Genetic modulation of cognitive flexibility and socioemotional behavior in rhesus monkeys

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In human and nonhuman primates, structural variants of the gene encoding the serotonin transporter [5-hydroxytryptamine transporter (5-HTT)] affect the transcription and functional efficacy of 5-HTT. Prior work has shown that structural variants differentially affect function of the amygdala and ventromedial prefrontal cortex (VMPFC), regions important for the regulation and expression of emotion. However, relatively little is known about the impact of 5-HTT allelic variants on cognition. To address this question, we tested rhesus monkeys carrying orthologous structural variants of 5-HTT on a battery of tasks that assess cognitive flexibility, reward processing, and emotion. Here we show that rhesus monkeys carrying two copies of the short allele (SS) of the rhesus 5-HTT gene-linked polymorphic region (rh5-HTTLPR) show significantly reduced cognitive flexibility as measured by two tasks in the battery: object discrimination reversal learning and instrumental extinction. Monkeys with the SS genotype also displayed alterations in socioemotional behavior. Genotype variation was not related to visual perceptual abilities, valuation of food rewards, or the ability to express a wide range of defensive responses. Although emotional alterations associated with 5-HTT variation have been described as the primary phenotype, the present study reports differences in at least one type of cognitive flexibility, which has not been described previously. Because behaviors modulated by the 5-HTTLPR are a subset of those dependent on the VMPFC, analysis of structural and functional correlates of gene variation in this region may inform the nature of the genetic modulation of cognition.

aggression | extinction | orbital prefrontal cortex | reversal learning | serotonin

The neurotransmitter serotonin plays a central role in emotion, as evidenced by brain serotonergic abnormalities in emotional disorders and the therapeutic efficacy of drugs targeting this system. The gene encoding the serotonin transporter [5-hydroxytryptamine transporter (5-HTT)], which regulates serotonergic turnover via extracellular clearance, contains a length polymorphism in the promoter region that is present in many anthropoid primates (1). *In vitro* functional analyses of the 5-HTT gene-linked polymorphic regions (5-HTTLPR) in humans and rhesus monkeys demonstrate lowered transcriptional activity associated with short (S) compared with long (L) alleles (2). Human S-allele carriers are at greater risk for anxiety and depression, especially after periods of stress and adversity (3). In addition, genomic imaging studies have shown that healthy, nondepressed individuals carrying one or more S alleles show altered brain responses to emotionally laden stimuli (4–6) and possess altered functional connectivity of neural circuitry essential for the expression and regulation of emotion (7, 8), namely, the amygdala and ventromedial prefrontal cortex (VMPFC), defined broadly to include both ventral (i.e., orbital) and medial sectors of prefrontal cortex (PFC). Furthermore, structural differences in volume and gray matter density in a number of frontal, limbic, and cerebellar regions also differ as a function of 5-HTT genotype (6, 8). The finding that S-allelle carriers also exhibit altered neurophysiological responses to neutral stimuli, as well as to positive and negative ones, suggests that 5-HTTLPR variation influences neural systems beyond those regulating emotion (6, 9).

A parallel literature demonstrates that serotonergic manipulations also affect cognitive functions mediated specifically by the VMPFC. For example, just as monkeys and humans with VMPFC damage exhibit a robust impairment in cognitive flexibility, as measured by object discrimination reversal learning (ODRL) and related tasks such as extinction of an instrumental response (10–14), marmoset monkeys with 5-HT depletion within the PFC (15) and humans with dietary tryptophan depletion (16) exhibit impaired performance on ODRL. The effects of serotonin depletion within the marmoset PFC are both behaviorally and neurochemically specific. Whereas selective 5-HT depletion within the PFC disrupts one kind of cognitive flexibility mediated by the VMPFC, ODRL, it has no effect on another kind of cognitive flexibility mediated by lateral frontal cortex, attentional set shifting (17). In addition, serotonin depletion, but not dopamine depletion, limited to the orbital portion of the VMPFC reproduces the deficit on ODRL (18). Other tests of inhibitory control and decision making are disrupted by 5-HT manipulations as well. Marmoset monkeys with 5-HT depletion within VMPFC are slow to learn a detour-reaching task, and this deficit, like the one in ODRL, is associated with perseverative responding (19). In addition, rats with systemic 5-HT depletion choose more small, immediate rewards relative to controls on a temporal discounting task (20) and humans with acute dietary tryptophan depletion are impaired on probabilistic choices in an experimental gambling task (21). Together, these findings implicate serotonergic mechanisms within the VMPFC in at least one type of cognitive flexibility and hint at a broader role in inhibitory control and in decision making related to probabilistic choices.

