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Behavioral and Neurobiological Characteristics Influencing Social Hierarchy Formation in Female Cynomolgus Monkeys

Natallia V. Riddick¹, Paul W. Czoty¹, H. Donald Gage², Jay R. Kaplan³, Susan H. Nader¹, Michelle Icenhower¹, Peter J. Pierre¹, Allyson Bennett¹, Pradeep K. Garg², Sudha Garg², and Michael A. Nader^{1,2,*}

¹Department of Physiology & Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC

²Department of Radiology, Wake Forest University School of Medicine, Winston-Salem, NC

³Department of Pathology, Wake Forest University School of Medicine, Winston-Salem, NC

Abstract

Socially housed monkeys have been used as a model to study human diseases. The present study examined behavioral, physiological and neurochemical measures as predictors of social rank in sixteen experimentally naïve, individually housed female cynomolgus monkeys (*Macaca fascicularis*). The two behavioral measures examined were novel-object reactivity (NOR), as determined by latency to touch an opaque acrylic box placed in the home cage, and locomotor activity assessed in a novel open-field apparatus. Serum cortisol concentrations were evaluated three times per week for four consecutive weeks, and stress reactivity was assessed on one occasion by evaluating the cortisol response to adrenocorticotrophic hormone (ACTH) following dexamethasone suppression. Measures of serotonin function included whole blood serotonin (WBS) concentrations, cerebrospinal fluid (CSF) concentrations of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) and brain serotonin transporter (SERT) availability obtained using positron emission tomography (PET). After baseline measures were obtained, monkeys were assigned to four social groups of four monkeys per group. The two measures that correlated with social rank were CSF 5-HIAA concentrations, which were significantly higher in the animals who eventually became subordinate and latency to touch the novel object, which was significantly lower in eventual subordinate monkeys. Measures of serotonin function did not change as a consequence of social rank. These data suggest that levels of central 5-HIAA and measures of novel object reactivity may be trait markers that influence eventual social rank in female macaques.

Keywords

social rank; serotonin; cerebrospinal fluid; hormones; PET imaging; serotonin transporters

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Address Correspondence to: Michael A. Nader, Ph.D., Department of Physiology and Pharmacology, Wake Forest University School of Medicine, 546 NRC, Medical Center Blvd., Winston-Salem, NC 27157-1083, PH: 336-713-7172, FAX: 336-713-7180.

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INTRODUCTION

Nonhuman primates living in social groups have been used to study psychiatric diseases, drug abuse, atherosclerosis and the neurobiology of behaviors such as aggression and impulsivity (Kaplan et al., 1982; Morgan et al., 2002; Shively et al., 1998). Social rank (an individual's relative position within a dominance hierarchy) has been shown to have significant effects on susceptibility and resistance to diseases, indicating a powerful influence of the environment (e.g., Abbott et al., 2003; Kaplan, 2004; Morgan et al., 2002; Sapolsky, 2005; Virgin and Sapolsky, 1997). The study of social rank and health in nonhuman primates has been frequently related to different levels of stress. When received chronically, stress can increase risks for numerous diseases (Sapolsky, 2005). In addition, dominance hierarchies produce marked inequalities in division of resources (e.g. food, space, access to sexual partners, etc.); thus, an animal's social rank can profoundly affect its quality of life and its health (Kaplan, 1987; Sapolsky, 2005), including increased risk for coronary artery atherosclerosis, poor ovarian function, and adrenal hypersecretion of cortisol (Kaplan et al., 19867; Shively et al., 1986, 1997). Studies assessing disease states as a function of social rank have typically examined the effects of "state" variables, that is, characteristics attributable to environmental circumstances. In order to more fully understand the role of the environment, including social rank, on disease states, other investigators have examined potential "trait" variables, that is, pre-existing characteristics that may underlie an individual's likelihood of occupying a high or low social rank. Such information is critical in understanding the interactions between the individual and the social context. The present study focused on several potential predictors of social rank in adult female cynomolgus monkeys (*Macaca fascicularis*), including behavioral measures associated with reaction to novelty, hormone concentrations and measures of serotonin (5-HT) neurotransmission.

