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Polymorphisms in the serotonin reuptake transporter gene modify the consequences of social status on metabolic health in female rhesus monkeys

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Abstract

Individuals vary substantially in their vulnerability to physical and psychosocial stressors. The causes of such variation in susceptibility to stress are poorly understood, but are thought to relate in part to genetic factors. The present study evaluated the extent to which polymorphisms in the gene encoding the serotonin reuptake transporter (5HTTLPR or SERT) modulated physiologic responses to the imposition of psychosocial stress (social reorganization and subordinate social status) in female rhesus monkeys. Forty females, drawn from the middle ranking genealogies of several large social groups, were reorganized into eight groups containing 5 monkeys each; four groups were comprised entirely of animals homogeneous for the long promoter variant in the SERT gene (*l/l*), while the other four groups had monkeys with at least one allele of the short promoter variant (*l/s or s/s*). Females were sequentially introduced into these new groups in random order and dominance ranks were established within several days. During the ensuing 6 weeks, dominant monkeys exhibited elevated rates of aggression while subordinates displayed high rates of submission. Notably, females with the s-variant SERT genotype, collapsed across social status positions, exhibited the highest overall rates of both aggression and submission. Although neither social status nor SERT genotype influenced morning cortisol concentrations, glucocorticoid negative feedback was reduced significantly in subordinate compared to dominant females irrespective of genotype. All animals lost weight and abdominal fat across the experiment. However, decreases were greatest in subordinates, regardless of genotype, and least in dominant females with the l/l genotype. Serum concentrations of insulin, glucose, and ghrelin decreased significantly during the group formation process, effects that were independent of genotype or social status. In contrast, social status and genotype interacted to influence changes in serum concentrations of leptin and triiodothyronine (T3), as dominant, l/l females had the highest levels while subordinate s-variant females had the lowest levels. The order in which a female was introduced to her group generally predicted her eventual social rank. However, rank was additionally predicted by pre-experimental T3 and abdominal fat values, but only in the l/l animals.

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While these findings must be replicated with a larger sample size, the data suggest that the s-variant SERT genotype confers increased vulnerability to the adverse effects of psychosocial stress associated with subordinate status while the l/l genotype benefits the most from the absence of stress conferred by dominant social status. These findings suggest that genetic factors modify the responses of monkeys to social subordination and perhaps other psychosocial stressors.

Keywords

Social status; 5HTTLPR; Psychosocial stress; Metabolism

1. Introduction

It is often suggested that occasional activation of neuroendocrine stress–response systems is adaptive, but that frequent or prolonged activation can adversely affect health. Hence, an acute release of glucocorticoid hormones in response to stress promotes cognitive and physiological responses that are protective in the midst of a short-term challenge. In addition to stimulating gluconeogenesis, glucocorticoids increase appetite and locomotor activity, responses that would normally constitute adaptive mechanisms for survival [1]. In contrast, chronic activation of stress–response mechanisms can elevate blood pressure, exacerbate the development of atherosclerosis, adversely alter carbohydrate metabolism, reduce immune function, and disrupt the reproductive axis [2–6].

Emerging evidence suggests that genetic factors may modulate individual differences in responses to stress and perhaps, the adverse health consequences associated with chronic stress exposure [7,8]. Variation in serotonin (5HT) neurotransmission is one such factor, as 5HT can limit responses to acute stress [9], and its activity is compromised during chronic stress [10, 11]. Indeed, the short length variant in the promoter region of the gene that encodes the serotonin reuptake transporter (5HTTLPR or SERT) is associated with diminished transcriptional activity compared to the long allele [12,13], and people with the short allele have increased incidences of anxiety and depression in response to life stressors [14–18]. Individuals who are homozygous for the long variant (*l/l*) show a greater response to SSRIs than those with either one or two short alleles (s/l, s/s) [19]. Length variations with reduced transcriptional activity are present in other primates, including rhesus monkeys (*Macaca mulatta*) [12,20]. Indeed, an interaction exists between an animal's genotype and rearing environment, as CSF levels of the 5HT metabolite 5-hydroxyindoleacetic acid (5HIAA) are lower in peer-raised monkeys with the short allele (either *s/s* or *l/s*) compared to *l/l* animals; in contrast, 5HIAA is similar in mother-reared monkeys, regardless of genotype [20]. Also, targeted disruption of the SERT gene [21] increases anxiety as well as glucocorticoid and adrenocorticotropic hormone (ACTH) responses to stressors in mice [22,23]. Similarly, peer-reared monkeys with the *s* allele exhibit a greater ACTH response to social separation than monkeys with an *l/l* genotype [24].

