

Behavioral Depression and Positron Emission Tomography–Determined Serotonin 1A Receptor Binding Potential in Cynomolgus Monkeys

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Context: Current animal models of depression are inadequate to further our understanding of depression. New models that allow for analysis of cognitive function and sex differences are needed.

Objective: To characterize serotonin 1A (5-HT_{1A}) receptor binding potential (BP) and its relationship with specific characteristics of behavioral depression in cynomolgus monkeys.

Design: A 23-month case-control study.

Setting: Small social groups in the laboratory.

Subjects: Seventeen adult female cynomolgus monkeys.

Main Outcome Measures: Serotonin 1A receptor BP was examined by positron emission tomography using the radioligand 4,2'-(methoxyphenyl)-1-[2'-(N-2'-pyridinyl)-p-fluorobenzamido]ethylpiperazine in the raphe, amygdala, hippocampus, and anterior cingulate cortex in monkeys characterized by behavioral observation as depressed or not depressed. Aggression, submission, affiliation, pathologic behaviors, and activity levels were determined by behavioral observation. Heart rate and hypothalamic-pituitary-adrenal function were also determined.

Results: Throughout the brain areas examined, there was a reduction in 5-HT_{1A} BP in depressed monkeys. The 5-HT_{1A} BP in the amygdala and hippocampus was associated with aggression and submission. Friendly interaction, grooming, and locomotion were associated with 5-HT_{1A} BP in the left cingulate cortex, whereas attention directed toward the environment was associated with 5-HT_{1A} BP in the right cingulate cortex. The 5-HT_{1A} receptor BP was inversely associated with heart rate in the raphe, left cingulate, and right amygdala.

Conclusions: This is the fourth in a series of studies that suggest that depressive behavior in adult female cynomolgus monkeys is similar to that observed in humans. It has been observed in 2 large groups of monkeys randomly selected from feral populations, suggesting that the capacity for depression is inherent in the species. This animal model holds promise to further our understanding of the basic mechanisms of affective behavior, the neuropathophysiologic characteristics of depression and the cognitive dysfunction that accompanies them, genetic and environmental factors that may affect depression risk, and the role of reproductive function in the excess depression risk in women.

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THE LIFETIME PREVALENCE OF major depressive disorder in women is nearly twice that in men (21.3% vs 12.7%).¹ Sex differences in prevalence occur during the reproductive years. Several biological processes may contribute to this increased risk, including hormonal fluctuations related to reproductive function and sensitivity to these hormonal fluctuations in brain systems that mediate mood states. Sensitivity to hormonal fluctuations manifests as excess risk of depression associated with changes in reproductive system function during premenstrum, pregnancy, post partum, and perimenopause.^{2,3} In addition, women are more likely than men to respond to stress with depression.⁴

Animal models have been used routinely to study the neurobiologic characteristics of depression and to test the efficacy of pharmacotherapies. Nearly all of this work has been performed in male rodents despite the early observation of sex differences in adaptation to chronic restraint stress.^{5,6} Sex differences have also been observed in the efficacy and adverse effects of the commonly prescribed selective serotonin reuptake inhibitors (SSRIs).⁷ Deficits in attention and effortful information processing are characteristic of depression, and a common adverse effect of SSRIs is cognitive dysfunction,^{8,9} which was not anticipated by tests of efficacy in rodent models. Recently, the validity and utility of currently available animal models of depres-

sion have been questioned, and the need for more and better animal models using sophisticated imaging techniques has been emphasized.^{10,11}

Historically, nonhuman primate (*Macaca* species) models have been widely used to study depression, primarily in infants.¹² Price¹³ was the first to postulate changes in affect with changes in social dominance relationships, a model of specifically adult depression. He perceived the depressive behavior that accompanies loss of status as adaptive because it decreases the likelihood that the subordinate will fight back, and he recognized the evolutionary implications of depression in nonhuman primates.^{13,14} Price and coworkers^{15,16} observed that subordination stress alters behavior and physiologic processes and they formalized their thinking about the relationship between social status and depression in a social competition hypothesis of depression. Depression has an inverse relationship with socioeconomic status in the human population as well.¹⁷ It has been hypothesized that it is the stress of low socioeconomic status that increases the risk of disease.¹⁸

In keeping with Price and colleagues and owing to the need for an animal model of depression in females, we studied the relationship between social status and mood-related behaviors in cynomolgus monkeys (*Macaca fascicularis*). Socially subordinate female cynomolgus monkeys live in an environment that is similar to that of human beings of low socioeconomic status. They receive more aggression, are more vigilant, are groomed less, and spend more time alone (ie, out of arm's reach of another monkey) than their dominant counterparts and thus appear socially stressed.¹⁹ Perhaps due in part to this apparently stressful social environment, subordinates also have many physiologic characteristics that are risk factors for or correlates of disease. Subordinate females are hypercortisolemic and dyslipidemic and have a high heart rate (HR) response to stress, impaired ovarian function, more coronary and carotid atherosclerosis, and reduced striatal dopamine D₂ receptor binding potential (BP) relative to dominants.¹⁹⁻²⁴