Macaques establish linear hierarchies based on outcomes of agonistic interactions (e.g., Kaplan et al., 1982). Variables such as body weight, age and kinship have been shown to affect eventual social rank (Cawthon-Lang, 2006; Gartland, 1968; Sapolsky, 2005). For example, in male cynomolgus monkeys, body weights prior to social housing were significantly correlated with eventual social rank, with heavier monkeys becoming the most dominant (Morgan et al., 2000). Such an outcome is consistent with observational studies indicating that physical appearance is an important variable in determining social rank in rhesus macaques (Bernstein and Mason, 1963; Bernstein et al., 1974; for review see Bernstein, 1991). The relationship between body weight and eventual social rank in female monkeys has not been explored. Other than body weight, the only other measure that predicted eventual social rank in our earlier study in male monkeys was locomotor activity in a novel environment (Morgan et al., 2000). In the present study, we examined this variable in female monkeys and extended the behavioral measures to include latency to touch a novel object. Other variables that have been examined as predictors of social rank in male monkeys include cortisol concentrations, which generally do not predict future social rank (e.g., Mendoza et al., 1979; Goo and Sassenrath, 1980; Morgan et al., 2000; but see Golub et al., 1979).

Finally, the present study extended our assessment of predictors of social rank to include measures of 5-HT function. The 5-HT system has been implicated in a variety of social behaviors such as grooming, approaching, feeding, as well as impulsive aggression and impulsivity (Kaplan et al., 2002; Miczek et al., 2002, 2004a,b; Pattij and Vanderschuren, 2008). To assess 5-HT activity, the present study examined whole blood 5-HT (WBS) levels (Kinnally et al., 2006; Unis et al., 1997), cerebrospinal fluid (CSF) levels of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA; Higley et al., 1991, 1992, 1996a,b; Higley and Linnoila, 1997; Kaplan et al., 2002; Westergaard et al., 1999; Yodyingyuad et al., 1985)

and measures of 5-HT transporter (SERT) availability using positron emission tomography (PET; Frankle et al., 2005) in brain areas rich in SERT-containing terminals. Each 5-HT marker was examined in all monkeys allowing for comparisons of all dependent variables in a repeated-measures experimental design. We assessed whether these measures predicted eventual social rank (i.e., trait variables) and whether they changed after social group formation (i.e., state variables). Because recent work from our laboratory reported that measures of dopamine receptor availability were influenced by menstrual cycle phase (Czoty et al., 2008), PET imaging and endocrine experiments were conducted while monkeys were in the follicular phase.

METHODS

Subjects

Sixteen experimentally naïve adult female cynomolgus monkeys (*Macaca fascicularis*) 9–17 years old served as subjects. All 16 were originally from Indonesia; eight animals were previously in a non-invasive study within the Wake Forest University Primate Center. While in quarantine, monkeys were fed *ad libitum*; their weights at the end of this period were used as an indication of free-feeding weights. After transfer to the laboratory, monkeys were weighed weekly and, to facilitate concurrent operant behavioral training involving food reinforcement (data not shown), body weights were maintained at approximately 95% of free-feeding weights by limiting daily access to food (Purina Monkey Chow LabDiet #5038 and fresh fruit); animals had unlimited access to water. Monkeys were singly housed in cages in which they had visual, olfactory and limited manual contact with conspecifics. Quadrant cages (Model #PR242732I4, Allentown Caging Inc., Allentown, NJ) were equipped with removable metal partitions that separated the four animals into individual quadrants of the cage. Each quadrant measured $0.71 \times 0.84 \times 0.84$ m. When partitions were removed, the total living space for the pen measured $0.71 \times 1.73 \times 1.73$ m. Each animal was trained to sit calmly in a primate chair (Primate Products, Inc., Redwood, CA), as described previously (e.g., Morgan et al., 2000; Czoty et al., 2005).

The duration of the menstrual cycle was initially assessed by daily vaginal swabs over several months (see Czoty et al., 2008). The first day of bleeding was considered to be indicative of menses and was counted as day 1 of the cycle. Once a complete cycle of approximately 28 days was observed, experiments were scheduled to occur during days 2–10 (the follicular phase). Experiments scheduled to occur in luteal phase were conducted on days 19–26 of the menstrual cycle. To confirm that monkeys were in the follicular phase during PET experiments, on the day of a PET study, 3 ml of blood was drawn from the femoral vein and centrifuged (Beckman Coulter, GPR Centrifuge) at 4° C for 30 min at 3000 rpm. The serum was aspirated into an Eppendorf tube and stored at –30° C. Progesterone levels were assessed by the Biomarkers Core Laboratory of the Yerkes National Primate Research Center of Emory University in Atlanta, GA. Progesterone levels < 4 ng/ml were indicative of follicular phase. Animal housing and handling and all experimental procedures were performed in accordance with the 2003 National Research Council *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* and were approved by the Animal Care and Use Committee of Wake Forest University. Environmental enrichment was provided as outlined in the Animal Care and Use Committee of Wake Forest University Non-Human Primate Environmental Enrichment Plan. This included toys, mirrors and food enrichment, which was delivered via multiple types of foraging devices (Challenger balls, PVC cups, PVC tubes) and provided to each monkey a minimum of 2 times/week during their 2 hour feeding period while they are separated for feeding.