Numerous experimental paradigms have been used to explore the effect of chronic stress on animal physiology and behavior. Perhaps the most ethologically relevant for human beings have been those focusing on the mammalian proclivity to form social status hierarchies and on the stress often associated with hierarchy formation. Status hierarchies are a major organizing feature in many rodent and primate species and individual differences in social status have been identified as a predictor of health outcomes in human populations [7]. For example, low status in employment or social networks places individuals at increased risk for cardiovascular disease [25] and immune disorders [26]. In socially housed rhesus monkeys, frequent harassment from dominant individuals results in a lack of environmental control that delays or prevents access to food, shelter, and preferred social partners while increasing the risk of

wounding at the hands (and teeth) of higher ranking monkeys trying to preserve their dominant status [27,28]. Within a status hierarchy of monkeys the clearest physiological sign of increased stress on the part of subordinates is a sustained elevation in glucocorticoid release [29–31], which is accompanied in females by reproductive suppression, bone loss, psychopathology, and increased risk of cardiovascular disease [14,15,16].

We recently observed that, among a cohort of adult female rhesus monkeys drawn from the mid-ranking matrilines of several social groups, individuals possessing the short allele variant (*l/s* or *s/s*) had lower body weights and adiposity measures, lower serum concentrations of leptin and insulin in response to a fast, and reduced 5HT release in response to provocative stimuli in comparison to their l/l counterparts; animals with the short allele variant also displayed subtle dysregulation of the limbic–hypothalamic–pituitary–adrenal (LHPA) axis [32]. The present study, using this same cohort of animals, was designed to determine whether the foregoing SERT polymorphisms affect the pattern of physiological responsivity to the psychosocial stress, represented here by individual differences in social status produced during new group formation. Specifically, we tested the hypothesis that social subordination in the presence of the short allele variant in the SERT gene would reduce glucocorticoid negative feedback and adversely change body mass and hormones associated with energy balance.

2. Materials and methods

2.1. Subjects

Subjects were forty adult female rhesus monkeys (*M. mulatta*) that, with the exception of one female, were members of one of five breeding groups located at the Yerkes National Primate Research Center Field Station, Emory University. These groups contained multiple adult females, juveniles, and two to three adult males each. Animals were housed in outdoor compounds with attached indoor quarters as described previously [33]. One female was a member of a smaller breeding group, housed in an indoor–outdoor run with other adult females, a male, and juvenile offspring. Groups were fed standard monkey chow (Ralston Purina Company, St. Louis MO) twice-daily *ad libitum* and supplemented daily with seasonal fresh fruit and vegetables. All procedures were approved by the Emory University Animal Care and Use Committee in accordance with the Animal Welfare Act and the U.S. Department of Health and Human Services “Guide for Care and Use of Laboratory Animals.”

Females were selected on the basis of three criteria: 1) parity; 2) dominance status within their respective group; and 3) SERT genotype. Hence, all females were multiparous adults and were between 7 and 10 years of age. First pregnancy for females in our breeding colony typically occurs at 4 years of age [34]. Secondly, only animals from the middle portion of the dominance hierarchy were selected [27]. Because the five groups used varied in the number of adult animals (>3 years) from 19 to 66, we divided the group into thirds and focused on only those in the middle portion of the hierarchy. Dominance status was determined from the outcome of dyadic interactions [35], based on 10 h of observation of each group over a 4-week period. Following confirmation of parity and dominance status, monkeys were screened for SERT polymorphisms [12,32]. Based on these results, we chose 20 females with an *l/l* genotype and 20 with either an *l/s* or *s/s* genotype, as the heterozygote produces a similar phenotype as the homozygous genotype (*s/s*) on most [20,36] though not all measures [37]. Four females of each genotype were identified in each of 5 groups. The female living in the small breeding group had an *l/l* genotype. In our cohort of selected females, nineteen were *l/s* and one was *s/s* and are hereafter referred to as s-variant. Following selection, veterinarians within the Division of Clinical Veterinary Medicine at the Primate Center ovariectomized females.

2.2. Group formation process

Females, who had been studied previously in their natal groups [32], were removed from their natal groups to form eight, five-member groups. Four groups were comprised entirely of females with an *l/l* SERT genotype and four were comprised of monkeys with either the *l/s or s/s* genotype. Furthermore, animals in the new groups had not lived with each other previously (see Table 1). Although all monkeys were middle ranking in their natal groups, they occupied the full range of ranks in their new groups (one to five). Groups were formed using indoor–outdoor runs in which each area measured approximately 144 ft^2 (12 × 12 ft). The group formation process was modified from established procedures that introduced animals simultaneously [38]. Rather, we chose a staged introduction process that involved as a first step the placement of two animals together in two adjacent indoor–outdoor pens. After 24 h access to two runs the space was reduced to one indoor–outdoor run. A third female then was placed into the adjacent run where she had visual access (via a Plexiglas door) to the just-established pair. Twenty-four hours later, the third female was introduced to the pair by again reducing the available space to one run. This procedure was repeated until all five animals were together in one run, where they were confined for the remainder of the study. Females were added to groups in random order. As new females were added to each group, behavioral data were collected throughout the day *ad libitum* to assess the degree of aggression and to intervene, if necessary.

