Social and Environmental Influences on Blood Serotonin Concentrations in Monkeys

Michael J. Raleigh, PhD; Michael T. McGuire, MD; Gary L. Brammer, PhD; Arthur Yuwiler, PhD

- Dominant male adult vervet monkeys have whole-blood serotonin concentrations approximately twice those of subordinate adult males. We examined the effects of spontaneous and induced changes in social status, temporary isolation from the social group, and membership in single male groups on whole-blood serotonin concentrations. We found that in male vervet monkeys, elevated blood serotonin concentration is a state-dependent consequence of active occupation of the dominant male social position, and we believe that a reinterpretation of the significance of hyperserotonemia in humans may be warranted.

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Biologic studies in psychiatry are often restricted to the examination of peripheral tissues. Consequently, whether and how abnormalities in these tissues reflect CNS function are of considerable importance. A peripheral measure of interest to psychiatric researchers is blood serotonin because alterations in its concentration have been reported in a variety of disease states. Hyperserotonemia has been observed in a substantial subset of autistic children,1,2 mentally retarded persons without aminoacidemias,3 and chronic schizophrenics in whom the elevation can be associated with enlarged ventricles.4 Because blood serotonin concentration is dependent on peripheral tryptophan metabolism and platelet physiology, its elevation in different clinical populations may result from diverse mechanisms. As with any clinical variable, studying the mechanisms underlying hyperserotonemia in different disease states may be more revealing than the observation of its association with a variety of diseases.

The relationship between blood and brain serotonin levels has yet to be fully specified. Under some conditions, changes in blood serotonin levels parallel changes in brain serotonin levels. For example, both are similarly affected by pharmacologic interventions that augment precursor availability (eg, tryptophan loading5,6), that inhibit biosynthetic enzymes (eg, p-chlorophenylalanine treatment7), that disrupt serotonin storage (eg, fenfluramine hydrochloride administration8,9), or that alter enzymes catalyzing serotonin degradation (eg, treatment with the monoamine oxidase inhibitor clorgyline10). However, in other conditions such as carcinoid syndrome, blood and brain serotonin levels do not appear to be associated.3 Furthermore, because blood serotonin is stored exclusively in platelets, an association between blood and brain serotonin levels would not necessarily be expected in diseases of platelet physiology.11,12

An important issue in assessing the significance of hyperserotonemia is whether its incidence is trait or state dependent. In normal men, blood serotonin concentrations are remarkably stable over long periods of time (up to ten years13). This observation suggests that blood serotonin concentrations are trait dependent. Among autistic children, blood serotonin levels exhibit somewhat more intradividual variability, although this variation may be largely due to maturational changes.2 In partial contrast, there are reports that in Huntington's chorea14 and motor neuron disease,15 blood serotonin levels are state dependent and in motor neuron disease, correlate with the severity of the clinical condition. Alterations in blood serotonin concentrations also appear to be associated with the onset of migraine attacks.16 Interpretation of these studies is complicated in part because they are largely cross sectional across populations rather than longitudinal within subjects. Nevertheless, they are compatible with the view that blood serotonin concentrations can be state as well as trait dependent.

While investigating the contributions of serotonergic mechanisms to the mediation of overt social behavior in captive groups of adult vervet monkeys (Cercopithecus aethiops sabaeus), we examined the behavioral effects of tryptophan, 5-hydroxytryptophan, p-chlorophenylalanine, and the monoamine oxidase inhibitor clorgyline, alone and in combination.17 To verify the effectiveness of these pharmacologic interventions, we measured blood serotonin levels before, during, and after drug treatment. We noted...
that prior to drug treatment, in each of six groups, one male had blood serotonin levels about twice those of the other males. In each group this male was identifiable behaviorally as the dominant male. Subsequently this association between dominant male social status and high blood serotonin concentration was confirmed in 11 additional stable multimale groups. In groups of captive vervet monkeys, male social status may be manipulated behaviorally. Removal of the original dominant male leads to the emergence of another behaviorally defined dominant male. Return of the original dominant male within ten weeks of his removal results in the interdominant male resuming his original subordinate position. Thus, the trait or state dependency of the association between dominant male social status and an elevated blood serotonin level in vervet monkeys may be determined experimentally. In this article, we report on the intraindividual stability of blood serotonin concentrations among adult male vervet monkeys in multimale social groups under conditions of stable social relationships, and on the effects of a variety of socioenvironmental influences on blood serotonin concentration. Our investigations on the mechanisms underlying the elevated blood serotonin levels in dominant vervet monkeys will be reported elsewhere.