Social housing procedure and rank determination

After arriving in our laboratory, monkeys were individually housed for approximately 27 months while baseline behavioral (locomotor activity and novel object reactivity, NOR), neurochemical (5-HT function) and hormonal measures (menstrual cycle, cortisol concentrations and adrenal responsiveness) were obtained. Monkeys lived in social groups of four monkeys per pen. The initial placement of monkeys into groups was counterbalanced and based on body weight. Monkeys were ranked from 1–16 based on body weight and each of the 4 heaviest were randomly assigned to groups 1–4; the next 4 heaviest were then randomly assigned, etc. On the first day of social housing the steel partitions were removed and the animals were allowed to interact. Monkeys were monitored closely for the first 72 hr after placement into social groups. For the next 5 days, partitions were replaced at approximately 4:15PM and monkeys remained separated overnight. This precaution was taken to prevent injury occurring when laboratory personnel were not present. After 1 week, monkeys were socially housed overnight and separated each morning, during which time monkeys were fed. Thus, time spent socially housed was approximately 22 hours per day. In rare cases of an injury that required veterinary care or other routine veterinary procedures, the entire pen was individually housed overnight (or until the injury healed).

For each group, social status was determined using the outcome of agonistic encounters (Kaplan et al., 1982). From week 2 through week 12 of social housing, two observers separately conducted a total of 34–36 observation sessions per pen (3 observations/week per pen). Each 15-min session began immediately after partitions were removed in the afternoon. Aggressive, submissive and affiliative behaviors were recorded according to an ethogram described previously (Kaplan et al. 1991; see Morgan et al. 2000, Table I) utilizing Noldus Observer software (Noldus Information Technology; Wageningen, The Netherlands). In these focal group sessions, both initiators and recipients of behaviors were recorded. The monkey in each pen aggressing towards all other monkeys and submitting to no one was ranked #1 (most dominant). The monkey aggressing at everyone except the #1-ranked monkey and submitting only to the #1-ranked monkey was ranked #2, etc. The monkey designated #4 displayed low levels of aggression and submitted to all other monkeys in the pen. Thus, a transitive, linear hierarchy was established in each pen.

Experiment 1. Behavioral measures

Locomotor activity—Monkeys were placed in a $3 \times 2 \times 1.75$ m open-field apparatus for 15 min to assess locomotor activity in a novel environment. The open-field apparatus was divided into 9 zones. Activity was recorded by 2 small cameras built into the top panels of the apparatus and a side-view camera set up on a tripod outside the apparatus. The primary dependant variable was number of crossings between compartments and was scored from the video.

Novel object reactivity—On the day of the test, the monkey in the quadrant adjacent to the test subject's home cage was removed, the test subject was moved to the neighboring cage and the partition was replaced. An opaque box ($30.5 \times 20.3 \times 20.3$ cm) made of black Plexiglas was placed in the subject's home cage. The partition was removed and activity was filmed for 15 min. Latency to touch the object was scored from the video. If the animal did not touch the box within 15 min a score of 900 sec was assigned.

Experiment 2. Measures of cortisol concentrations and adrenal responsiveness

Serum cortisol—Blood samples (2 ml) were collected three times per week from each monkey, one day at 4:00 PM and two days at 7:30 AM, for 4 consecutive weeks. The samples were drawn by percutaneous stick from the femoral vein while the animal was

awake and seated in a primate chair. The blood was centrifuged at 3000 rpm for 30 min and the serum was aspirated into an Eppendorf tube and stored at -20°C until analysis.

Adrenal responsiveness—Assessment of adrenal responsiveness was performed in the follicular phase of the menstrual cycle in all monkeys during individual housing and repeated in dominant and subordinate monkeys after 3 months of social housing. While the monkey was seated in a primate chair, a blood sample was drawn from the femoral vein at 11:00 AM the day before the experiment to serve as a baseline. At 7:00 AM the following morning the animal was injected with 0.5 mg/kg dexamethasone (DEX) i.m. in her home cage. Four hours later, the monkey was seated in a primate chair and a baseline blood sample was obtained (time -15). Fifteen min later synthetic ACTH (Cortrosyn, 10 ng/kg) was injected into the saphenous vein followed by 3 ml sterile saline flush. Blood samples were drawn from the femoral vein 15- and 30-min following the Cortrosyn injection. Blood samples were centrifuged for 30 min at 3000 rpm. The serum was then aspirated into an Eppendorf tube and stored at -20°C until analysis. All blood samples were analyzed by the Biomarkers Core Laboratory of the Yerkes National Primate Research Center.