2.3. Outcome measures

The intent of this analysis was to determine whether the effect of psychosocial stress (new group formation and attendant changes in social status) on activation of the LHPA axis [39, 40] was influenced by SERT genotype. Because corticotropin-releasing hormone (CRH) is known to be anorexic [41,42], we focused on metabolic and body weight-related endpoints. During the month prior to the group formation process, we obtained height and sagittal abdominal diameter (SAD) measurements for all individuals. The same measurements were made 7 weeks following the group formation. All samples were collected following anesthesia with ketamine given intramuscularly at 10 mg/kg. To determine height, animals were placed in a supine position with the legs straightened on a long piece of paper and a mark was made at the top of the head and at the bottom of the heel. Two investigators measured the distance between points using vernier calipers and the mean of these measurements was calculated for an animal's height. SAD was determined by measuring the distance from back to the top of the abdomen at the level of the navel while animals were in a supine position [43]. As with height, SAD was calculated as the mean of two independent measurements. Body weights were obtained on the day a female was removed from her natal group prior to relocation to the new housing unit and weekly thereafter.

All females were trained prior to the start of the study for conscious venipuncture using previously validated methods [33,44]. This technique allowed five animals within a specific group to be captured, sampled, and returned to their housing within 10 min. While it is possible that stress-related hormones can be elevated by the venipuncture procedure in acclimated subjects, the amount of time that elapsed between the removal from the housing unit to collection of the sample was unrelated to the variance in either cortisol (r^2 =0 to 8%) or ACTH (r^2 =0 to 6%).

Serum samples were obtained from each female the morning she was removed from her natal group prior to relocation to the new housing unit. Subsequent samples were obtained 24 h later and then at weekly intervals for 7 weeks. All samples were obtained between 0800 and 0900 h, following the morning feeding. In addition to measuring cortisol, a number of hormones were assayed to assess the relationship between SERT polymorphisms and stress responsivity. These included leptin and ghrelin, which provide peripheral signals of adiposity, energy

balance, and food intake [45]. Insulin and glucose were measured as indices of energy homeostasis [46]. Finally, the thyroid hormones, triiodothyronine (T3) and thyroxine (T4), were measured, as these hormones are decreased by reduced food intake [47] and exposure to chronic stressors [48].

In addition to measuring morning samples for cortisol, LHPA reactivity to the group formation process was also assessed by a dexamethasone suppression test 4 weeks after the last female had been added to the new group. In this assessment, plasma samples were obtained at 0900, 1200, and 1730 h followed by a dexamethasone injection (0.25 mg/kg, IM). Post treatment plasma samples were obtained at 0900 and 1200 h the next day to assess glucocorticoid negative feedback on both pituitary ACTH and adrenal cortisol release. The degree of suppression by dexamethasone was determined by comparing hormone values at 0900 (15.5 h) and 1200 (18.5 h) following dexamethasone to similar times prior to treatment. Ten females (two groups) were tested at the same time.

Behavioral data were recorded by means of *ad libitum* scans across the first week of group formation. Following the addition of the final female to each group, the *ad libitum* scans were continued and complemented by formalized, one hour sampling periods during which all occurrences of affiliative, anxiety-like, and agonistic behavior were recorded using a Palm PDA and the “Hands Obs” program developed by the Center for Behavioral Neuroscience [49]. Data were collected in the format of actor–behavior–recipient, except for solitary behaviors (no recipient). “Affiliative” behavior included the initiation of grooming or proximity. “Aggressive” behavior was defined by grabs, bites, slaps, threats, and chases. Avoidance, grimaces, cowers, and squeals indicated “submission”, while “anxiety” was comprised of body shakes, yawns, scratching, and self-grooming. After each 1-h session, the file was downloaded to a desktop computer for error checking and summarization for analysis. Two 1-h sessions were conducted during each of the next 6 weeks on each of the eight groups. For analysis, data from the two weekly sessions were collapsed into a weekly mean for each female. Behavior was recorded by three previously trained observers who had an inter-observer reliability of >92%.

Dominance status was determined by the outcome of unequivocal dyadic agonistic interactions [27]. Using previously described conventions [50], females were categorized as dominant (ranks 1 and 2) or subordinate (ranks 3, 4, and 5) for analysis. Using this approach, group sizes were: dominant, l/l genotype n=8; dominant, s-variant genotype n=8; subordinate, l/l genotype n=12; and subordinate, s-variant genotype n=12 (see Table 1).