We studied behavioral depression, defined as a slumped or collapsed body posture accompanied by a lack of responsiveness to environmental events, in 42 socially housed females for 2 years (**Figure 1**).²⁵ Subordinates were more likely than dominants to display depressed behavior.²⁶ In subsequent studies,²⁷⁻³¹ it was observed that monkeys displaying behavioral depression had other behavioral and physiologic characteristics in common with human depression, including reduced body fat, low levels of activity, suppressed ovarian function, low insulin-like growth factor I levels, dyslipidemia, high 24-hour and overnight HRs, and hypothalamic-pituitary-adrenal (HPA) axis disturbances. To our knowledge, the neurobiologic characteristics of this monkey model of stress-associated depression have not yet been described.

The 5-HT_{1A} receptor is implicated in major depressive disorder. Results of positron emission tomography (PET) studies of 5-HT_{1A} receptor BP, using WAY-100635, in nonmedicated patients with major depressive disorder suggest that 5-HT_{1A} BP is decreased in several cortical regions (mediotemporal, orbitofrontal, anterior cingulate, insula, and dorsolateral prefrontal cor-

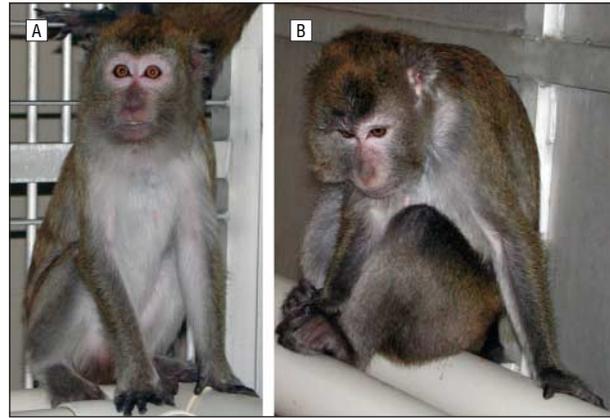


Figure 1. An alert monkey (A) compared with a monkey in depressed posture (B). These photographs were taken by the same photographer at approximately the same distance from each monkey. The nondepressed monkey (A) was alert and orienting toward the photographer (a potential threat), whereas the depressed monkey (B) seemed oblivious to the presence of a human being. The depressed monkey (B) was sitting in a slumped or collapsed body posture, with open eyes directed downward, and was unresponsive to environmental events.

tex). Laterality in these effects was observed in the mediotemporal, ventral anterior cingulate, and dorsolateral prefrontal cortex.^{32,33}

In the experiment reported herein, 5-HT_{1A} receptor BP was evaluated using PET in the raphe nucleus, amygdala, hippocampus, and cingulate cortex of monkeys that varied by degree of depressive behavior, and the relationships between 5-HT_{1A} BP in these neuroanatomic regions and key behavioral and physiologic characteristics of depression were determined.

METHODS

SUBJECTS

Thirty-six adult female cynomolgus monkeys (*M fascicularis*) (estimated age based on dentition, 8-20 years) were imported from the Institut Pertanian Bogor, Bogor, Indonesia. During the study, 9 animals died, resulting in 27 animals completing the protocol. Behavioral depression was associated with an increased mortality rate (correlation between days lived and depression: $r = -0.41$; $P \leq .02$). Causes of death followed no discernible pattern; for example, 2 monkeys died of trauma inflicted by cage mates, 2 did not recover from sedation, and 1 became ketotic and died. Depression in humans is also associated with increased (nonsuicide) mortality rates.³¹ The 5-HT_{1A} BP was studied using PET in 17 monkeys chosen based on good health history and to represent the complete range of time spent in the depressed posture and social status. Nine of the 17 monkeys were from the lower third and 8 were from the upper third of the range of time spent in the depressed posture. Eight of the 17 monkeys were socially dominant, and 9 were subordinate. All procedures involving primates were conducted in compliance with institutional, state, and federal laws governing the use of primates in laboratory settings.

The animals were part of an experiment to evaluate the comorbidity of depression and cardiovascular disease risk factors. Thus, for 24 months the animals consumed an atherogenic diet, which contained 0.28 mg of cholesterol per calorie (approximately equal to human consumption of 500 mg/d) and 42.4% of calories as fat.