**SUBJECTS AND METHODS**

**Subjects**

The subjects were adult male vervet monkeys. All were ferally reared, had fully erupted canines, were free of diarrhea and other diseases, weighed between 5.4 and 8.5 kg, and had been in captivity for at least five months prior to the onset of the present studies. They were maintained on isoniazid-free commercial monkey chow supplemented once or twice weekly with fresh fruit. Chow and water were available ad libitum.

As shown in Table 1, four studies were conducted using subjects housed in the following six conditions: stable multimale groups (study 1); multimale groups in which spontaneous shifts in dominance occurred (study 2); behaviorally manipulated multimale groups (study 2); multimale groups from which the dominant and a subordinate male were temporarily isolated (study 3); multimale groups from which the dominant male was temporarily isolated (study 3); and stable single-male groups (study 4). Ten stable multimale groups were used in study 1. They lived in indoor-outdoor enclosures ranging in size from 4 x 3 x 2 m to 30 x 24 x 3 m. Each of these groups contained three or more adult males, three or more adult females, and their immature offspring. Four of these groups subsequently had spontaneous shifts in dominance relationships and were used in study 2. This study also used nine behaviorally manipulated groups, each of which originally contained three adult males and three adult females. These groups were housed in 4 x 3 x 2-m enclosures. One part of study 3 used seven multimale groups from which the dominant and one subordinate male were simultaneously isolated. When isolated, these 14 animals were housed individually in 3 x 2 x 1-m cages and were unable to see or be seen by members of their original groups. All of these seven groups contained three or more adult males, two or more adult females, and their offspring. One of these groups was originally used as one of the ten stable multimale groups. Study 3 also used three other multimale groups from which the dominant male was temporarily isolated in an individual cage containing a one-way mirror that enabled him to see but not be seen by members of his group. These three groups originally contained at least two males, two females, and their offspring. In study 4, four single-male groups each containing three or more adult females were used. After the male was added to these groups, no changes in group composition occurred.

**Methods**

**Social Status.** — In the stable and behaviorally manipulated multimale groups, the behaviors described in Table 2 were recorded. Social status was assessed by noting the subject’s success in dyadic internmale aggression. Subject A was regarded as successful in a dyadic agonistic encounter with subject B if B submitted to or avoided A when A threatened B, displayed to B, or engaged in contact aggression with B. In each group the male with the highest percentage of success in internmale dyadic encounters was regarded as the dominant male. All remaining adult males were regarded as nondominant or subordinate. In these studies, the only behavioral distinction made was between the dominant male and the subordinate males. No attempt was made to rank order the subordinate males. Every dominant male won at least 91% of his dyadic internmale agonistic encounters with outcomes, and no subordinate male won more than 48% of his dyadic agonistic encounters. For all groups, intergroup vigilant behavior was scored.

**Interrater Reliability.** — Interrater reliability was measured before and during the present studies, as described in detail elsewhere. Briefly, for the six behaviors (threatening, displaying, engaging in contact aggression, submitting, avoiding, and being vigilant) recorded in these studies, interrater reliability between