2.3.1. Laboratory assays—Serum or plasma levels of cortisol were determined by radioimmunoassay (RIA) with a kit from Diagnostic Systems Laboratory (Webster TX). Using 25 μl, the assay has a range from 0.5 to 60 μg/dl with an inter-and intra-assay coefficient of variation (CV) of 4.9% and 8.7%, respectively. Plasma ACTH was determined with a commercially available kit from DiaSorin (Stillwater MN). Using 100 μl, the assay has a range from 8.5 to 476 pg/ml with an inter-and intra-assay Cv of 9.83% and 6.84%, respectively. Serum leptin was measured by RIA using a commercially available kit (Linco, St. Louis MO). Assaying 100 μl, the assay has a range of 0.5 to 100 ng/ml. Intra-assay CVs were 6.84% and inter-assay were 7.24%. Serum insulin was assayed with a kit from Diagnostics Products Corporation (DPC, Los Angeles, CA) having a sensitivity of 3 to 372 IU/L and an inter-and intra-assay CV of 9.02% and 5.87%, respectively. Serum glucose was determined by a commercially available colorimetric enzyme assay (Stanbio Laboratory, Boerne TX), having a range from 0 to 27 mmol/L and inter-and intra-assay CVs of 2.12% and 4.21%, respectively. Serum T4 was assayed with a kit from DPC having a sensitivity of 0.5 to 24 μg/dl and inter-and intra-assay CVs of 10.46% and 6.96%, respectively. Serum T3 was assayed with a kit from DPC having a range from 0 to 600 ng/dl and inter-and intra-assay CVs of 11.17% and 2.06%,

respectively. Active (acylated) ghrelin concentrations were measured by an ELISA following the sample collection protocol to minimize ghrelin degradation (ALPCO, Boston MA). The assay has a range from 1.96 to 250 pg/ml with an inter-and intra-assay CV of 4.47% and 5.50%, respectively.

2.4. Statistical analyses

Analysis of variance models evaluated the main and interaction of social status, SERT genotype, and time in a 2×2 factorial design. Simple main effects were used to determine group differences at baseline and week 7. If interaction terms were significant, Bonferonni post hoc tests identified how groups differed using the error terms for those pairs. Data were transformed if nonhomogeneity of variance was present. Analysis of baseline measures addressed two hypotheses: whether the variance in a given measure was related to SERT genotype *prior* to the formation of the new groups and whether these measures were related to, or predictive of, the dominance status position acquired by the animal. To address this second question specifically, stepwise multiple regression was performed separately for each SERT genotype to determine what variables accounted for social dominance acquired. Statistical tests having a probability of $p<0.05$ were considered significant.

3. Results

3.1. Behavior

Groups were formed with minimal contact aggression and an absence of wounding. The top two ranking animals within each group emerged unambiguously within the first week of the introductions following the introduction of the 5th female. Formalized observations, begun after the last female was added to each group, indicated that rates of affiliation declined significantly over the ensuing 6 weeks (Table 2; $F_{5,180}=7.42$, $p<0.01$) in a pattern that was significantly influenced by the interaction of status and genotype ($F_{5,180}=4.01$, $p<0.01$). However, a consistent effect of status and genotype was not evident across the 6 weeks, as rates of affiliation were similar among the four groups during weeks 1, 4, and 6 yet varied significantly at other times with dominant, s-variant females having highest rates on weeks 2 and 3 and subordinate, l/l females the highest during week 5. In contrast, the time females spent engaged in affiliative behavior (sitting in proximity or grooming) decreased over time ($F_{5,180}=2.30$, $p=0.05$) but was not influenced by a main effect of status ($F_{1,36}=0.19$, $p=0.67$) or genotype ($F_{1,36}=0.50$, $p=0.49$) or their interaction with weeks ($F_{5,180}=0.91$, $p=0.47$).

As illustrated in Table 2, overall rates of aggressive behavior initiated by females were significantly higher in dominant females ($F_{1,36}=8.76$, $p<0.01$). Furthermore, overall rates of aggression were also higher in females with the s-variant genotype ($F_{1,36}=5.57$, $p=0.02$) and the pattern of aggressive behavior showed a significant week by genotype interaction ($F_{5,180}=4.06$, $p<0.01$), with s-variant females, collapsed across social status positions, having higher rates of aggression on weeks 1, 3, and 5. While rates of submissive behavior exhibited by females were significantly higher in subordinate compared with dominant females ($F_{1,36}=27.13$, $p<0.01$), the pattern over the 6 weeks of observation was significantly influenced by genotype ($F_{5,180}=2.38$, $p=0.04$), with higher rates shown by s-variant females on weeks 1, 3, and 5. Finally, rates of anxiety-like behavior increased significantly over the 6 weeks ($F_{5,180}=3.55$, $p<0.01$) but were not affected by status ($F_{1,36}=0.44$, $p=0.51$) or genotype ($F_{1,36}=0.96$, $p=0.33$).

3.2. LHPA responsivity

Morning (0800–0900 h) cortisol concentrations varied significantly during the 7-week period ($F_{6,216}=22.68$, $p<0.01$) but were not significantly affected by SERT genotype ($F_{6,216}=1.88$, $p=0.09$) or attained social status ($F_{6,216}=1.14$, $p=0.34$) (Fig. 1). Serum cortisol rose

significantly during the first day of the introduction before declining significantly by the end of the first week. However, levels through week 7 remained significantly elevated above pre-introduction, baseline values for all females. See below.