Because rhesus monkeys in our laboratory had been administered ODRL, instrumental extinction, and other tests as part of a program examining the neural substrates of affective processing (10, 11, 22), we retrospectively evaluated their cognitive abilities with respect to 5-HTTLPR variation. We considered data for the five tasks that had been administered to all monkeys: ODRL, extinction, reactions to a rubber snake, reactions to a human intruder, and reinforcer devaluation. Standard tests of ODRL and instrumental extinction were included to measure cognitive flexibility. Both tasks tax the ability of monkeys to choose objects in the face of changes in reward contingency. We used the snake test as an assay of emotional responsiveness because rhesus monkeys show innate defensive responses to both fake and real snakes (23). Thus,

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Abbreviations: 5-HTT, 5-hydroxytryptamine transporter or serotonin transporter; 5-HTTLPR, 5-HTT gene-linked polymorphic regions; PFC, prefrontal cortex; VMPFC, ventromedial PFC; ODRL, object discrimination reversal learning.

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this test required no formal training. Similarly, we used the human intruder task because the presence of an unfamiliar adult human has been shown to elicit a constellation of behavioral responses in monkeys (24), many of them different from those elicited by a fake snake. The hallmark responses include defensive freezing (especially in the no eye contact condition) and both submission and aggression (especially in the stare condition). The behaviors elicited in response to an unfamiliar human, like those elicited by the snake, are held to be unconditioned responses; they are present early in life and reflect long-term emotional disposition (25). Finally, we included the reinforcer devaluation task to broaden our assessment of cognitive and affective processing. Unlike ODRL and extinction, in which monkeys choose objects according to reward contingency, reinforcer devaluation requires choosing objects according to reward value. Importantly, the neural substrates for reinforcer devaluation and ODRL are at least partially nonoverlapping; the former is dependent on the integrity of the amygdala, whereas the latter is not (26). Thus, the five tests taken together probed several aspects of cognitive, reward, and emotional processing.

Given the effects of manipulating serotonergic activity on object reversal learning, we predicted an effect of rh5-HTTLPR variation on ODRL and the related test of instrumental extinction. Although functional imaging studies in humans have provided strong evidence in support of amygdala hyperreactivity in response to threatening or negative stimuli in human S-allele carriers (4–6, 27), a prediction was less secure for the snake and human intruder tests. This is because emotional responses to a snake and human intruder target the expression of largely unconditioned defensive and aggressive responses, as opposed to the learning or context-dependent regulation of emotion. Finally, because the activity of neurons in VMPFC reflects the current biological value of foods gained from predictive cues (28, 29) and 5-HTTLPR variation in humans has been found to modulate incentive processes (30), we speculated that performance on the reinforcer devaluation task, too, would be modulated by genotype.

Results

For consistency with the ANOVA using repeated measures (across-test and between-subjects analyses below), parametric tests were used throughout. It should be noted, however, that because of the small number of subjects, nonparametric analyses also were conducted. In all cases, nonparametric tests yielded statistically identical results to those found by using parametric tests.

Analysis Across Tests. A 3×4 ANOVA with repeated measures on four of the five tests (ODRL, extinction, exposure to fake snake, and reinforcer devaluation) and between-subjects analysis of genotype (SS, SL, and LL) revealed a significant main effect of test ($F_{3,21} = 41.56$, $P < 0.01$), a significant main effect of genotype ($F_{2,7} = 8.60$, $P = 0.01$), and a significant test by genotype interaction ($F_{6,21} = 5.78$, $P < 0.01$). A single number could not be generated for each monkey in the human intruder task; analysis of the data from this task required a more complex, repeated-measures design. Accordingly, results for this test are considered separately (see below).