<table>
<thead>
<tr>
<th>Study</th>
<th>Housing</th>
<th>No. of Groups</th>
<th>Adult Males/Group</th>
<th>Comments</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Stable multimale</td>
<td>10</td>
<td>≥3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Spontaneous dominance changes</td>
<td>4</td>
<td>3</td>
<td>Previously used in study 1</td>
</tr>
<tr>
<td>2</td>
<td>Behaviorally manipulated multimale</td>
<td>9</td>
<td>3</td>
<td>One group excluded from analysis</td>
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<tr>
<td>3</td>
<td>Multimale, dominant and subordinate isolated</td>
<td>7</td>
<td>3</td>
<td>Subjects isolated without visual contact with group members</td>
</tr>
<tr>
<td>3</td>
<td>Multimale, dominant and subordinate isolated</td>
<td>3</td>
<td>2</td>
<td>Subjects isolated with visual contact with group members possible</td>
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<td>4</td>
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<tr>
<th>Behavior</th>
<th>Reliability</th>
<th>Description</th>
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<tbody>
<tr>
<td>Threaten</td>
<td>0.89</td>
<td>Jerking head, opening mouth, staring, retracting ears, lunging toward</td>
</tr>
<tr>
<td>Contact aggress</td>
<td>0.93</td>
<td>Slapping, pushing, pulling fur, biting</td>
</tr>
<tr>
<td>Display</td>
<td>0.86</td>
<td>Exaggerated bouncing, circling movement with tail over back; jerking penis in sagittal plane</td>
</tr>
<tr>
<td>Avoid</td>
<td>0.94</td>
<td>Retreating from threat, turning or moving away</td>
</tr>
<tr>
<td>Submit</td>
<td>0.86</td>
<td>Squealing, screaming, hopping backward, pawing ground</td>
</tr>
<tr>
<td>Be vigilant</td>
<td>0.94</td>
<td>Scanning environment outside enclosure, shaking fence with rigid tense posture, threatening neighboring groups</td>
</tr>
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observer A and observer B was assessed by dividing the number of times a behavior was scored by both observers by the number of times it was scored by either one or both. As indicated in Table 2, for all behaviors, interrater reliability was at least 86%.

**Blood Collection Procedures.**—Subjects were deprived of fruit for at least 48 hours and fasted overnight prior to blood collection. Subjects were rendered tractable with an intramuscular 7- to 12-mg/kg dose of ketamine hydrochloride (a procedure that does not affect blood serotonin concentration), and blood samples were obtained by femoral venipuncture within 15 minutes of ketamine administration. Two to four milliliters of blood was collected into a sterile plastic syringe, immediately transferred to a tube containing edetic acid, inverted, and stored on ice. Within 90 minutes of collection, blood samples were transferred from the edetic acid tubes to polypropylene tubes and frozen either at -70°C or in liquid nitrogen until assayed one to four months later. Neither circadian nor seasonal rhythms in whole-blood serotonin concentration have been observed in vervet monkeys, and in our study all blood samples were obtained between 6:30 and 8:00 AM.

Blood serotonin concentration was determined by the flourometeric method of Yuwiler et al. All samples were run in duplicate. A linear regression equation was obtained for each subject by the least-squares method and was used to determine blood serotonin concentration, which was expressed as nanograms per milliliter and was corrected for percentage hematocrit by a coefficient of 24.8% (range, 21.3% to 27.8%). A second coefficient, 1.17% (range, 1.05% to 1.28%), was used to correct for percentage nitrogen in blood serum.

**Experimental Design and Data Analysis.**—Four studies were completed. Study 1 assessed the intrindividul stability of blood serotonin concentration in behaviorally stable multimale groups. Blood serotonin concentration was measured in 13 males living in 2 groups. For each subject, blood serotonin concentrations were determined on at least five occasions. For each group, consecutive samples were obtained at least 11 days apart, and samples were obtained over a minimum of 90 days (mean, 128 days). The intrindividul stability was determined by obtaining intrindividul Pearson product-moment correlations between the separate sample points (e.g., first-second, first-third, second-third) and by calculating intrindividul coefficients of variation across the five sample points.

Study 2 assessed the effects of spontaneous and induced changes in social status on blood serotonin concentrations. In one part of these studies, four previously stable multimale groups (of study 1) underwent a dominance reversal experiment. In this experiment, a dominant male became subordinate and a formerly subordinate male became dominant. Blood serotonin concentrations were measured at least four times before and three times after these status changes. The effects of these status changes on blood serotonin level were assessed by a two-group (formerly dominant and formerly subordinate) by two-condition (before and after status change) analysis of variance (ANOVA) with repeated measures.