Cortisol and ACTH concentrations just prior to dexamethasone administration did not vary significantly by social status ($F_{1,36}$ =0.39, p=0.54; $F_{1,36}$ =1.46, p=0.24, respectively) or SERT genotype ($F_{1,36}$ =0.12, p=0.74, $F_{1,36}$ =0.5, p=0.82, respectively). However, following dexamethasone exposure, cortisol was suppressed to a significantly greater extent in dominant as compared to subordinate monkeys, irrespective of SERT genotype (Fig. 2; Status: $F_{1,36}$ =4.23, p=0.04; SERT genotype: $F_{1,36}$ =0.37, p =0.54). A similar pattern was observed with respect to plasma ACTH concentrations (Status: $F_{1,36}$ =4.75, p =0.04; SERT genotype: $F_{1,36}$ =0.14, p =0.91).

3.3. Anthropometric measures

Initial body weights varied significantly by SERT genotype (Fig. 3; $F_{1,36}$ =15.78, p<0.01) as well as the interaction between genotype and a female's attained dominance status ($F_{1,36}$ =5.91, p =0.02). That is, *l/l* females that became dominant were significantly heavier prior to the group formation than other females that did not differ significantly among each other. This interaction was again significant at week 7 ($F_{1,36}$ =6.24, p=0.02), as dominant *l/l* females had significantly higher body weights than s-variant dominant and all subordinate animals. Assessment of weekly changes in body weights indicated that all females lost weight during the 7-week period (Fig. 3, $F_{6,210}$ =10.54, p < 0.01). This decrease was significantly less in dominant compared with subordinate animals ($F_{1,36}$ =7.36, p=0.01) and was not influenced by a status–genotype interaction ($F_{1,36}$ =0.95, p=0.33). The pattern of weight loss between dominant and subordinate females also varied significantly over the 7 weeks ($F_{6,210}$ =4.60, p < 0.01), as dominant females lost the most weight during the first week, while weights declined in subordinates through week 4 after which they stabilized (Fig. 3). It must be noted that although the loss in body weight in dominant s-variant females was not significantly different than in dominant, l/l females (Fig. 3), their body weight at week 7 was significantly lower due to lower baseline, pre-introduction weights.

Heights at baseline did not vary between *l/l* and s-variant dominant (76.4±0.9 vs. 77.1±0.9 cm) and subordinate females (77.2±0.7 vs. 76.4±0.8 cm; $F_{1,36}$ =0.81, p =0.37) and did not change following group formation (data not shown). In contrast, the measure of adiposity (SAD) at baseline was significantly influenced by an interaction of genotype and social status (Fig. 4; $F_{1,36}$ =5.88, p=0.02), with *l/l* animals that became dominant showing significantly more adiposity than other groups. This significant interaction between status and genotype was also evident at week 7 ($F_{1,36}$ =7.05, p =0.01). The significant impact of social status was evident in change in adiposity from baseline to week 7 ($F_{1,36}$ =4.78, p =0.03), as dominant females showed a smaller decrease in SAD. Consideration of the interaction of status and genotype did not affect this pattern ($F_{1,36}$ =0.38, p =0.54).

3.4. Metabolic indices

Fig. 5 shows that serum leptin concentrations varied significantly by the interaction of genotype and status over the course of the study. Baseline levels of leptin were significantly higher in females with the *l/l* genotype ($F_{1,36}$ =13.55, p < 0.01). The significant interaction of social status and genotype ($F_{1,36}$ =12.57, p < 0.01) was evident as a result of the group formation, as leptin concentrations by week 7 were significantly higher in dominant females with the *l/l* genotype compared to all other groups whereas s-variant subordinate animals had the lowest concentrations. The change in serum leptin over the course of the study was significantly influenced by the status–genotype interaction ($F_{1,36}$ =5.72, p=0.02), as dominant, *l/l* females

showed an increase, s-variant females who became dominant had little change, and subordinate females, regardless of genotype showed the greatest decrease.

Other metabolic hormones were not affected in the same manner as leptin (Table 3). Insulin concentrations at baseline did not vary significantly by genotype ($F_{1,36}$ =2.60, p =0.12) or eventual status ($F_{1,36}$ =2.95, p=0.09). However change in insulin over the 7 weeks was significantly affected by genotype ($F_{1,36}$ =6.90, p =0.01) but not a status by genotype interaction ($F_{1,36}$ =2.34, p =0.14). These changes from baseline produced insulin concentrations at week 7 that were significantly higher in dominant compared with subordinate females ($F_{1,36}$ =6.61, p = 0.01), regardless of genotype ($F_{1,36}$ = 0.185, p = 0.67). Glucose levels at baseline ($F_{1,36}$ =0.34, p =0.56) and week 7 ($F_{1,36}$ =0.53, p =0.99) were not influenced by status or genotype but levels did decline significantly in all females ($F_{1,36}$ =12.86, $p < 0.01$). Similarly, ghrelin concentrations at baseline ($F_{1,36}$ =0.12, p =0.73) and week 7 ($F_{1,36}$ =2.68, p =0.11) were also unaffected by a status–genotype interaction but concentrations decreased significantly in all females during the 7 weeks ($F_{1,36}$ =4.83, p =0.03).