ODRL. The groups did not differ in the number of errors scored in acquiring a single, novel discrimination problem, before any reversal ($F_{2,7} = 1.96$, $P = 0.21$). Groups differed significantly, however, in the total number of errors required to reach criterion when reinforcement contingencies of the discrimination pair were reversed ($F_{2,7} = 7.49$, $P = 0.02$). Fisher's post hoc comparisons revealed that monkeys with the SS genotype made significantly more errors than those with either the LL ($P < 0.01$) or SL ($P = 0.02$) genotype (see Fig. 1*A*).

Instrumental Extinction. There was a marginally significant effect of genotype on the number of unrewarded displacements ($F_{2,7} = 4.25$, $P = 0.06$). Fisher's post hoc comparisons revealed that monkeys with the SS genotype performed significantly more unrewarded object displacements than did monkeys with the LL genotype ($P = 0.02$; see Fig. 1*B*).

Behavior During Exposure to a Rubber Snake. There were two measures of behavioral reactions to the fake snake: (*i*) mean cumulative duration of defensive responses and (*ii*) latency to reach over the snake to retrieve a piece of food. As shown in Fig. 1*C*, there was no effect of genotype on the duration of defensive behaviors displayed during exposure to a fake snake ($F_{2,7} = 0.60$, $P = 0.57$). A 3×5 repeated-measures ANOVA on food-retrieval latencies with within-subjects factor of session (session 1–5) and between-subjects factor of genotype (SS, SL, LL) likewise revealed no significant effect of genotype ($F_{2,7} = 1.49$, $P = 0.29$) and no significant session by genotype interaction ($F_{8,28} = 0.94$, $P = 0.50$). There was, however, a significant effect of session reflecting the tendency of all monkeys, regardless of genotype, to decrease their food-retrieval latencies with continued exposure to the snake ($F_{4,28} = 2.58$, $P = 0.05$).

Responses to Reinforcer Devaluation. The first phase involved acquisition of a set of 60 object-discrimination problems. There was no effect of genotype on the number of trials or errors scored in learning the 60 pairs (trials, $F_{2,7} = 0.07$, $P = 0.94$; errors, $F_{2,7} = 1.21$, $P = 0.35$). The second phase required monkeys to choose between objects associated with foods of different value, achieved by devaluing one food through selective satiation. As shown in Fig. 1*D*, there was no effect of genotype on the ability of monkeys to choose objects appropriately after changes in food value ($F_{2,7} = 1.67$, $P = 0.26$).

Behavior During the Presence of a Human Intruder. The measures of responses to the human intruder were mean cumulative durations of behaviors listed in Table 1. For each category of behavior (aggression, defense, submission, approach, and other), a 3×3 ANOVA with repeated measures on condition (Alone, No Eye Contact, and Stare) and between-subject analysis of genotype (SS, SL, LL) was conducted. Main effects of genotype on aggression (mild and high aggression combined) and "other behaviors" were found (aggression: $F_{2,7} = 4.8$, $P = 0.049$; other: $F_{2,7} = 43.7$, $P < 0.01$). An analysis of total aggression (Fisher's protected least-significant difference, all conditions collapsed) showed monkeys with the SS genotype displayed significantly more aggression across all conditions in the human intruder task relative to monkeys with either the SL ($P = 0.03$) or LL ($P = 0.03$) genotype (Fig. 2).

Discussion

Effects of rh5-HTTLPR Variation on Cognition and Emotion. Although there has been much emphasis on emotional alterations associated with 5-HTTLPR variation (31, 32), our results indicate that these effects extend to at least one type of cognitive flexibility. Rhesus monkeys homozygous for the S allele (SS) displayed significantly poorer performance on both ODRL (Fig. 1*A*) and instrumental extinction (Fig. 1*B*) compared with monkeys with either one or two copies of the L allele. Although there are several possible mechanisms that might underlie this cognitive modulation (33), the effects of serotonin depletion in the frontal cortex of monkeys suggests that the difficulty lies with inhibiting responses to the previously rewarded object, as opposed to learning the new status of the previously unrewarded object (18). In any event, rh5-HTTLPR variation appears to influence cognitive functions outside the affective domain. Importantly, there was neither an effect of genotype on acquisition of the initial discrimination problem (before reversal) nor an effect of genotype on acquisition of a large set of visual discrimination problems administered in phase 1 of the reinforcer devaluation task (see *Results*). These findings indicate