Experiment 3. Measures of serotonin function

Whole blood 5-HT (WBS) measures—WBS was measured in each monkey in the follicular phase of the menstrual cycle while individually housed ($n=16$) and in dominant and subordinate monkeys following 3 months of social housing. Blood samples were obtained from the femoral vein while the animal was awake and seated in a primate chair. The blood samples were placed into EDTA-coated vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) and centrifuged at room temperature for 10 min at 1300 rpm to obtain platelet-rich plasma (PRP). The PRP-supernatant (200 μl) was then transferred into another tube with 800 μl of physiological saline and the total solution was centrifuged at 4°C for 10 min at 6300 rpm. The supernatant was then discarded and 200 μl of double-distilled water was added to the pellet. The final solution was stored at -80°C until analyzed by Dr. Richard R. Yeoman at the Oregon National Primate Research Center.

Cerebrospinal fluid (CSF) measures—While monkeys were individually housed, CSF was collected by a cervical puncture from 12 monkeys, once during the follicular phase and once during the luteal phase of a single menstrual cycle (i.e., two weeks apart) while the animals were anesthetized with ketamine (10 mg/kg, i.m.). These samples were used to assess whether 5-HIAA levels differed across menstrual cycle phases. In the remaining four monkeys, who were not cycling regularly at this time, two samples were taken two weeks apart. When it was determined subsequently that 5-HIAA levels did not differ across menstrual cycle phase (see Table I), data from the two samples were averaged for each monkey, including those that were not cycling, for use in determining whether CSF 5-HIAA levels predicted eventual social rank. Following social housing, CSF was collected from four dominant and four subordinate animals during the follicular phase. The back of the skull was shaved and cleaned with betadine and 95% alcohol. A 25-G, 1.5-inch needle was inserted into cisterna magna and approximately 2 ml of CSF was collected within 10 min of induction of anesthesia. CSF was then immediately transferred to chilled vacutainer tubes on ice. Samples were centrifuge at 4°C for 30 min at 3000 rpm. Aliquots were transferred to microcentrifuge tubes for storage at -30°C until analysis. The samples were assayed by Dr. John Mann at the New York State Psychiatric Institute.

PET imaging procedures—Prior to the first PET imaging study, magnetic resonance imaging (MRI) scans were acquired for each monkey under ketamine (20 mg/kg, i.m.) anesthesia (for details see Czoty et al., 2008). Images were used to anatomically define spherical regions of interest (ROI); data are shown for the caudate nucleus, putamen,

anterior cingulate cortex (ACC), thalamus, hippocampus and cerebellum. PET scans to measure SERT availability were conducted using [^{11}C]-3-amino-4-(2-dimethylaminomethylphenylthio) benzonitrile ([^{11}C]DASB) during the follicular phase of the menstrual cycle, which was confirmed from blood samples taken prior to the PET study and analyzed for levels of progesterone. These regions were chosen because human postmortem studies suggest high to moderate densities of SERT (e.g., Mash et al., 2000) and significant occupancy by SERT compounds associated with improved anxiety scores (Kent et al., 2002). Serotonin has also been shown to influence basal ganglia function (e.g., Dray, 1981); a dopamine-rich region previously shown to be sensitive to changes in social rank (Morgan et al., 2002; Czoty et al., 2005). Scans were conducted in all monkeys prior to social group formation and again at least 3 months after formation of social groups in the four dominant and four subordinate monkeys.

Prior to the 90-min PET scan, the monkey was anesthetized with 10 mg/kg ketamine and transported to the PET Center, where she was intubated. Anesthesia was maintained by 1.5% isoflurane; this level of anesthesia dose not affect measures of [^{11}C]DASB availability (Milak et al., 2005). The monkey was placed in the scanner and a catheter was inserted by percutaneous stick into a vein. The venous catheter was used for [^{11}C]DASB administration and for administration of lactated ringers solution (5 ml/kg/hr) for fluid replacement throughout the study. To assure that the monkey did not move during the PET study, a paralytic (0.07 mg/kg vecuronium Br, i.v.) was administered 15 min before the start of the scan and respiration was maintained on a ventilator. Supplemental doses of vecuronium Br (0.1 mg/hr) were administered throughout the study. During the scan, the temperature of the monkey was maintained through the use of a heating pad (temperature of the circulating water = 40° C). The following vital signs were monitored constantly throughout the scanning procedure: heart rate, blood pressure, respiration and body temperature. PET scans were performed using a primate microPET P4 scanner (Siemens/CTI Concorde) specifically designed for small-animal imaging (see Tai et al., 2001). Time-activity curves were generated for radiotracer concentrations in ROIs defined on each subject's co-registered MRI. Distribution volumes ratios (DVR) were calculated using the cerebellum as the reference region and the graphical method of Logan et al. (1996). For all regions, the right and left sides were then averaged and the DVR in each ROI was calculated.