In the other part of study 2, changes in social status were experimentally elicited in the nine groups by the removal and return of the original dominant male. These groups were observed for a 70-day baseline period, a 60- to 70-day period after removal of the original dominant male from the group, and a 50- to 65-day reunion period after the original dominant male had been returned. During the removal period, in each group, one of the two former subordinate males became dominant. In eight of nine groups, this interim dominant male reasserted his subordinate position in the reunion period. In the ninth group this initially subordinate male remained dominant in the reunion period. Data from this ninth group were excluded from analysis. The other nine originally subordinate males remained subordinate in all periods. For dominance, blood serotonin concentration was measured at least five times in each period. The effects of this gain (from subordinate to dominant) and loss (from interim dominant to subordinate) of status on blood serotonin concentration were assessed by a two-group (always subordinate and interim dominant) by three-condition (baseline, removal, and reunion) ANOVA with repeated measures.

During the removal period of study 2, the nine originally dominant males were divided into three groups of three males each, and each group was combined with a previously formed group of three females. Six of the nine originally dominant males (two from each group) became subordinate. On return to their original groups, eight of these nine males regained their dominant status. Only data from these eight groups are reported. The effects of this loss of and subsequent gain in status on blood serotonin concentration were assessed by a two-group (always dominant and interim subordinate) by three-condition (baseline, removal, and reunion) ANOVA with repeated measures.

Study 3 examined the effects of temporary isolation on blood serotonin levels. In one part of this study, the dominant and a subordinate male from seven groups were isolated without visual access to their groups for 21 to 28 days. These animals were subsequently returned to their groups. Blood serotonin concentration was measured at least twice before isolation, three times during isolation, and twice after return to their groups. The effects of this temporary isolation were assessed in a two-group (dominant and subordinate) by three-condition (before, during, and after isolation) ANOVA with repeated measures.

In the other part of this study, three dominant males were isolated from their groups for 16 days. When isolated, the animals had visual access to their groups through a one-way mirror and were able to initiate displays and threats to other group members. Blood serotonin was measured before, six times during, and after isolation. The effects of this procedure on blood serotonin concentration were assessed by comparing these animals with three dominant males who remained in their groups. Data were analyzed by a two-group (temporarily isolated and remaining in group) by three-condition (before, during, and after isolation) ANOVA with repeated measures.

Study 4 examined the effects of membership in a one-male group on blood serotonin concentrations. Each of four males who had been dominant in multimale groups was placed in a previously formed group of three adult females. Subjects were the only adult male in these groups for at least 60 days, and for each subject blood serotonin concentration was measured at least three times before the subject became a member of a one-male group and five times when it was a member of the one-male group. The effects of being in a one-male group were assessed by a paired t test.

**RESULTS**

**Study 1**

Whole-blood serotonin concentrations in subjects living in stable multimale groups exhibited little intrindividul variation. For no subject did whole-blood serotonin concentration vary more than 19% from the median of all sample points. For each of the 33 subjects, all sample values were within 12% of the mean. This intrindividul stability was reflected in the low intrindividul coefficient of variation across the five sample points (mean, 6.1%; range, 2.2% to 9.6%) and, as shown in Table 3, by the high intrasubject Pearson product-moment correlation across all pairs of sample points (r = .91 for all pairs of sample points).