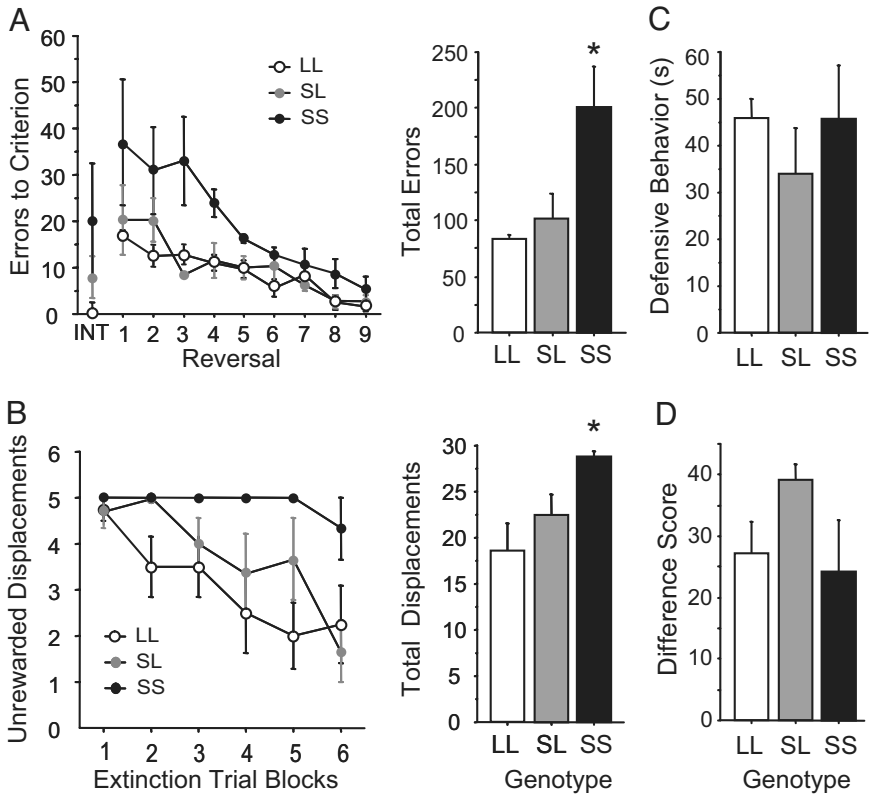

Fig. 1. Effects of rh5-HTTLR variation on cognitive flexibility, emotion, and reward processing. (*A*) Group mean errors (±SEM) in initial learning of a single visual discrimination problem (INT) and to complete nine successive reversals of ODRL (*Left*) and total errors summed across the reversals (*Right*; SS vs. LL: *, $P < 0.01$; SS vs. SL: *, $P = 0.02$). (*B*) Group mean number (±SEM) of unrewarded object displacements across blocks of five trials on a test of instrumental extinction (*Left*) and total number summed across the blocks (*Right*; *, $P = 0.02$ for SS vs. LL). (*C*) Group mean cumulative duration (sec) of defensive behaviors (±SEM) elicited during exposure to a rubber snake (NS). (*D*) Group mean cumulative difference scores (±SEM) representing shift in choices of objects after devaluation of the associated food reinforcer (NS). LL, monkeys homozygous for the rh5-HTTLPR long allele ($n = 4$); SS, monkeys homozygous for the rh5-HTTLPR short allele ($n = 3$); SL, monkeys carrying one each of the short and long alleles ($n = 3$); NS, no significant effect of genotype.

not only that visual perceptual abilities are unaltered, but also that not all types of visual learning are affected.