Drugs and Chemicals

DEX (10 mg/ml) and Cortrosyn (250 $\mu\text{g}/\text{ml}$ prepared on the day of the study) were purchased from the North Carolina Baptist Hospital Pharmacy. [^{11}C]DASB was prepared as previously described (Wilson et al., 2000) with slight modification: [^{11}C] methyl triflate was reacted with 0.5 mg of 3-amino-4-(2-methylaminomethylphenylsulfanyl)-benzonitrile (desmethyl-DASB) in 300 μL of acetonitrile. The reaction mixture was purified using reverse-phase HPLC. The radiochemical yields ranged between 20 – 30% with >95% radiochemical purity. The specific activity of [^{11}C]DASB was 4.73 ± 0.13 mCi at the time of injection.

Statistical Analysis

Data are displayed for individual monkeys and as group means $\pm$ SEM. Data describing body weight and data from all pre-social housing experiments except the DEX/cortrosyn study were analyzed using one-way analysis of variance (ANOVA) between the three groups (dominant, intermediate and subordinate); significant main effects or interactions were followed with a Newman-Keuls multiple comparisons post-hoc test. Because some monkeys did not touch the novel object and were thus assigned a score of 900, a (non parametric) Spearman's correlation was used to determine the relationship between latency to touch the novel object and CSF 5-HIAA levels. Data from the DEX/cortrosyn experiment

were analyzed using a two-way ANOVA (social rank and time) as the main effects. Social housing data for WBS, CSF, and SERT availability were analyzed using unpaired t-test between dominant and subordinate groups. Correlations between measures were conducted by Pearson correlation. In all cases, significance was accepted at the 95% level of confidence ($p < 0.05$).

RESULTS

Experiment 1. Behavioral measures

Weight and age of the monkey—The body weight recorded immediately prior to social housing changed by less than 1% on average over the 3 months following social group formation, did not change significantly for the duration of the study (data not shown) and did not correlate significantly with social rank. There was no significant correlation between age or weight and any dependent variable examined in the present study, including social rank.

Locomotor activity and novel object reactivity—Locomotor activity ranged from 15 to 30 crossings over 15 min and did not differ as a function of eventual social rank (Fig. 1, left panel). However, latency to touch the novel object (Fig. 1, right panel) differed significantly according to eventual social rank ($F(2,13)=5.225$; $p < 0.05$). Post-hoc t-tests revealed that eventual dominant animals' latencies to touch the novel object was significantly longer ($p < 0.05$) than those of the eventual intermediate and subordinate animals. This relationship was observed in all pens. Latency to touch the novel object and locomotor activity scores were not significantly correlated.

Experiment 2. Measures of cortisol concentrations and adrenal responsiveness

Serum cortisol—Mean cortisol concentrations were significantly higher in the AM compared to the PM samples (32.06 ± 2.23 vs. 20.79 ± 2.95 $\mu\text{g/dl}$, respectively; $t(3)=6.636$, $p < 0.01$), but did not differ significantly as a function of eventual social rank (data not shown). During the first week of social housing, PM cortisol concentrations increased in all but three monkeys, but were not different in dominant and subordinate monkeys (see Fig. 2).

ACTH challenge—In assessments of adrenal responsiveness (Fig. 2, top panel), two-way ANOVA revealed a main effect of time ($F(3,39)=42.75$, $p < 0.0001$), but no significant main effect of eventual rank and no significant interaction between eventual social rank and time. There were no significant differences in cortisol levels as a function of eventual social rank after DEX suppression or after an ACTH challenge as a function of eventual social rank. Moreover, following 3 months of social housing, the extent to which ACTH elevated cortisol levels did not differ between dominant and subordinate monkeys (Fig. 2, bottom panel).