EXPERIMENTAL DESIGN

The monkeys were housed in stable social groups of 4 animals each for 23 months. Behavior and menstrual cycles were documented throughout this period. The following assessments were performed: HR was assessed during months 10 to 12, HPA axis function (dexamethasone suppression test, corticotropin challenge test, and corticotropin-releasing hormone challenge test) was assessed during months 16 to 20, and 5-HT_{1A} BP was assessed using PET during months 19 to 23.

SOCIAL STATUS

Social status was determined monthly throughout the experiment by recording the outcomes of aggressive interactions between cage mates. The animal in each social group to which all members submitted was designated the first-ranking monkey. The animal to which all but the first-ranking monkey submitted was designated the second-ranking monkey, etc. As in previous experiments,³⁴ social status hierarchies were stable across time.

SOCIAL BEHAVIOR

Affiliative and agonistic behavior, and behavioral time budgets, including time spent in the depressed posture, were recorded for 15 minutes weekly using the focal animal technique. The frequencies of the following behaviors were recorded: bite and slap/grab (together considered extreme aggression involving physical contact); stare threat, open-mouth threat, chase, and displace (together considered mild aggression); scream, scream threat, crouch, and flee (together considered extreme submissive behavior); and lip smack, grimace, submissive present, and move away (together considered mild submission). The duration of affiliative behaviors was recorded, including grooming, sitting close (within monkey arm's reach), in body contact, or alone (see Shively et al³⁵ for operational definitions). Time spent depressed, defined as a slumped or collapsed body posture accompanied by a relative lack of responsiveness to environmental stimuli to which other monkeys are attending,²⁶ was recorded while monkeys were in body contact, close, or alone (Figure 1). Interobserver reliability was 93%.

REPRODUCTIVE SYSTEM FUNCTION

The monkeys were trained to present themselves for vaginal swabbing to detect menses and for femoral venipuncture to collect blood samples for progesterone assay 3 times a week. Serum progesterone level was assessed using a kit from Diagnostic Products Corp (Los Angeles, Calif) as previously described.³⁶ These data were used to determine timing of the follicular phase for PET.

Heart rate was recorded by means of telemetry. After capture and sedation with ketamine hydrochloride (10 mg/kg), each monkey was outfitted with a nylon mesh protective jacket over a portable electrocardiogram telemetry unit. After overnight recovery from sedation, HR recording was initiated the next morning (at approximately 7 AM) and continued for 24 hours.³⁰ Night-time HR was calculated as the mean HR from 9 PM to 5 AM, which includes the nadir of the diurnal HR curve and a time when no research or care staff are active in the monkey building.

HPA FUNCTION

Dexamethasone suppression tests were performed as previously described.³¹ Blood samples were obtained within 5 minutes of capture and sedation with ketamine hydrochloride (10

mg/kg). The difference between the first and second morning cortisol levels (percentage suppression) was calculated as an indicator of sensitivity to negative feedback. Baseline cortisol level (first morning sample) and percentage suppression were used as dependent variables. The cortisol response to corticotropin challenge was determined as previously described.³¹ Area under the cortisol curve was used as the dependent variable. The response to corticotropin-releasing hormone challenge was assessed as previously described.³¹ Circulating corticotropin and cortisol levels were measured 15, 30, 45, and 60 minutes after corticotropin-releasing hormone injection. The areas under the cortisol and corticotropin curves were used as dependent variables. Interassay coefficients of variation for all clinical chemistry values were less than 7%.

POSITRON EMISSION TOMOGRAPHY

4, 2'-(Methoxyphenyl)-1-[2'-(N-2'-pyridinyl)-p-fluorobenzamido]ethylpiperazine ([¹⁸F]MPPF), a high-affinity, reversible, and selective ligand for the 5-HT_{1A} receptor (K_d 5-HT_{1A} 0.34, α_1 113, α_2 >1000, β >1000, dopamine D₂ 19, 5-HT₂ 37nM), was used as the radioligand.³⁷⁻³⁹ The radio-synthesis of [¹⁸F]MPPF followed the procedure described by Le Bars et al.⁴⁰

