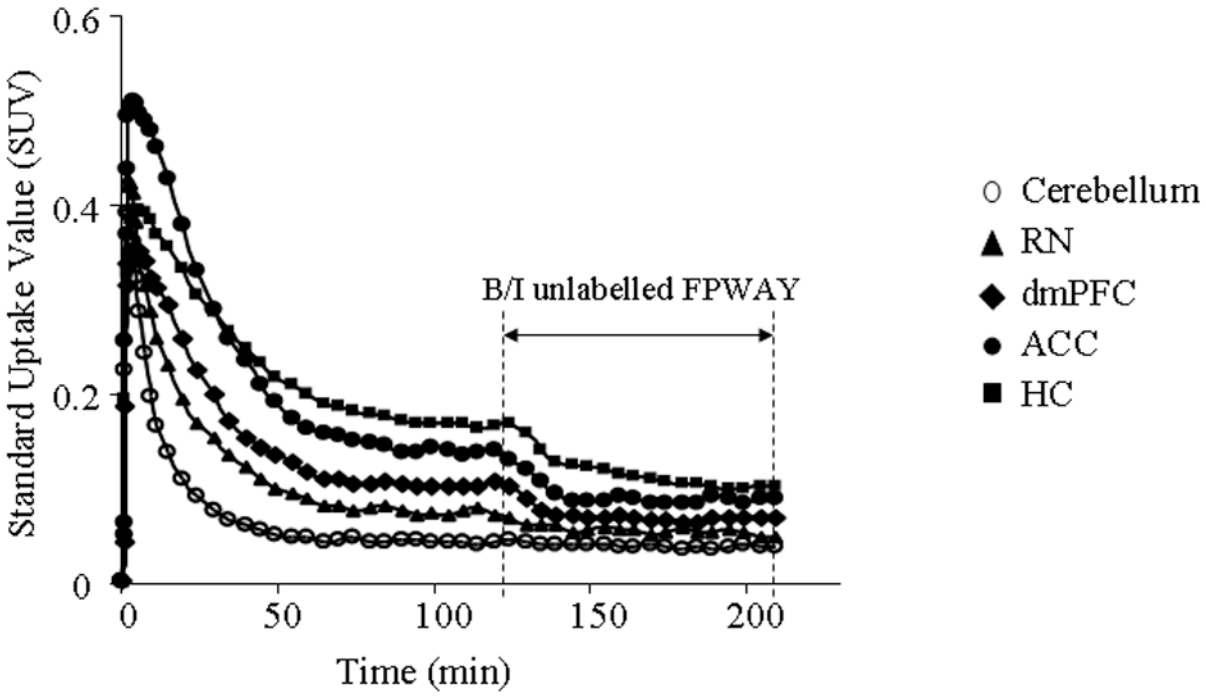

Figure 2b

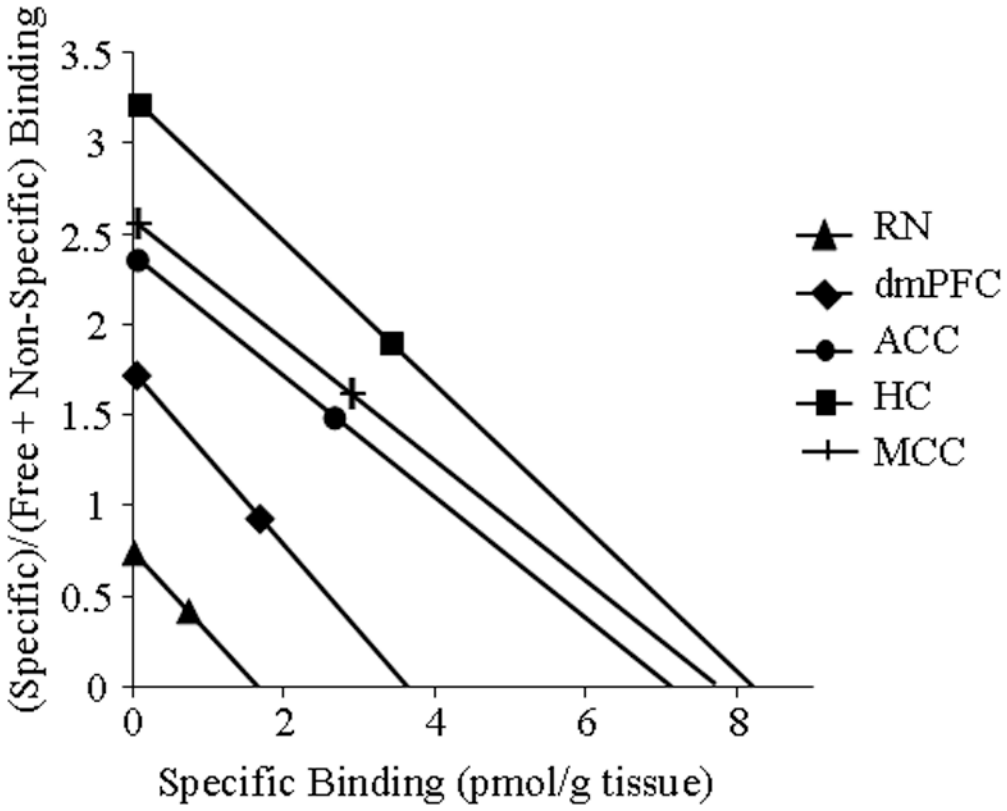

Figure 2.

Time-activity curves (TAC) (a) and Scatchard plots (b) from brain areas of a single animal. A: Y-axis - standard uptake value (SUV) (fraction of injected dose/g body weight/g tissue), X-axis – time after [^{18}F]FPWAY administration onset. [^{18}F]FPWAY was injected as a bolus followed by a constant infusion (B/I) to reach radioactivity equilibrium distribution. 120 min after the B/I of [^{18}F]FPWAY, unlabelled FPWAY was co-injected as bolus plus constant infusion (B/I) for another 90 min to reach a second equilibrium state. B: Two points for Scatchard analysis were obtained from TAC, averaging the data from 90 to 120 min after the start of [^{18}F]FPWAY injection; second point - 50 to 90 min after the beginning of unlabelled FPWAY administration, respectively (see Methods for details). HC - hippocampus, MCC - medial cingulate cortex, ACC - anterior cingulate cortex, AMY - amygdala, dmPFC - dorsomedial prefrontal cortex, and RN - raphe nuclei (midbrain).