Experiment 3. Measures of serotonin function

Whole-blood 5-HT levels (WBS)—While individually housed, WBS levels were highly variable between subjects, ranging from 101–1337 ng/ml (data not shown). There were no significant differences in WBS concentrations in monkeys as a function of eventual social rank, with mean values for eventual dominant, intermediate and subordinate groups of 554 ± 98.81 , 611 ± 150.15 and 463 ± 184.54 ng/ml, respectively. Three months after social housing, these values had not significantly changed in dominant and subordinate monkeys, with means of 477.25 ± 101.89 and 329 ± 76.99 ng/ml, respectively.

CSF concentrations of 5-HIAA—In twelve normally cycling monkeys, CSF concentrations of 5-HIAA did not significantly differ as a function of menstrual cycle phase

(Table I). Two samples were collected two weeks apart in four other monkeys who were not cycling normally. CSF 5-HIAA levels in those samples were similar to those in the normally cycling monkeys (subject #6805: 375.04 and 318.36, #6820: 453.54 and 404.6; #6818: 394.22 and 345.99; #6802: 346.79 and 338.06 pmol/ml). For determining the ability of social rank to predict future social rank, the two samples were averaged for each of the 16 monkeys. A one-way ANOVA indicated that CSF concentrations of 5-HIAA taken while individually housed differed significantly as a function of eventual social rank ($F(2,13)=4.42$; $p=0.034$; Fig. 3, left). Post-hoc analyses indicated that 5-HIAA concentrations were significantly lower in monkeys that would become dominant compared to eventual subordinate and intermediate monkeys ($p<0.05$). This relationship was observed in three of the four pens. In the fourth, CSF 5-HIAA levels were similar. In addition, there was a significant negative correlation between baseline CSF 5-HIAA concentrations and latency to touch the novel object ($r=-0.57$; $p<0.05$). Three months after establishing social groups, 5-HIAA levels did not significantly change from individual-housing baselines and remained significantly lower in dominant compared to subordinate monkeys ($t(6)=2.86$; $p<0.05$; Fig. 3, right).

SERT availability as measured by PET—There was a high level of uptake of [^{11}C]DASB and a linear rate of washout from all regions of interest. In the reference region, the cerebellum, there was low level of [^{11}C]DASB uptake and rapid rate of washout. For all regions of interest, there were no differences between left and right sides, so mean DVR data are shown (Fig. 4). SERT availability did not differ between eventually dominant and subordinate animals in caudate nucleus, putamen, hippocampus, thalamus, and anterior cingulate cortex (Fig. 4, top panel). Following 3 months of social housing, SERT availability did not significantly differ as a function of social rank (Fig. 4, bottom panel). There was not a significant correlation between CSF 5-HIAA levels and SERT availability, nor was SERT availability significantly correlated with any behavioral measure.

DISCUSSION

The primary goal of the present study was to examine several behavioral, physiological, and neurochemical measures as predictors of eventual social rank in female cynomolgus monkeys (i.e., trait variables) and to determine if these measures changed after social group formation (i.e., state variables). These measures included age, body weight, locomotor activity in a novel environment, latency to touch a novel object, HPA-axis function and 5-HT activity (peripheral and central). The only behavioral measure that was predictive of eventual social rank was a greater latency to touch a novel object among future dominant animals. We also found that CSF concentrations of the 5-HT metabolite 5-HIAA, but not other measures of 5-HT activity, predicted eventual social rank, and that no 5-HT measure changed after the establishment of stable social hierarchies.

The present study extends earlier research in male cynomolgus monkeys (Morgan et al., 2000) in which reliable predictors of social rank were the weight of the monkeys prior to social group formation and locomotor activity. Age, cortisol and testosterone measures were not significantly correlated. In the present study, neither weight nor locomotor activity in a novel environment was predictive of eventual social rank in female monkeys. The range of weights of the females prior to social housing was 2.4–3.7 kg, which was a narrower range than the males' weights (3.2–6.4 kg; Morgan et al., 2000), perhaps obscuring a significant correlation. Regarding locomotor activity, the apparatus was modified from the original study in males and is larger and allows for more vertical activity, which may preclude us from making direct comparisons between the two studies. In addition, it is possible that sex differences exist in locomotor activity and are due to ovarian hormone fluctuations. In females, novelty and stimulant-induced locomotor behavior varied as a function of estrus

cycle in rats (Davis et al., 2008; Sell et al., 2002), suggesting a source for sex differences in this dependent measure. To our knowledge, no studies have addressed this question in nonhuman primates. Overall, these findings suggest that physiological and behavioral variables that can be considered traits that predict social rank in male cynomolgus monkeys (body weight and locomotor activity) are not predictive in females.