Before the start of the PET study, each monkey was sedated (ketamine hydrochloride, 10 mg/kg intramuscularly) and intubated with a 5.0 tracheal tube, and anesthesia was maintained throughout the entire procedure with 1.5% isoflurane (Modulus Gas Anesthesia machine; Madison, Wis, Ohio Medical). A 20-gauge intravenous catheter was inserted into the femoral artery and the saphenous vein of the opposite leg. The monkey was positioned on the gantry of the scanner with the canthomeatal line perpendicular to the scanner axis. Heart rate, blood pressure, respiration rate, oxygen saturation, and body temperature were continuously monitored. Test subjects were given an intravenous bolus injection of the radiotracer (4-6 mCi [148-222 M β q]), and arterial blood samples were collected into preheparinized tubes for analysis. The first 50 samples (0.3 mL, 1-125 seconds after intravenous injection) were obtained at 2.5-second intervals using an automatic blood sampler (Ole Dich Instruments, Hvidovre, Denmark). The remaining 1.5-mL samples were obtained manually (at 3, 5, 7.5, 10, 15, 20, 30, 60, 90, and 120 minutes). Samples were centrifuged and plasma aliquots of 50 to 100 μ L were counted in a calibrated Packard Cobra II Auto-Gamma Counter (Packard, Meriden, Conn). The correction of arterial blood curves for the presence of metabolites was accomplished using a solid-phase extraction-high-performance liquid chromatography procedure,⁴¹ and metabolite analysis followed.⁴²

Images were acquired using a PET scanner (Advance NXi; GE Healthcare, Bucks, England). In a single scan, the Advance NXi provides 35 transverse sections with 4.25-mm center-to-center spacing over a 15.2-cm axial field of view. The transaxial resolution of the scanner ranges from 3.8 mm at the center of the field of view to 7.3 mm radial and 5.0 mm tangential at a radius of 20 cm when reconstructed with a ramp filter. Its axial resolution ranges from 4.0 mm at the center to 6.6 mm at a radius of 20 cm when reconstructed with a ramp filter. The sensitivity of the scanner is 1200 kcps/ μ Ci per cubic centimeter when operated in 3-dimensional mode.⁴³

After the subject was placed in the scanner, a 5-minute transmission scan was acquired in 2-dimensional mode using 2 rotating rod sources containing an equilibrium mixture of germanium Ge 68-gallium Ga 68. The monkey received the bolus dose of [¹⁸F]MPPF (2.6-5.0 mCi [96.2-185.0 M β q]), and a 120-minute dynamic acquisition scan was acquired.³⁸ The emission scan was acquired in 3-dimensional mode (ie, with the septa

retracted). Image reconstruction of the 3-dimensional data was performed using the 3-dimensional reprojection method with full quantitative corrections.⁴⁴ The transmission scan data were smoothed using a 4-mm Gaussian filter transaxially and then segmented.⁴⁵ The emission data were then corrected for attenuation and reconstructed into 128×128 matrices using a Hanning filter with a 4-mm cutoff value transaxially and a ramp filter with an 8.5-mm cutoff value axially.

The PET images were registered to the animal's magnetic resonance images (MRIs) using the automated image registration algorithm⁴⁶ after extracting the brain from the MRI.⁴⁷ Regions of interest (ROIs) were defined on coregistered MRIs. The ROIs were determined by the consensus of 2 investigators (C.A.S. and D.P.F.) who inspected the coregistered MRI-PET images with reference to brain atlases.^{48,49} Primary placement of the ROIs was performed using the coregistered MRI with slight modifications for inaccuracies in image registration guided by the coregistered PET image. As in previous studies with [¹⁸F]MPPF,^{50,51} distribution volumes were calculated for each ROI using their tissue time activity curve and the metabolite-corrected blood data in conjunction with the Logan graphical technique.⁵² The distribution volume ratio,⁵³ a measure of 5-HT_{1A} BP, was then determined in 11 ROIs: the left and right whole amygdala, the whole hippocampus (including the dentate gyrus, the hippocampus proper, and the subicular formation), the cingulate gyrus (3 adjacent sites in area 24a just rostral to the genu of the corpus callosum), and the midbrain raphe nucleus (dorsal raphe nucleus, just ventral to the aqueduct of Sylvius in the midbrain). Two ROIs were measured in the cerebellum (where 5-HT_{1A} BP is low), averaged, and used as the reference region for calculation of the distribution volume ratios. Positron emission tomography was performed during the first 8 days of the menstrual cycle, when ovarian steroids are relatively low and quiescent.

ANALYSIS

The distribution of behavioral depression was divided into tertiles: no time spent depressed ($n=6$), a modest amount of time spent depressed ($<1\%$ of time; $n=6$), and the highest amounts of time spent depressed ($1\%-4\%$ of time; $n=5$). Initially we evaluated the relationship between behavioral depression and 5-HT_{1A} BP over all brain areas using a 3 (levels of depression) \times 11 (brain areas) analysis of variance. The overall analysis of variance was followed by 3 (levels of depression) \times 2 (brain areas) analyses of variance to evaluate laterality in the hippocampus, cingulate cortex, and amygdala. The average of the 3 ROIs in the cingulate cortex was used for this analysis. Correlations were used to measure the degree of association between 5-HT_{1A} BP in each area and other behaviors, HPA axis function, and HR. Pearson r was used for all variables except social status, for which the nonparametric Spearman ρ was used. The α level was set at $P=.05$, and reported P values are the result of 2-sided tests.