Thyroid hormones were also differentially affected during the group formation process. As shown in Table 3, serum T4 was not affected significantly by a status–genotype interaction at baseline ($F_{1,36}$ =0.55, p=0.46) or week 7 ($F_{1,36}$ =0.67, p=0.42) and did not change significantly over the course of the study ($F_{1,36}$ =2.68, p =0.11). In contrast, T3 concentrations at baseline were significantly higher in females who became dominant versus those who became subordinate (Fig. 5; $F_{1,36}$ = 5.55, p=0.02), a pattern not influenced by the interaction with genotype ($F_{1,36}$ =2.45, p =0.13). The decrease in T3 concentrations over the 7 weeks was significantly influenced by genotype ($F_{1,36}$ =4.57, p=0.04) and not status ($F_{1,36}$ =0.65, p=0.43), with s-variant females showing the greatest decline. Consequently, by week 7, T3 levels varied significantly by status ($F_{1,36}$ =8.43, p<0.01) and genotype ($F_{1,36}$ =4.28, p=0.04), with dominant *l/l* females having the highest levels and subordinate s-variant females the lowest levels (Fig. 5). This effect was also reflected in T3:T4 ratios (Table 3). While baseline ratios of T3 to T4 did not vary by genotype and social status ($F_{1,36}$ =0.21, p =0.65), ratios at week 7 were significantly higher in dominant compared to subordinate females ($F_{1,36}$ = 9.52, p<0.01), and this difference was not influenced by genotype ($F_{1,36}$ =0.48, p =0.83).

3.5. Predictors of social status

Stepwise multiple regression analyses were performed separately for each SERT genotype to determine what measures obtained at baseline predicted ranks acquired during the group formation process. Predictors included the order a female was added to the group (1, 1, 3, 4, 5); age; body weight; SAD; BMI; insulin; baseline and Day 1 cortisol concentrations; and serum T3. The analysis of the s-variant females indicated that 43.9% of the variance in the rank a female acquired was accounted for by the order she was introduced to the group ($F_{1,18}$ =14.11, p=0.001). None of the other variables added significant predictability to the model. In contrast, analysis of the *l/l* females indicated that 70.3% of the variance in the acquired rank was accounted for by SAD, T3, and order in which she was introduced ($F_{3,16}$ = 12.64, $p < 0.01$). Indeed, SAD alone accounted for 44.8% of the variance and the inclusion of T3 accounted for an additional 16% of the variance. Because the order females were added to the groups was done randomly, there was no relationship between order added and a female's SAD or T3 for either the *l/l* groups (r_{18} = − 0.041; r_{18} = − 0.073, respectively, $p > 0.05$) or s-variant females (r_{18} = − 0.315, r_{18} =0.202, respectively, $p > 0.05$).

4. Discussion

The purpose of this study was to determine whether the SERT genotype would affect behavioral and physiological responses in female rhesus monkeys to the formation of new social groups and the attainment of specific social status positions. Because groups were homogeneous for

the SERT genotype, we could determine how genotype interacted with dominance status to affect the outcome measures. The data clearly show that social status positions attained during the group formation process significantly affected a number of behavioral, neuroendocrine, and metabolic factors in a predictable fashion, with subordinate females showing more submissive behavior, reduced glucocorticoid negative feedback, reduced body mass, and increased evidence of negative energy balance. With the exception of LHPA activity, SERT genotype modified the effects of social status on the frequency of agonistic behavior, changes in anthropometric measures, and circulating concentrations of metabolic hormones, as well as influenced what factors best predicted the social ranks attained by each female during the group formation process.

Rates of affiliation decreased throughout the study period and although the specific pattern was affected by a status by genotype interaction, no consistent group differences emerged. High rates of affiliation in the immediate interval following the introductions likely functions to establish social bonds and acquisition of dominance positions [38], whereas the latter, of course, is maintained by aggression. While rates of aggressive and submissive behavior were largely influenced by social status, the main effect of genotype was also significant for aggressive behavior and the pattern of both aggressive and submissive behavior across the study was significantly affected by genotype, with s-variant females showing higher rates during three of the 6 weeks. Although this observation could imply that the establishment of the dominance hierarchy took longer in groups comprised of s-variant females, we have no evidence that this was the case. Rather, the increased aggression suggests the s-variant females may be more impulsive in this socially challenging situation. While we did not characterize central 5HT activity, previous studies indicate that the s-variant allele in SERT gene is associated with reduced 5HT tone [32,51,52] and reduced central 5HT activity is associated with increased impulsivity and aggression [53,54] as well as hostility in humans [55,56]. Thus, the significantly higher rates of aggression in s-variant dominants compared to *l/l* dominant animals and the higher rates of submissive behaviors in s-variant animals suggest a difference in reactivity inherent to these genotypes. Continued assessment of these animals, using standardized tests of impulsivity, will confirm this possibility. Whereas rates of anxiety-like behavior did not vary by genotype and social status, these behaviors increased over time. It is possible that as the novelty of the new group wanes, evidence of more anxiety-like behavior will not only emerge in subordinate females [57] but in s-variant females, regardless of social status [18].