In contrast to locomotor activity, NOR was predictive of eventual social rank in female monkeys. In a recent study examining locomotor activity and a behavioral measure of impulsivity (a 5-choice serial reaction test), Dalley et al. (2007) found that subjects determined to be more impulsive had lower locomotor activity. Taken together, both findings suggest that locomotor activity in a novel environment reflects a behavior that is different from impulsivity. In the present study, we hypothesized that latency to touch the novel object would reflect a trait measure related to temperament and that eventual subordinate monkeys would be more reactive (i.e., have lower latencies). Highly impulsive rhesus monkeys have been shown to occupy lower positions in the social dominance hierarchy and are more sensitive to the reinforcing effects of alcohol (Higley et al., 1992, 1996a,b). Similarly, high impulsivity is characteristic of human cocaine abusers (Moeller et al., 2001, 2002). We found that more reactive monkeys were more likely to become subordinate when socially housed, extending earlier work in well-established social groups to include this behavior as a trait marker. In addition, NOR latencies correlated with central 5-HT activity as indicated by low CSF 5-HIAA levels, which is consistent with earlier work in nonhuman primates (Higley et al., 1996a,b; Raleigh et al., 1980, 1983; Westergaard et al., 1999).

CSF 5-HIAA concentrations have been shown to predict social rank in female rhesus and pigtail monkeys (Westergaard et al., 1999), but in a direction opposite to the present results. Westergaard et al. (1999) reported a direct relationship between CSF 5-HIAA concentrations and eventual social rank. In the present study, we found that individually housed female monkeys' CSF 5-HIAA levels were higher in eventual subordinate monkeys. The differences in results between the two studies may be that Westergaard et al. (1999) used adolescent female macaques that were approximately 2 years old, while monkeys in the present study were adults between 9–17 years old. This may suggest that developmental changes occur within 5-HT systems and that relationships apparent at a young age may be reversed as the subject ages. It is worthy of note, however, that we have observed a similar association between social rank and NOR in a group of male cynomolgus monkeys younger than the females used in the present study ($n = 21$, mean age = 10.3 ± 3.2 years, unpublished data). Further studies using a within-subject design will be needed to more directly address this important issue.

As was observed in individually housed male monkeys (Morgan et al., 2000), cortisol levels were not predictive of eventual social rank. In the earlier study, plasma cortisol levels were only measured once; in the present study cortisol was measured three times per week for 4 weeks, but significant differences related to eventual social rank and cortisol levels were not observed at any time point, nor were differences observed after stable social group formation. Similarly, adrenal responsiveness, as assessed using DEX suppression and ACTH administration was not predictive of social rank, nor were there differences following the establishment of stable social groups. A recent study from our group reported transient differences in cortisol levels in male monkeys early in social hierarchy formation (Czoty et al., in press). In that study, eventual subordinate monkeys had significantly higher evening cortisol levels compared to eventual dominant monkeys over the first three days of social housing, but by the fourth day no differences were observed (Czoty et al., in press). In addition, following social housing and DEX suppression, ACTH-induced cortisol release was significantly greater in subordinates (Czoty et al., in press). Taken together, these data

suggest that social rank-related differences in basal cortisol concentrations are transient and that compensatory mechanisms are rapidly recruited that normalize circulating cortisol concentrations. In addition, it appears that there may be sex differences in adrenal responsiveness, with males being more sensitive than female macaques. It should be cautioned, however, that this conclusion is likely to be limited to the precise conditions employed in the present study.

Several measures of 5-HT function, which have been implicated in social behavior in nonhuman primates (Kaplan et al., 2002; Raleigh et al., 1983), were also examined in these female monkeys. WBS is purported to be an indirect measure of central 5-HT reuptake (Fekkes et al., 1997; Mann et al., 1992). WBS has been shown to be higher in adolescent boys with conduct disorder and increased aggression (Unis et al., 1997). In animal studies, WBS levels were higher in less impulsive marmosets as exhibited by more inhibition in response toward a stranger (Kinnally et al., 2006), as well as dominant male vervet monkeys (Raleigh et al., 1983). In the present study, WBS was not predictive of eventual social rank nor was it different between dominant and subordinate monkeys during social housing. Moreover, WBS did not correlate with central measures of 5-HT activity, CSF 5-HIAA or SERT availability. It is not clear whether the lack of sensitivity of WBS in the present study compared to earlier work is related to sex differences or the duration of social housing (which was only 3 months in the present study). Nonetheless, this measure was insensitive to housing conditions in adult female monkeys.