**Study 2**

In four groups used in study 1, a spontaneous change in domin¬

ance relationships occurred. Prior to this change the initially
dominant males won about 85% of their dyadic intermale aggres¬
sive encounters (range, 92% to 100%). After this change, the new
dominant males won a similar percentage of such encounters (range, 91% to 98%). As shown in Table 4, the transition from subordi¬

nate to dominant position was accompanied by a rise in
whole-blood serotonin concentration, while concentrations in the
four initially dominant males that became subordinate declined.
There was a significant interaction between status (initially domi¬
nant or initially subordinate) and time (before or after dominance shifts) (P1,6) = 85.37, P < .001. During the periods of social stabil¬

ity both before and after the dominance shifts occurred, blood
serotonin concentrations showed marked intrindividul stability (intrasubject Pearson product-moment correlations both before and after dominance changes in these eight animals were r = .95, n = 8, P < .05).
As shown in Table 5, similar changes in whole-blood serotonin concentration occurred in the eight groups in which dominance relationships were experimentally altered. Concentrations in each of the three periods (baseline, removal, and reunion) exhibited little intraindividual variation (intraindividual r = .92, n = 16, P < .01 for each period). Accordingly, the effects of the induced status change on serotonin concentration were assessed by using each subject's mean concentration for each period. After removal of the initially dominant male, one of the two initially subordinate males became dominant. When dominant, these males were behaviorally indistinguishable from the initially dominant males. They won about 94% of their intermale aggressive encounters and were vigilant at the same rate as the initially dominant males. In the reunion period these animals resumed their subordinate positions after reintroduction of the initially dominant male. The other eight initially subordinate subjects (one per group) remained subordinate throughout all three periods. As Table 5 shows, blood serotonin concentrations in the eight subjects who became dominant in the removal period increased about 60%. In contrast, blood serotonin concentrations were unchanged in those animals that had remained subordinate. The interaction between status (always subordinate or initially dominant) and period (baseline, removal, reunion) was significant (F[2,28] = 8.12, P < .001).

Comparable alterations in blood serotonin concentration were shown by the initially dominant subjects in these eight groups. As Table 6 shows, three of these subjects were dominant in all periods and they had unchangingly high concentrations of blood serotonin. The five males that became subordinate during the removal period manifested a parallel 40% decline in blood serotonin concentration. The interaction between status (always dominant and interim subordinate) and period (baseline, removal, and reunion) was significant (F[2,12] = 23.47, P < .001).

**Study 4**

The effects of living in a one-male group on the whole-blood serotonin concentration were assessed in four subjects that previously had been dominant in multimale groups. As shown in Table 7, in the single-male groups these subjects continued to engage in intergroup vigilant behavior at the same rate as when they were in multimale groups but obviously were not submitted to or avoided by other male group members. These subjects showed little intraindividual variability in blood serotonin concentration either when in their multimale groups (mean coefficient of variation, 3.5%; range, 2.2% to 5.1%) or when in the single-male groups (mean coefficient of variation, 3.8%; range, 2.5% to 6.9%). Consequently, the effects of living in a one-male group were assessed by using each subject's mean blood serotonin concentration for each living condition. Living in a one-male group resulted in a significant decline in mean blood serotonin concentration (±SEM) from 968±41 ng/mL to 606±51 ng/mL (t = 14.9, df = 9, P < .001 for correlated samples).

**COMMENT**

These results indicate that, in adult male vervet monkeys, whole-blood serotonin concentrations can be influenced by social and environmental factors. In both single-male or multimale groups, whole-blood serotonin concentrations were altered by social factors, including removal and reunion of animals of different social status.
concentrations show little intraindividual variation as long as dominance relationships are stable. Dominant males have substantially higher whole-blood serotonin concentrations than do subordinate males or males living in single-male groups. Spontaneous and induced gains or losses in social status are accompanied by corresponding increases or decreases in whole-blood serotonin concentrations. Temporary isolation, with or without visual access to group members, reduces whole-blood serotonin concentrations in dominant males to levels exhibited by subordinate males. Males in single-male groups continue to engage in inter troop vigilant behavior, and temporarily isolated males continue to threaten and display to their group members. The elevated blood serotonin concentrations exhibited by dominant males therefore are probably contingent on both initiating aggressive and receiving submissive behavior from other adult male group members. In any case, among male vervet monkeys, elevated blood serotonin concentration is, at least in part, a state-dependent consequence of active occupation of the dominant male social position.