Given that there are several types of cognitive flexibility, future studies need to assess the impact of reduced transcriptional activity of 5-HTT associated with the 5-HTTLPR short allele on this domain more broadly. Although attentional set shifting, which depends on lateral portions of PFC, does not appear to be affected by serotonergic manipulations, other tests of cognitive flexibility that should be examined include response reversal, which depends on portions of medial PFC (34); detour reaching, which depends on serotonergic innervation of the VMPFC (19); and rule implementation and reversal, which also depends on PFC (35, 36), among others.

The effects of rh5-HTTLPR variation on expression of aggression is consistent with findings in humans, nonhuman primates, and rodents that have emphasized a role for serotonergic modulation of emotion (37), including aggression (38). For example, serotonin transporter knockout mice display abnormal emotional behaviors on a variety of tasks (39) and show both altered fronto-amygdalar morphology and impairments in fear extinction recall (40). At the same time, emotional responses and food-retrieval latencies exhibited by our monkeys in the presence of a fake snake, as well as their responses to changes in reinforcer value, were all unaffected by genotype (Fig. 1 *C* and *D*), even though these behaviors also depend on the integrity of the VMPFC (10, 22). If these results can be replicated in a prospective study with a larger sample size, they would indicate a highly specific and dissociable effect of rh5-HTTLPR variation on cognitive functions mediated by the VMPFC.

Comparison with Earlier Studies. Prior studies have compared rhesus monkeys heterozygous for the long and short alleles (SL) with those homozygous for the long allele (LL); in these groups, an influence of the rh5-HTTLPR on the central nervous system typically emerges only in the context of environmental stressors (2, 38, 41, 42). Yet our findings, which reveal a difference between monkeys homozygous for the short allele (SS) and those carrying one or two copies of the long allele (LS and LL), suggest that there is an influence of rh5-HTTLPR on cognition independent of environmental factors. Because the monkeys we studied were purchased from domestic breeding colonies, the details of their rearing histories are unknown to us. Accordingly, we cannot rule out the possibility that environmental factors such as stress contributed to the genetic influence on cognition we report. The strong possibility remains, however, that our results reflect the impact of carrying two copies of the short allele (i.e., allele load), rather than an effect of gene–environment interaction. If so, then perhaps environmental stressors act to unmask effects in heterozygotes. These questions should be addressed directly by studying the impact of rh5-HTTLPR variants on cognition in monkeys with known pedigrees and rearing history.

Effects of Genotype Variation on Serotonergic Function. The short allele of the rh5-HTTLPR is associated with reduced gene expression, which should translate to a lower 5-HTT density and increased synaptic 5-HT. It therefore is puzzling that the effects of carrying the S allele in rhesus monkeys mirror to some extent the cognitive inflexibility and impulsivity observed after serotonin depletion in frontal cortex of marmoset monkeys (18, 19), findings that are

Table 1. Behaviors analyzed during exposure to a fake snake and human intruder

Behavior	Description
Mild aggression	
Frown	Wrinkles or moves eyebrows up and down
Ears back	Flattens ears against head
Yawn	Opens mouth wide, baring upper teeth
High aggression	
Head/body lunge	Thrusts head or body forward
Cage shake	Shakes cage
Mouth threat	Opens mouth slightly, exposing lower teeth
Defense	
Freezing	Motionless for 3 sec or more
Startle	Jerks suddenly
Eye/head aversion	Avoids eye contact, shifts gaze or whole head
Piloerection	Hair stands on end
Move away	Retreats from the stimulus
Submission	
Lip smack	Purses, and alternatively closes and opens lips
Grimace	Mouth closed, pulls lips backward exposing teeth
Presentation	Presents its hindquarters with tail up
Approach	
Look at	Makes eye contact
Move toward	Shifts body forward, closer to stimulus
Touch	Handles with hand or foot
Take/eat reward	Picks up or mouths the food reward
Other behaviors (not directed towards the stimulus)	
Manual exploration	Handles any part of its surrounding
Oral exploration	Licks or mouths any part of its surrounding
Locomotor stereotypies	Activities, such as circling, hopping, repeated 3 or more times
Self-directed activities	Scratches, grooms, holds, etc. any part of its body
Look away	Looks away while engaged in behavior not directed towards stimulus
Teeth gnashing	Chewing motion without food in mouth
Miscellaneous	Engages in any peculiar activity not described above