RESULTS

GENERAL NEUROANATOMIC PATTERNS of 5-HT_{1A} BP

To determine the distribution of MPPF binding in normal brain, we examined the sum of the frames after 19 minutes of 4 of the PET studies of nondepressed animals because these frames give a useful representation of specific binding. Cortical binding was most intense along the midline dorsal to the corpus callosum and in the anterior portion of the lateral sulcus. The midline label extended the entire length of the cingulate gyrus.

Throughout this region, the entire dorsoventral extent of the midline dorsal to the corpus callosum was labeled, although the intensity was greatest rostral to the anterior commissure. In 2 of the animals, several millimeters of the most medial portions of the dorsolateral cortex, corresponding to area 6, were also labeled. Intense labeling was also observed in the most rostral portion of the insula, including the agranular and rostral dysgranular fields, at the level of the rostral pole of the corpus callosum. Additional label was apparent throughout the entire extent of the insula and in the region of the superior temporal sulcus. Subsequent autoradiographic studies (D.P.F., unpublished data, 2006) have shown that MPPF labels virtually the entire cortex. The label is densest in layers I and II, with additional less dense label in layer VI. It seems that this distribution and density of receptors is not sufficient to be detectable in our PET scans unless several regions of the cortex are in close proximity to each other, as they are along the dorsal midline, and within the lateral and superior temporal sulci. It was generally not possible to resolve specific labeling dorsal to the lateral sulcus, even within the sulci there. It is not clear why this label was not visible on PET.

Additional label was observed in the amygdala and the hippocampus. This label was visible beginning at the level of the optic chiasm, which corresponds to the most rostral border of the amygdala, and continued caudally through the entire extent of the hippocampus. It was not possible to resolve individual amygdalar nuclei or hippocampal fields. Binding was also observed in the most rostral portions of the raphe (most likely corresponding to the dorsal raphe nucleus) but appeared faint relative to the aforementioned areas.

DEPRESSION AND 5-HT_{1A} BP: OVERALL ANALYSIS

The relationship between behavioral depression and 5-HT_{1A} BP was evaluated using a 3 (levels of depression) \times 11 (ROIs) analysis of variance (**Figure 2**). The main effect for depression was significant ($F_{2,14}=9.13$; $P<.003$), suggesting that depressed animals have lower 5-HT_{1A} BP than their nondepressed counterparts across all brain areas measured. There were differences in 5-HT_{1A} BP among brain areas (repeated-measures $F_{10,140}=37.96$; $P<.001$); however, there was no difference in the relationship between 5-HT_{1A} BP and depression by brain areas (levels of depression \times brain areas interaction: $F_{20,140}=1.02$; $P=.44$), suggesting that the same relationship between 5-HT_{1A} BP and depression was observed in all brain areas.

LATERALITY

Because there is potential for side-to-side differences in the relationships between depression and 5-HT_{1A} BP, the left and right hemispheres were compared in the hippocampus, amygdala, and cingulate cortex (3 levels of depression \times 2 sides). There were no effects of side in the amygdala ($F_{1,14}=0.27$; $P=.61$), hippocampus ($F_{1,14}=0.42$; $P=.53$), or cingulate cortex ($F_{1,14}=0.13$; $P=.72$). There were also no side \times level of depression interactions (amyg-