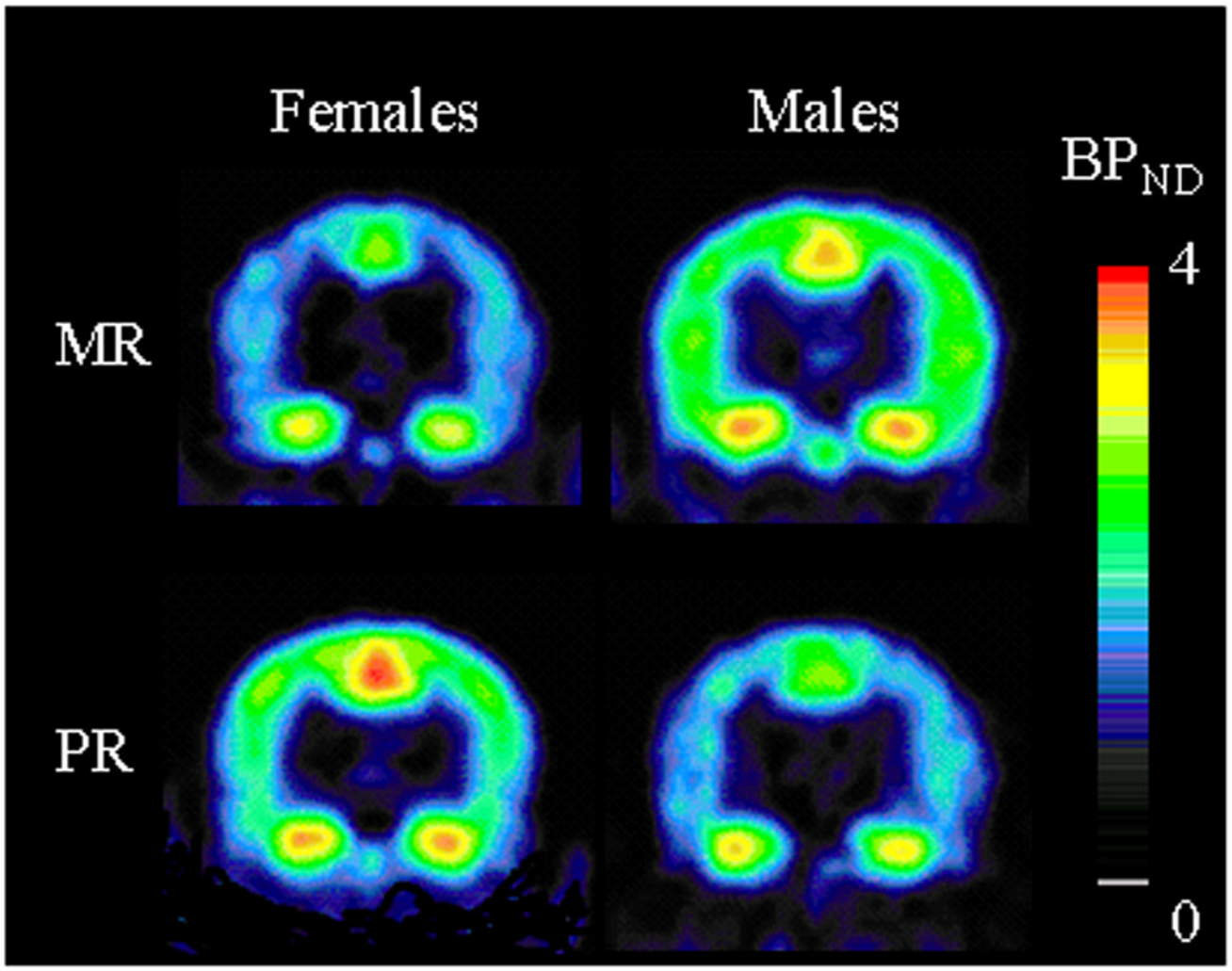

Figure 3.
Coronal view of BP_{ND} parametric maps obtained with [18F]FPWAY in four representative animals. Top row: MR animals - bottom row: PR animals - left column: females - right column: males. Images correspond to coronal slices at the level 15–16 mm from the interaural line (36). Pseudocolor bar represents BP_{ND} values (arbitrary unit).

Table 1

Demographic and physiological measures

Measures	Female MR	Female PR	Male MR	Male PR
Age (months)	25.80 ± .86	25.60 ± .25	24.17 ± .48	26.00 ± .78
Body weight (kg) *	3.32 ± .21	3.10 ± .07	3.61 ± .12	3.74 ± .09
CSF 5-HIAA (pmol/ml) #	281.66 ± 24.26	212.73 ± 8.68	232.76 ± 34.54	256.35 ± 11.60

Results are presented as mean ± s.e.m.

* Body weight was significantly smaller in females compared to males (ANOVA, $p<0.005$).

Baseline CSF 5-HIAA concentrations were significantly lower in PR compared to MR monkeys (ANOVA, $p<0.05$).