generally consistent with the observation of increased impulsivity associated with low 5-HT in humans and nonhuman primates (43, 44). Currently, however, there is no consensus on the effect of 5-HTTLPR variants on the expression of 5-HTT in the brain. Although some *in vivo* imaging studies in humans have found an association of the S allele with reduced 5-HTT binding (45, 46), others have found no such association (47, 48). To our knowledge, the impact of the rh5-HTTLPR on 5-HTT density has not been evaluated in rhesus monkeys. Consequently, the full impact of carrying the S allele on 5-HT neurotransmission in the primate brain has yet to be elucidated.

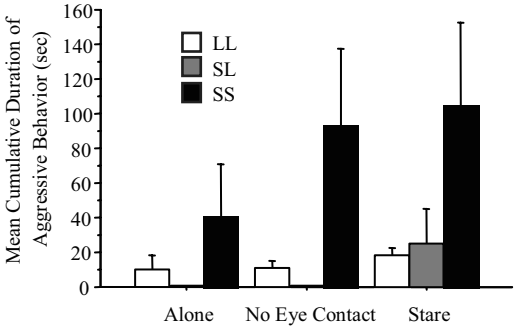

Fig. 2. Mean cumulative duration in seconds (±SEM) of aggressive behavior in the three different conditions of the Human Intruder task (Alone, No Eye Contact, and Stare) by genotype (SS, SL, and LL). Aggression includes behaviors in both mild aggression (frown, ears back, yawn) and high aggression (head/body lunge, cage shake, and mouth threat) categories. More aggression was observed in monkeys with the SS genotype across all three conditions of the task. Abbreviations are defined in the Fig. 1 legend.

One possible explanation for the puzzling set of observations is that the relative loss of 5-HTT function in S-allele carriers during development leads to changes in the function or sensitivity of 5-HT receptor subtypes. For example, it has been proposed that reduced 5-HT_{1A} receptor binding in S-allele carriers (49) produces a relative insensitivity to 5-HT in the synapse, which in turn yields reduced serotonergic modulation (see refs. 32 and 37 for review). Thus, paradoxically, increased amounts of 5-HT in the synapse during development could yield loss of signal in circuits by using serotonergic neurotransmission.

Neural Underpinnings of rh5-HTTLPR Variation Effects on Cognition and Emotion. As indicated earlier, difficulties with ODRL, instrumental extinction, and increased aggression are all associated with VMPFC but not amygdala damage or dysfunction (10, 22). In addition, our results are consistent with the clinical literature: damage to or dysfunction of VMPFC, but not amygdala, has been linked to clinical reactive aggression and acquired sociopathy (50, 51). Accordingly, the behavioral phenotype of monkeys homozygous for the S allele points to alterations in the functional connectivity of VMPFC with regions such as the rhinal (i.e., perirhinal and entorhinal) cortex and the caudate nucleus, both of which are essential for ODRL (52, 53) and likely interact with VMPFC in mediating this behavior. At the same time, the neuropsychological findings do not rule out the possibility that our results reflect an effect of the 5-HTTLPR on amygdala function, as demonstrated by functional genomic studies (7, 8). Given our current lack of knowledge regarding the potential functional interactions between amygdala and VMPFC in the rhesus behaviors that are modulated by

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genotype, clarification regarding a potential amygdala contribution awaits direct empirical investigation.

Rhesus monkeys not only exhibit functionally similar 5-HTT polymorphisms but also possess PFC anatomy similar to that of humans (54). Accordingly, they afford a valuable model for investigating the structural and functional correlates of the behavioral phenotype associated with the S-allele polymorphism in humans (38). In addition, given that serotonin function in rhesus monkeys is modulated by an interaction of rh5-HTTLPR variation and rearing experience (2), rhesus monkeys also provide a means of elucidating the gene–environment interactions affecting the development of VMPFC and related circuitry that is essential for both cognitive flexibility and the adaptive expression and regulation of emotion.