We chose to form the groups so that they were homogeneous for genotype. It is entirely possible that different patterns of behavior during the group formation process would have emerged had groups consisted of both l/l and s-variant genotypes, particularly if s-variant females showed more impulsivity. Furthermore, this point underscores the fact that social behavior between animals in a group is not independent; thus, it is not surprising that the weeks during which s-variant females showed higher rates of aggression, rates of submission were also increased. While our intent was to describe the general behavioral phenotype of animals during the formation of these groups, additional assessments using standardized tests of anxiety or sequence analysis of spontaneous social behavior may show how SERT genotype influences the behavior of females at different social status positions.

The data provided evidence that the LHPA axis was differentially activated in subordinate females. Morning serum cortisol levels did not vary amongst females, supporting previous data that social subordination is unrelated to morning cortisol values in newly formed groups [58, 59] but may predict higher baseline levels in long-term, established groups [58]. Cortisol concentrations rose in all females within 24 h of the introduction and remained elevated throughout the 7-week period. These data corroborate other findings [40] and show that, even in the absence of severe aggression, the group formation process was a psychosocial stressor

to all females. However, as had been reported previously [60,61], glucocorticoid negative feedback was reduced significantly in subordinate animals, regardless of genotype. This test of glucocorticoid resistance accurately reflects changes in glucocorticoid receptor binding in the hippocampus in response to a chronic stressor in a number of animal models [62,63] and is predictive of stress-induced affective disorders in humans [64]. Although other studies of rhesus macaques show that the s-variant allele in SERT gene is associated with greater LHPA reactivity [24,32], the approach used in the present study did not find differences between subordinate females with the *l/l* and s-variant genotypes. It is possible that that genotype differences in subordinate females will emerge with time.

Assessment of the anthropometric and metabolic data lends support for the hypothesis that the group formation process was a stressor for all females, although the consequences were mitigated by SERT genotype in several cases. Studies using the visible burrow system in rats clearly show the adverse metabolic effects of subordination [29–31] and complimentary studies using repeated restraint stress provides similar data [41]. All females in the present study lost body weight over the course of 7 weeks but the decrease was greater in subordinate females, regardless of SERT genotype. These rank-related differences in body weight are similar to those observed following the formation of groups of juvenile macaques [65]. The most parsimonious explanation for this effect is that food intake was suppressed during this time period; however, other rodent data suggest that the maintenance of lower body weights resulting from repeated restraint stress is not explained by persistent hypophagia but reflects an adaptation to a new body weight set point [41]. Although food intake was not measured, circulating concentrations of glucose and active ghrelin decreased significantly, independent of social status and genotype, during the 7-week period. Plasma ghrelin increases with diet-induced weight loss [66] whereas excess glucocorticoids resulting from Cushing's disease or exogenous prednisone treatment suppresses ghrelin secretion in humans [67]. Although glucocorticoids can increase food intake under certain conditions [68], CRH is anorexic [69, 70] and likely participates in stress-induced weight loss in rats [71]. However, it is unclear whether any of these effects are mediated through ghrelin. The effect of the group formation process on serum insulin followed that of the changes in body weight, as concentrations were significantly lower in subordinate compared with dominant females at week 7, regardless of SERT genotype. However, concentrations fell from baseline in *l/l* females but rose in s-variant females, indicating more studies are needed to determine whether social status—5HTTLPR differences in insulin secretion are due to differences in food intake or glucocorticoid-induced decreases in insulin sensitivity [72].

While the change in body weight during the 7-week assessment was explained by social rank attained and not genotype, body weights at the end of the assessment were significantly higher in dominant *l/l* females compared to all other females. Because these animals started at a higher body weight, this observation is not that compelling. However, measures of SAD, as an index of adiposity, showed a similar rank-dependent pattern, with dominant *l/l* females being least affected by the new group formation and subordinate females, regardless of genotype, experiencing the greatest loss in abdominal fat. While serum leptin was higher at baseline in *l/l* females, the response to the group formation differed by rank attained and genotype with highest levels in dominant *l/l* females lowest in subordinate, *s*-variant females. As found in rodent models of psychosocial stress [31], these differences in serum leptin are likely explained by parallel changes in adipose tissue rather than an acute change in energy balance. While glucocorticoids can redistribute fat to central stores [73], all females in the present study had low measures of abdominal diameters except dominant *l/l* females. Thus, this redistribution may depend on preexisting fat stores and food consumption. Taken together, these data indicate that *l/l* females who become dominant are affected less metabolically than others and the effect of dominance is not the same for s-variant females. Furthermore, the leptin data indicate that s-variant females are more responsive to the adverse effects of group formation than *l/l* females

who become subordinate. Although we saw no genotype differences in glucocorticoid negative feedback, we hypothesize that these effects of SERT genotype are due to differences in reactivity to the social environment [20,74], perhaps the result of a disruption in 5HT regulation of central CRH circuits conferred by the short allele variant.