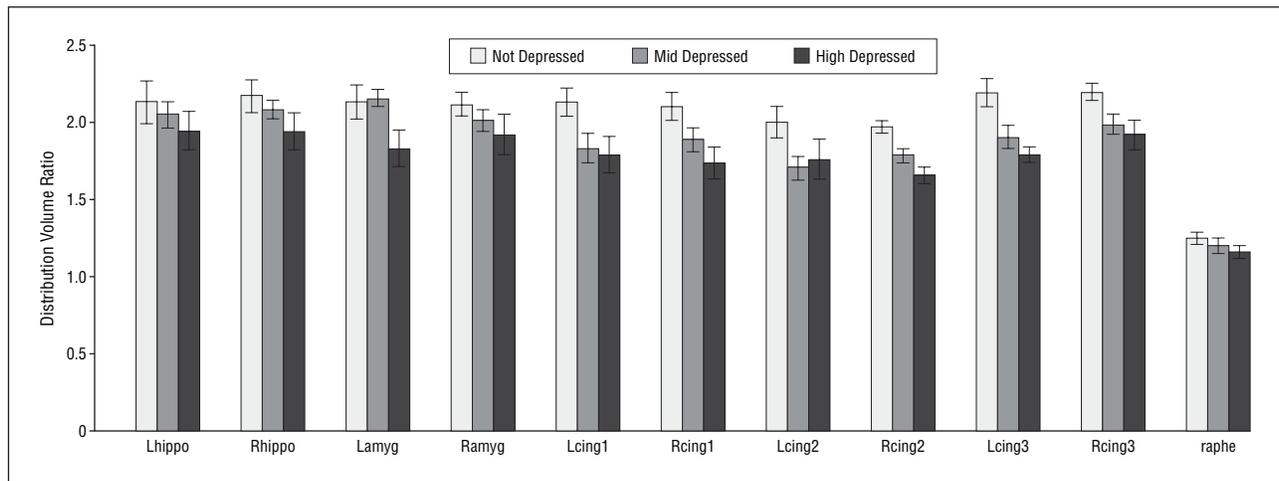


Figure 2. Serotonin 1A (5-HT_{1A}) binding potential (BP) and time spent in the depressed posture. The main effect for depression was significant ($F_{2,14}=9.13$; $P<.003$). There were differences in 5-HT_{1A} BP between brain areas (repeated-measures $F_{10,140}=37.96$; $P<.001$). There was no difference in the relationship between 5-HT_{1A} BP and depression by brain areas (levels of depression \times brain areas interaction: $F_{20,140}=1.02$; $P=.44$). Amyg indicates amygdala; cing, cingulate; hippo, hippocampus; L, left; and R, right. Error bars represent SD.

dala: $F_{2,14}=1.86$; $P=.19$, hippocampus: $F_{2,14}=0.10$; $P=.91$, and cingulate cortex: $F_{2,14}=0.39$; $P=.68$), suggesting that relationships between 5-HT_{1A} BP and depression were equivalent in both hemispheres.

RELATIONSHIPS BETWEEN 5-HT_{1A} BP AND BEHAVIOR, HPA, AND AUTONOMIC NERVOUS SYSTEM FUNCTION

Depression is not a unitary biological phenomenon; instead, it involves widespread disturbances in many systems. Thus, we reasoned that 5-HT_{1A} BP may be differentially associated with various behavioral and physiologic characteristics that may be perturbed in depression. For this reason, correlations were calculated between behavior and physiologic indices of depression and 5-HT_{1A} BP in each brain region in all animals (**Table**).

CORRELATIONS BETWEEN BEHAVIORAL AND PHYSIOLOGIC CHARACTERISTICS

Percentage of time spent depressed was significantly associated with rates of mild submission (+), social status (-), and adrenal cortisol levels at baseline (+) and in response to a corticotropin challenge (-). Note that HPA axis function and social status were not significantly associated with 5-HT_{1A} BP in any brain region (Table).

5-HT_{1A} BP AND AGGRESSION

There is long-standing interest in the relationship between aggression and depression because suicide may be conceived of as a self-aggressive act, and aggression and serotonergic function have been repeatedly associated in human studies and animal models. The 5-HT_{1A} BP in the right amygdala was negatively associated with the frequency of submission and vigilant scanning (behaviors characteristic of subordinates) and positively associated with initiating agonistic interactions, whereas 5-HT_{1A} BP in the left amygdala was positively associated with rates

of aggression (Table). Thus, there seems to be lateralization in specific aspects of aggressive behavior, that is, one side relating more to initiating aggressive interactions and having low rates of submission and the other relating more to aggressive behavior frequency. The 5-HT_{1A} BP in the right hippocampus, similar to that in the right amygdala, was negatively associated with the frequency of submissive behavior, whereas the left hippocampus was not significantly associated with any measure of social behavior or stress.

ACTIVITY AND FRIENDLY BEHAVIOR

Activity levels and the initiation of friendly behavior seem reduced in depression (psychomotor retardation in human beings and activity levels in monkeys).³¹ The 5-HT_{1A} BP in the left anterior cingulate was positively associated with affiliation (initiating friendly interactions and percentage of time spent grooming) and activity (stereotypic locomotor and locomotor behavior) (Table). The 5-HT_{1A} BP in the right anterior cingulate, in contrast, was positively associated with the percentage of time spent engaging in stereotypic behaviors directed at the environment (eg, repeated perch patting and bar chewing) and in investigating (nonrepeated attention to an object, eg, licking a drop of water on the side of the cage or picking up a novel object). Self-directed behaviors (self-grooming or stereotypics such as biting and hair pulling) were not associated with 5-HT_{1A} BP in either cingulate cortex (data not shown).