Materials and Methods

Subjects. The subjects were 10 male rhesus monkeys (*Macaca mulatta*), purchased from domestic breeding colonies. Their weights ranged from 4.8 to 14.6 kg at the beginning of testing. Animals were housed individually and fed a diet of primate chow (#5038; PMI Feeds, St. Louis, MO) supplemented with fruit. Water was always available. The 10 monkeys comprised two cohorts that had served as controls in two different studies run concurrently. One cohort ($n = 6$) served as the unoperated control group in a series of studies assessing the effects of orbital PFC damage on affective processing (10, 11, 22), and the other cohort ($n = 4$) served as the unoperated control group in a parallel series of studies (11, 22, 26) assessing the effects of selective amygdala damage. The training histories of the monkeys were virtually identical. Indeed, the two studies had been designed so that results would be directly comparable across studies (11, 22). Four monkeys were homozygous for the long allele of the rh5-HTTLPR (LL), three were heterozygous (SL), and three were homozygous for the short allele (SS). Procedures were reviewed and approved by the National Institute of Mental Health Animal Care and Use Committee.

Test Apparatus and Materials. Behavioral testing took place in the Wisconsin General Testing Apparatus following described methods (11, 55). For ODRL and reinforcer devaluation, a test tray measuring 19.2 cm (width) × 72.7 cm (length) × 1.9 cm (height) containing two food wells spaced 290 mm apart was used. For extinction, a three-well tray with wells spaced 180 mm apart was used. For the snake test, a clear Plexiglas box measuring 11.4 cm (width) × 71.1 cm (length) × 11.4 cm (height) contained the objects. Several hundred junk objects, varying widely in size, shape, color and texture, were available for testing. Different objects were used in each task. Rewards consisted of one of the following six foods: a single 300-mg banana-flavored pellet (P. J. Noyes, Inc., Lancaster, NH), one-half peanut, a raisin, a sweetened dried cranberry (Craisins; Ocean Spray, Lakeville-Middleboro, MA), a fruit snack (Giant Food Inc., Landover, MD), or a chocolate candy (M&Ms; Mars Candies, Hackettstown, NJ).

Behavioral Testing. Five tests were administered: the first two assessed the ability of monkeys to respond to changes in reward contingency (ODRL and extinction); the third and fourth measured behavioral reactions to a rubber snake and an unfamiliar human; and the fifth assessed the ability of monkeys to respond to changes in reward value (reinforcer devaluation).

ODRL. All monkeys first learned a single visual discrimination problem. On each trial, two objects were presented, an S+ (baited with food) and an S− (not baited), one each overlying the two food wells. Monkeys were allowed to displace only one of the two objects and, if correct, to retrieve the food reward underneath. No correction trials were administered. The intertrial interval was 10 sec, and the left–right position of the S+ followed a pseudorandom order. Monkeys were given 30 trials per daily session at the rate of 5 sessions per week. After monkeys attained criterion (a score of at least 93% on day 1 followed by at least 80% on day 2) on the initial discrimination, the reward contingencies were reversed. This procedure was repeated until a total of nine serial reversals had been completed. The number of errors to criterion for each reversal as well as the total number of errors accrued across all reversals were analyzed.

Extinction. All monkeys first acquired an instrumental response. On each trial, a single object was presented over the central, baited well of the test tray. Monkeys were given a maximum of 30 sec to displace the object and to obtain the food reward underneath. Whether or not the monkeys displaced the object, the trial continued until 30 sec had elapsed. After acquiring this instrumental response (28 of 30 responses in 30 trials for 5 consecutive days), monkeys were given a session of extinction in which the procedure was the same in all respects except that no food was located underneath the object. On each trial, the experimenter scored whether the monkey displaced the object within the 30-sec time limit. Monkeys received 30 trials each separated by 15 sec. The total number of unrewarded object displacements in 30 trials was recorded and analyzed.

Responses to a Rubber Snake. Exposure to real or fake snakes provides robust emotional responses in monkeys without the need for formal training. On each trial, the clear Plexiglas box contained one of eight neutral objects, a rubber spider, or a rubber snake. Monkeys had the opportunity to reach over the stimuli to retrieve a food reward located at the far edge of the box. Monkeys were tested for a total of 10 trials per day for 5 sessions. A camera recorded a frontal view of the monkey; the duration of the behavioral reactions to the stimuli were analyzed by a trained observer blind to genotype. Specifically, behaviors within the "defensive" category (see Table 1) were summed and analyzed for mean cumulative duration (see *Videotape Scoring of Emotional Reactions*). Latencies to reach over the stimuli to retrieve the food also were scored from videotape.