PET imaging studies of dopamine D2 receptor availability have reported differences as a function of social rank in male and female monkeys (Grant et al., 1998; Morgan et al., 2002). Importantly, levels of D2 availability appear to be sensitive to changes in housing conditions, such that the transition from individual to social housing resulted in significant increases in D2 levels in dominant male monkeys but no change in subordinate monkeys (Morgan et al., 2002). The present experiment extended these studies to SERT availability. SERT availability was not predictive of eventual social rank and did not significantly change in any monkey following the establishment of stable social groups. These findings were surprising considering the observed rank-related difference in 5-HIAA levels. Based on earlier imaging work (see Laruelle, 2000), we hypothesized that SERT availability would vary with differences in extracellular 5-HT levels, which was suggested by the observed difference in 5-HIAA concentrations. One possible reason for the discrepancy is that it is presently unclear whether [^{11}C]DASB competes with endogenous 5-HT for the same SERT binding sites (Lundquist et al., 2005; Tabot et al., 2005; Yamamoto et al., 2007). Future microdialysis studies examining the neurochemical changes associated with social group formation would provide important information related to the role of this neurotransmitter system in social behavior. The lack of a difference in SERT availability between dominant and subordinate monkeys was also unexpected in light of studies showing differences in SERT availability, SERT mRNA levels, and polymorphisms of the SERT gene in humans and laboratory animals that correlated with differences in impulsivity or social stress (Berton et al., 1999; Filipenko et al., 2002; Frankle et al., 2005; Paaver et al., 2007). This may also be due to the regions studied. However, other brain regions related to impulsivity, such as nucleus accumbens and prefrontal cortex, are difficult to study with PET because their target receptor regions are small with respect to the PET scanner's image resolution. Future studies examining the functional nature of the 5-HT system, using indirect and direct-acting agonists and antagonists, may uncover important differences based on social rank.

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List of Abbreviations

5-HIAA	5-hydroxyindoleacetic acid
5-HT	serotonin
ACTH	adrenocorticotrophic hormone
ANOVA	one-way analysis of variance
CSF	cerebrospinal fluid
DA	dopamine
[¹¹C]DASB	[¹¹ C]-3-amino-4-(2-dimethylaminomethylphenylthio)benzonitrile
DEX	dexamethasone
DVR	distribution volume ratio
HPA	hypothalamus-pituitary-adrenal
MRI	magnetic resonance imaging
NOR	novel object reactivity
PET	positron emission tomography
PRP	platelet-rich plasma
ROI	region of interest
SERT	serotonin transporter
WBS	whole blood serotonin

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Figure 1.

Left: Number of grid-line crossings in a novel environment over 15 min in monkeys that eventually became dominant (Dom), intermediate (Inter) and subordinate (Sub) ranked monkeys. *Right:* Latency to touch a novel opaque acrylic box (in sec). Values shown are mean $\pm$ SEM for dominant (n=4), intermediate (n=8), and subordinate (n=4) monkeys.

*p<0.05

Figure 2.

Mean ($\pm$ SEM) cortisol concentrations ($\mu\text{g/dl}$) at baseline (24 hrs), following DEX suppression and 15 and 30 min after administration of 10 ng/kg Cortrosyn, while individually housed (*top*) and following stable social hierarchies (*bottom*).

Figure 3.

CSF 5-HIAA concentrations in female cynomolgus monkeys as a function of eventual social rank while individually housed (*left*) and following stable social group formation (*right*). Symbols represent individual subject data; horizontal lines represent group means for dominant (n=4), intermediate (n=8), and subordinate groups (n=4). All samples were obtained in the follicular phase. *p<0.05.

Figure 4. SERT availability as measured by $[^{11}\text{C}]\text{DASB}$ in individually housed monkeys (*top*) and following stable social group formation (*bottom*) in the caudate nucleus (Cd), putamen (Pt), hippocampus (Hip), thalamus (Tha) and anterior cingulate cortex (ACC). Values represent mean $\pm$ SEM for dominant (n=4) and subordinate (n=4) ranked monkeys.

Table I

Mean ($\pm$ SEM) concentrations of 5-HIAA (pmol/ml) in CSF as a function of menstrual cycle phase in individual monkeys.

Monkey	Follicular Phase	Luteal Phase
	5-HIAA	5-HIAA
6804	307.54	362.13
6812	310.94	264.53
7371	346.67	352.82
7373	198.25	182.33
7376	349.56	376.13
7377	333.67	287.04
7370	330.29	293.90
7372	390.13	341.40
7374	459.16	388.37
6816	254.59	247.12
6817	314.63	272.58
7375	346.31	339.19
Mean SEM	328.48 19.36	308.96 18.50