HPA AXIS AND AUTONOMIC NERVOUS SYSTEM FUNCTION

Depression is often accompanied by perturbations in HPA and autonomic system function. Whether these characteristics are associated specifically with 5-HT_{1A} BP is unknown. In this study, measures of HPA function were not associated with 5-HT_{1A} BP in any of the brain regions studied (Table). Heart rate averaged across 24 hours (data not

Table. Correlations Between 5-HT_{1A} Binding Potential and Behavioral and Physiologic Characteristics in 17 Adult Female Cynomolgus Monkeys*

	% of Time Depressed	Right Hippocampus	Right Amygdala	Left Hippocampus	Left Amygdala	Raphe	Right Cingulate	Left Cingulate
Agonistic behavior								
Contact aggression	-0.24	0.39	0.39	0.36	0.57	0.34	0.23	0.10
Mild aggression	-0.09	0.37	0.46	0.28	0.50	0.41	0.18	0.15
Total aggression	-0.10	0.37	0.46	0.28	0.50	0.41	0.19	0.15
Initiate aggression	-0.04	0.39	0.50	0.30	0.47	0.42	0.16	0.13
Extreme submission	-0.24	-0.22	-0.30	0.05	0.12	-0.16	0.16	0.00
Mild submission	0.49	-0.52	-0.63	-0.21	-0.38	-0.32	-0.18	-0.09
Total submission	0.10	-0.45	-0.56	-0.08	-0.12	-0.29	0.02	-0.05
Time vigilantly scanning, %	0.21	-0.40	-0.63	-0.11	-0.36	-0.41	-0.26	-0.23
Social status†	-0.60	0.36	0.43	0.26	0.24	0.12	0.30	0.01
Activity and friendly activity								
Time grooming, %	-0.22	-0.07	0.15	-0.47	-0.24	0.40	0.00	0.55
Initiate friendly	-0.14	0.00	0.11	-0.28	-0.15	0.46	-0.03	0.51
Time locomoting, %	-0.22	-0.06	0.03	-0.10	0.03	0.38	0.30	0.59
Time stereotypic locomoting, %	-0.18	-0.11	0.09	-0.44	-0.22	0.43	0.00	0.61
Time stereotypic to environment, %	-0.04	-0.22	-0.15	0.01	-0.01	-0.13	0.57	0.44
Time investigating environment, %	-0.25	-0.04	-0.01	0.20	0.17	-0.07	0.65	0.39
HPA axis and heart rate								
Cortisol response to corticotropin (AUC)	-0.52	0.06	0.04	0.19	0.30	0.11	0.19	0.12
Baseline cortisol	0.55	-0.22	0.03	-0.34	-0.35	0.11	0.00	0.23
Cortisol response in DST	0.25	-0.29	-0.21	-0.14	-0.31	-0.17	0.13	0.08
Suppression in DST, %	0.05	-0.19	-0.25	0.06	-0.10	-0.26	0.14	-0.08
Corticotropin response to CRH (AUC)	0.22	0.30	0.34	0.21	-0.06	0.01	0.15	0.02
Cortisol response to CRH (AUC)	0.21	-0.36	-0.12	-0.33	-0.18	-0.01	-0.08	0.20
Mean nighttime heart rate	0.01	-0.24	-0.57	0.06	-0.33	-0.56	-0.29	-0.48

Abbreviations: AUC, area under the curve; CRH, corticotropin-releasing hormone; DST, dexamethasone suppression test; HPA, hypothalamic-pituitary-adrenal; 5-HT_{1A}, serotonin 1A.

*All correlations are Pearson *r* except those with social status: *r*_{sv} = 0.48; *P* = .05, 2-tailed. Significant correlations are set in boldfaced type.

†Spearman *ρ*_{sv} = 0.48; *P* = .05, 2-tailed.

shown) and overnight was negatively associated with 5-HT_{1A} BP in the raphe nucleus, the left anterior cingulate cortex, and the right amygdala.

COMMENT

These results indicate lower 5-HT_{1A} BP in the raphe, amygdala, hippocampus, and anterior cingulate cortex of depressed monkeys relative to nondepressed controls. The effect of depression seemed to be associated with its severity because, in general, the more time an animal spent depressed, the lower its 5-HT_{1A} BP. The magnitude of the differences was greatest in the right and left anterior cingulate cortex (area 24a) and left amygdala. The results reported herein are consistent with results of studies of 5-HT_{1A} BP in humans with major depressive disorder and suggest a possible homologous behavioral abnormality in human and nonhuman primates.³²

Several issues should be considered in interpreting the present observations. First, PET has limited spatial resolution, which can make it difficult to visualize binding except when it is highly concentrated. Although 5-HT_{1A} receptors are concentrated in the outer layers (I-III) of the cortex, in specific nuclei of the amygdala, and in specific fields of the hippocampus, we had to measure the entire thickness of the cortex and the entire amygdala and hippocampal formation. This almost certainly resulted in lower 5-HT_{1A} BP than would be observed with

more tissue-specific approaches, such as postmortem autoradiographic studies. Likewise, the diffuse nature of the raphe nuclei likely resulted in a dilution of signal also. In addition, it is difficult to be confident in the lack of laterality differences in BP in the cingulate gyrus observed herein because this may be due to diffusion of signal to adjacent areas.