Table 2

B_{max} (pmol/mL) values measured by [^{18}F]FPWAY in PR and MR juvenile rhesus monkeys

ROI	Female MR	Female PR	% Difference in females	Male MR	Male PR	% Difference in males	% Difference MR - PR
HC	8.00 ± 2.07	5.46 ± 1.64	−31.75	13.30 ± 3.54	6.14 ± 2.15	−53.83	−45.44
MCC	5.98 ± 1.75	4.42 ± 1.07	−26.09	9.80 ± 2.11	5.61 ± 1.68	−42.76	−36.44
ACC	5.14 ± 1.34	3.84 ± .92	−25.29	8.20 ± 1.24	5.20 ± 1.62	−36.59	−32.22
AMY	4.34 ± 1.64	2.61 ± .96	−39.86	7.32 ± 1.73	5.38 ± 2.27	−26.50	−31.41
dmPFC	2.26 ± 0.79	2.96 ± .81	30.97	4.36 ± .86	3.31 ± 1.23	−24.08	−5.32
RN	1.48 ± 0.50	1.00 ± .20	−32.43	2.44 ± .44	1.38 ± .40	−43.44	−3.57

Data represent values averaged for right and left hemispheres for all animals in the group. HC - hippocampus, MCC - medial cingulate cortex, ACC - anterior cingulate cortex, AMY - amygdala, dmPFC - dorsomedial prefrontal cortex, RN – raphe nuclei (midbrain). Results are presented as mean ± s.e.m. % Difference is calculated as (mean MR values − mean PR values)/mean MR values*100. MANOVA across all ROIs showed a tendency for a significant rearing effect, $p<0.09$.

Table 3

K_D^{app} values (pmol/mL) measured by [^{18}F]FPWAY in PR and MR juvenile rhesus monkeys

ROI	Female MR	Female PR	% Difference in females	Male MR	Male PR	% Difference in males	% Difference MR - PR
HC	2.62 ± .70	1.50 ± .41	−42.75	4.08 ± 1.07	1.96 ± .72	−51.96	−48.36*
MCC	1.94 ± .56	1.22 ± .26	−37.11	2.84 ± .65	2.06 ± .62	−27.46	−31.38
ACC	1.90 ± .53	1.20 ± .26	−36.84	2.64 ± .47	1.98 ± .61	−25.00	−29.89
AMY	2.80 ± 1.17	1.69 ± .37	−39.64	4.28 ± 1.15	3.61 ± 1.67	−15.65	−25.07
dmPFC	1.60 ± .44	1.23 ± .26	−23.13	2.46 ± .46	1.84 ± .69	−25.20	−24.14
RN	1.72 ± .54	1.09 ± .17	−36.63	2.46 ± .31	1.83 ± .55	−25.61	−30.05

Abbreviations are as in Table 2. Results are presented as mean ± s.e.m. % Difference is calculated as (mean values for MR group − mean values values for PR group)/mean values for MR group *100. MANOVA across all ROIs showed a significant rearing effect, $p<0.05$.

* Rearing effect in the HC, ANOVA, $p<0.05$.

Table 4

BP_{ND} values of [^{18}F]FPWAY in brain regions in MR and PR juvenile rhesus monkeys

ROI	Female MR	Female PR	% Difference in females	Male MR	Male PR	% Difference in males	% Difference MR-PR
HC	3.05 ± .16	3.36 ± .19	10.16	3.08 ± .14	3.13 ± .13	1.62	5.91
MCC	3.03 ± .24	3.25 ± .19	7.26	3.31 ± .16	2.71 ± .15	−18.13^	−6.35
ACC	2.64 ± .22	2.95 ± .16	11.74	2.97 ± .14	2.47 ± .13	−16.84^	−4.04
AMY	1.59 ± .09	1.65 ± .09	3.77	1.67 ± .07	1.60 ± .12	−4.19	−0.86
dmPFC	1.43 ± .07	2.23 ± .22	55.94**	1.67 ± .13	1.67 ± .16	0.00	24.82*
RN	.80 ± .09	.80 ± .06	0.00	.91 ± .09	.77 ± .04	−15.38	−8.04

Abbreviations are as in Table 2. Results are presented as mean ± s.e.m. % Difference is calculated as (mean values for MR group − mean values for PR group)/mean values for MR group*100.

MANOVA across all ROI showed a significant rearing effect, $p<0.01$.

* Rearing effect in the dmPFC, ANOVA, $p<0.02$.

** Rearing effect in the female group, unpaired t-test, $p<0.04$.

^ Rearing effect in the male group, unpaired t-test, $p<0.04$.