Responses to a Human Intruder. Monkeys were placed in a wheeled transport cage, taken to a novel room, and left alone for 5 min (Alone condition). An unfamiliar adult male human entered the room and sat ≈2.5 m away from the test cage and presented his side profile to the monkey for 5 min without making direct eye contact (No Eye Contact condition). The human then left the room for 3 min. When the human returned, he turned to face the monkey and stared at the monkey directly (Stare condition) for 5 min, remaining motionless and projecting a neutral facial expression. All conditions were videotaped and scored for mean cumulative duration of behaviors (Table 1) by a trained observer blind to genotype, by using the same scoring methods as those used in the snake test (see *Videotape Scoring of Emotional Reactions*).

Videotape Scoring of Emotional Reactions. We used established methods for rating the emotional reactions of rhesus monkeys (56). Behavioral reactions to stimuli were videotaped and later classified into 25 activities (Table 1), defined to be exhaustive but not mutually exclusive (e.g., move toward and lip smack can co-occur). Because many behaviors do not occur as discrete events, cumulative duration was used as the main measure. Activities were collapsed into five nonoverlapping categories based on their ecological similarity: mild aggression, high aggression, defense, submission, and approach. A sixth category, "other behaviors," constituted any behavior not directed toward the snake or human intruder.

Reinforcer Devaluation. This test was carried out in two phases. In phase 1, all monkeys were familiarized with a large number of objects and their associated food rewards through training on a standard 60-pair concurrent discrimination learning task. Half of

the positive objects were rewarded with one type of food (food 1), and the remaining positive objects were rewarded with a different food (food 2). Criterion was set at a mean of 90% correct responses over 5 consecutive days (i.e., a minimum of 270 correct responses in 300 trials). We scored trials and errors accrued up to but not including the criterion run. To measure monkeys' abilities to link objects with food value, in phase 2, monkeys were required for the first time to choose between familiar rewarded objects from phase 1. The effects of reinforcer devaluation were assessed in four critical sessions involving only the S+ objects. Thirty pairs, each composed of one food-1- and one food-2-associated object, were presented for choice. On each trial, both objects were baited with the same foods that they had been paired with during learning. Monkeys were allowed to displace only one of the objects to obtain the food reward underneath. Two of the four critical test sessions were preceded by a selective satiation procedure intended to devalue one of the two foods. The other two sessions were preceded by no satiation procedure and served as baseline measures. The unit of analysis, the "difference score," was the change in choices of objects in the sessions preceded by selective satiation as compared with baseline sessions.

Genotyping. DNA was isolated from whole blood, collected from animals under ketamine anesthesia (10–15 mg/kg, IM), by using a standard salting out method (PureGene; Gentra Systems, Minneapolis, MN). Using a protocol modified from that of Lesch *et al.* (1), the rhesus macaque serotonin transporter gene promoter region was amplified from 25 ng of genomic DNA with oligonucleotide primers (stpr5, 5′-GGCGTTGCCGCTCTGAATGC; intl, 5′-CAGGGGAGATCCTGGGAGGG) in 15-μl reactions by using Platinum Taq and the PCRX Enhancer System kit, according to the manufacturer's protocol (Invitrogen, Carlsbad, CA). Amplifications were performed on a PerkinElmer thermocycler (9700) with one cycle at 96°C for 5 min followed by 30 cycles of 94°C for 15 sec, 60°C for 15 sec, 72°C for 30 sec, and a final 3-min extension at 72°C. Amplicons were separated by electrophoresis on precast, 10% polyacrylamide gels, and the short (*s*, 398 bp) and long (*l*, 419 bp) alleles of the rh5-HTTLPR were identified by direct UV fluorescent visualization after ethidium bromide staining, by using control amplicons for allele size determination.

Statistics. Statistical analyses were conducted by using Statview software.

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