Reductions in BP could result from either a reduction in the number of 5-HT_{1A} receptors available or a decrease in receptor affinity. Binding potential does not discriminate between these possibilities. Furthermore, reductions in receptor availability may be due to reduced receptor number or to increased binding of the endogenous ligand. Depression is thought to involve decreased serotonin action at the synapse, and Sargent et al³³ observed no difference in 5-HT_{1A} binding in patients before or during SSRI treatment, which should change synaptic serotonin concentrations. Thus, the second possibility seems to be less likely, although the current data set cannot discriminate between these 2 possibilities either.

Associations between dependent variables were calculated for the purpose of hypothesis generation. It has been known for some time that the amygdala is associated with aggression and that aggression is modulated by serotonergic function.⁵⁴⁻⁵⁶ Recent observations suggest that 5-HT_{1A} receptors may be associated with aggression. Parsey et al³⁷ observed inverse relationships be-

tween 5-HT_{1A} BP and aggressive trait (based on self-reported lifetime history of aggression) in the anterior cingulate, amygdala, dorsal raphe, and prefrontal cortex of 25 men and women. In the present study, we directly and objectively distinguished between rates of aggression and submission and the frequency of initiation of aggression. We found positive relationships between 5-HT_{1A} BP and rates of mild aggression and aggression involving physical contact in the left amygdala, whereas rates of submissive behavior were inversely related to 5-HT_{1A} BP in the right hippocampus and amygdala. In contrast, the frequency of initiating aggressive interactions was positively associated with 5-HT_{1A} BP in the right amygdala. There were some substantial, although non-significant, correlations for many agonistic behaviors in both amygdalae, suggesting the need for further studies to sort out potential laterality effects. There is longstanding interest in the relationship between aggression and depression because suicide may be conceived of as a self-aggressive act.^{58,59} These observations support a close neurobiologic relationship among 5-HT_{1A} BP, depression, and aggression in primates.

Anxiety is known to involve mechanisms mediated in the amygdala. Vigilant scanning is highly inversely correlated with social status³¹ and seems to represent fear or anxiety. This behavior was inversely correlated with 5-HT_{1A} BP in the right amygdala. The SSRI citalopram hydrobromide reduces conditioned fear responses through effects in the amygdala.⁶⁰ Further studies are needed to determine whether vigilant scanning is a reliable indicator of anxiety.

Little is known about the neurobiologic features of friendly behavior. Mehlman et al⁶¹ reported that cerebrospinal fluid 5-hydroxyindoleacetic acid concentrations are associated with affiliative behavior (time spent grooming and time spent close) in free-ranging rhesus monkeys. Knutson et al⁶² noted increased friendly social interaction among young adult people treated with an SSRI in a randomized double-blind experiment. Herein we observed positive correlations between time spent grooming and frequency of initiation of friendly behavior (eg, approaching and offering to groom or sitting in body contact) and 5-HT_{1A} BP in the left cingulate cortex. This relationship seemed to be unilateral, but laterality in relationships with behavior and cingulate 5-HT_{1A} BP should be assessed with caution because the left and right cingulate are neuroanatomically close and the camera resolution may not differentiate these areas with certainty.

The cingulate cortex also seems to be centrally involved in the neural control of autonomic function. In functional MRI studies, dorsal anterior cingulate cortex activation was associated with sympathetic modulation of HR, whereas ventral anterior cingulate cortex activation seems to be involved in parasympathetic modulation of HR.⁶³⁻⁶⁵ One article⁶⁶ suggests that the neural circuit controlling HR during negative affect includes the amygdala, whereas HR in response to positive stimuli does not. The 5-HT_{1A} receptor in ventral medullary sites, including the raphe pallidus, has been implicated in the control of HR.^{67,68} Herein, 5-HT_{1A} receptor BP was negatively associated with HR in the raphe nuclei, left cingulate cortex, and right amygdala. This is the first study,

to our knowledge, indicating that midbrain autonomic regulation may be mediated by 5-HT receptor function.

In conclusion, these observations suggest that there are common neurobiologic substrates for depressive behavior in human and nonhuman primates. This behavior pattern has been observed in 2 large groups (n=78) of adult female cynomolgus monkeys that were randomly selected from feral populations, suggesting that the capacity for depression is inherent in the species. Like human depressives, depressed monkeys seem to have widespread decreases in neural 5-HT_{1A} BP. This animal model holds promise to further our understanding of the basic mechanisms of affective behavior and the role of reproductive function in the excess risk of depression in women.

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