The biologic mechanisms underlying the linkage between blood serotonin concentration and social status are currently unknown. Whole-blood serotonin is largely derived from intestinal tryptophan metabolism and is contained in platelets that vary in size, number, serotonin uptake, and serotonin storage capacities. The differences in blood serotonin concentration between dominant and subordinate males might thus be due to alterations in tryptophan metabolism or platelet physiology. The extent to which any of these factors is susceptible to the hormonal and physiologic concomitants of dominant social status is unknown. Preliminary data indicate that the status-related differences in blood serotonin concentration observed in these studies are not due to differences in serum or plasma tryptophan concentration, platelet number, platelet size, or platelet monoamine or plasma amine oxidase activities. However, relative to subordinate males, dominant males appear to exhibit a much greater relative and absolute rise in whole-blood serotonin concentrations following an acute (20 mg/kg) tryptophan load. This observation suggests that there may be a status-related difference in tryptophan hydroxylation activity. An increased peripheral uptake and conversion of tryptophan in the hyperserotonemia of autism and schizophrenia was suggested by Freedman et al.

Previous investigations of the impact of social status on peripheral physiologic variables in nonhuman primates have focused on cortisol and testosterone concentrations. However, the relationship between these measures and male social status is controversial. For example, basal cortisol concentrations have been observed to be both positively and negatively correlated with male dominance. These apparent inconsistencies may result, in part, from methodologic differences in determining social status or from differences in housing conditions. Nevertheless, the lack of a convincing relationship between these variables and dominance may also be due to the extreme intraindividual variability in these measures. Cortisol and testosterone are released in a pulsatile fashion and exhibit circadian and circannual rhythms. In Old World monkeys, twofold to fourfold intra-animal variation in concentration in samples collected within 24 hours is common. This intra-animal variation in cortisol and testosterone levels contrasts with both the intraindividual stability of the whole-blood serotonin concentration and the persistent and consistent nature of dominance relationships in captive vervet monkeys. Among captive vervet monkeys, although the rate of intermale agonistic encounters may vary daily, the pattern of outcome is strikingly stable over long periods of time. Whether the association between whole-blood

<table>
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<th>Table 7.—One-Male Groups and Whole-Blood Serotonin Concentrations*</th>
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<tr>
<td><strong>Group</strong></td>
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<tr>
<td>Status</td>
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<tr>
<td>Vigilance, events/hr</td>
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<tr>
<td>Serotonin concentration, ng/mL</td>
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</table>

*Four males that were dominant in multimale groups were transferred to groups containing three females. Vigilance behavior was unchanged but serotonin concentrations declined significantly (P < .001 for correlated t test). Data are expressed as mean ± SE.
serotonin concentrations and male dominance generalizes to free-ranging vervets, to other species where dominance relationships shift more frequently, or to species with clear linear dominance hierarchies has yet to be determined. Nevertheless, recording the specific biologic and behavioral processes that account for the elevated blood serotonin concentrations exhibited by captive dominant male vervet monkeys is of interest in providing a model for investigating the mechanisms underlying hyperserotonemia. In addition, to the extent that blood and brain serotonin are related, vervet monkeys may be useful in defining the impact of social status on central serotonergic function. This potential relationship is being explored by measuring CSF 5-hydroxyindoleacetic acid (5-HIAA) level in basal conditions and following administration of probenecid with and without tryptophan loading.

The influence of social status and other socioenvironmental factors on blood serotonin concentration in humans has not been studied but may have clinical relevance. Our findings that blood serotonin concentration is correlated with a behavioral state suggest that the association between shifts in blood serotonin concentrations and clinical conditions in diseases such as Huntington's chorea, motor neuron disease, and bipolar depression needs to be investigated. However, conducting such studies in humans requires a more detailed characterization of human social status than is usually available. One of the characteristics of human societies is that each person is enmeshed in many social groups and many simultaneously occupy several dominant and subordinate status positions. Indeed, social influences on blood serotonin concentrations may be detectable only following major changes in life situations such as retirement, job demotion, extended personal crisis, or the assumption of leadership in a small, relatively closed group. We are currently studying some of these situations.

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