Assessment of the thyroid hormones also supports a gene by social status interaction. Serum concentrations of T3 but not T4 were significantly higher in dominant *l/l* females compared to all other groups by week 7. Within s-variant females, dominant females had higher levels than subordinate animals. Thyroid hormones are reduced by hypercortisolism associated with chronic disease [75] as well as restraint stress [76] and food restriction [77,78], effects that may be mediated through a reduction in hypothalamic TRH expression. However, other data indicate this decrease induced by food restriction [47] and stress [48] may occur independent of changes in TRH expression. Because we did not observe differences in T4, the decrease in T3 may be due to a disruption in type 2 deiodinase activity (D2). Glucocorticoids inhibit the conversion of T4 to T3 [79] although glucocorticoids can increase D2 activity in other models [80]. Plans are underway to examine specifically how social status affects each component of the HP-thyroid axis and how this is modified by SERT genotype.

A surprising observation resulting from this study was that SERT genotype influenced how ranks were attained. We expected that the order in which a female was introduced to a group would determine her eventual rank, as females introduced earlier in the sequential process would have more opportunity to establish alliances and maintain a higher rank. While this was the case for the four s-variant groups, order of introduction only accounted for a small amount of the variance in ranks attained for the *l/l* females. Indeed, abdominal obesity and T3 concentrations at baseline predicted significantly what ranks the *l/l* females attained. At baseline, estimates of body mass were significantly greater in *l/l* females who eventually became dominant compared to other females. On the other hand, T3 concentrations were higher in *l/l* females who became dominant compared to those who became subordinate. There was more variance in body mass measures and T3 concentrations in *l/l* females compared to s-variant females at baseline when social ranks were similar. Body weight has been shown to be a predictor of eventual dominant status in some reports [81] but not others [Bastian, 2003 No. 1810], suggesting that appreciably larger animals may elicit submissive responsiveness from smaller cage mates. One study [81] also found that greater reactivity to a novel environment predicted subordinate status. The significance of this observation for the present study is data showing thyroid hormones are lower in a number of affective disorders [82–84] and T3 can improve performance in novel, fear-evoking tasks by direct action on the hippocampus [85]. This implies high T3 concentrations, independent of its effects of metabolic rate, may enable individuals to deal more effectively with fear-invoking situations, such as group formations in the present study. An important question is whether rank and genotype-related differences in T3 are maintained as the novelty of the new group formation wanes and whether low T3 concentrations contribute to the behavioral phenotype of subordinate animals.

The present study provides an illustration of how the stress of social reorganization, experienced by all females, intensifies the psychosocial stress of social subordination. The data underscore the impact that social subordination can have on behavior and metabolic regulation. The adverse consequences of social subordination likely depend upon whether the subordinate animals are able to engage in coping responses [61], including social grooming and successfully attenuating aggression directed towards them [86]. However, the present study shows the importance of considering genetic differences that may predispose individuals to react differentially to a psychosocial stressor. While the new group formation was a stressor for all females, significant differences nevertheless emerged, with dominant females with the *l/l* SERT genotype mitigating the consequences of new group formation in those females attaining dominance status and the s-variant genotype exacerbating the consequences of social

subordination. Both leptin and T3 were affected in this fashion, as subordination-induced deficits were exacerbated in s-variant animals. Because 5HT systems in the raphe are targets of leptin [87,88] and recent data indicate that T3 down regulates 5HT autoreceptors, enhancing 5HT neurotransmission [89], these data suggest that 5HT mediated effects of leptin and T3 on behavior or physiology may be compromised in subordinate, s-variant females. Furthermore, as 5HT is a target of estradiol [90], it is possible that differences between *l/l* and *s*-variant females on these and other parameters may have been exacerbated in gonadally-intact females or those receiving estradiol replacement therapy. The importance of estradiol affecting status by SERT genotype interactions awaits empirical examination.

We must emphasize that our sample size is not adequate to determine the genetic contributions to behavior and physiology and it is entirely likely that the phenotypes examined in this study are influenced by many genes [91]. The data from the present study can best serve as the foundation for broader linkage and association analyses [92]. Nevertheless, our population of female rhesus monkeys will continue to be an invaluable resource to address the question of what factors predispose certain women to a higher instance of stress-related health disorders, particularly of the metabolic and reproductive axes.

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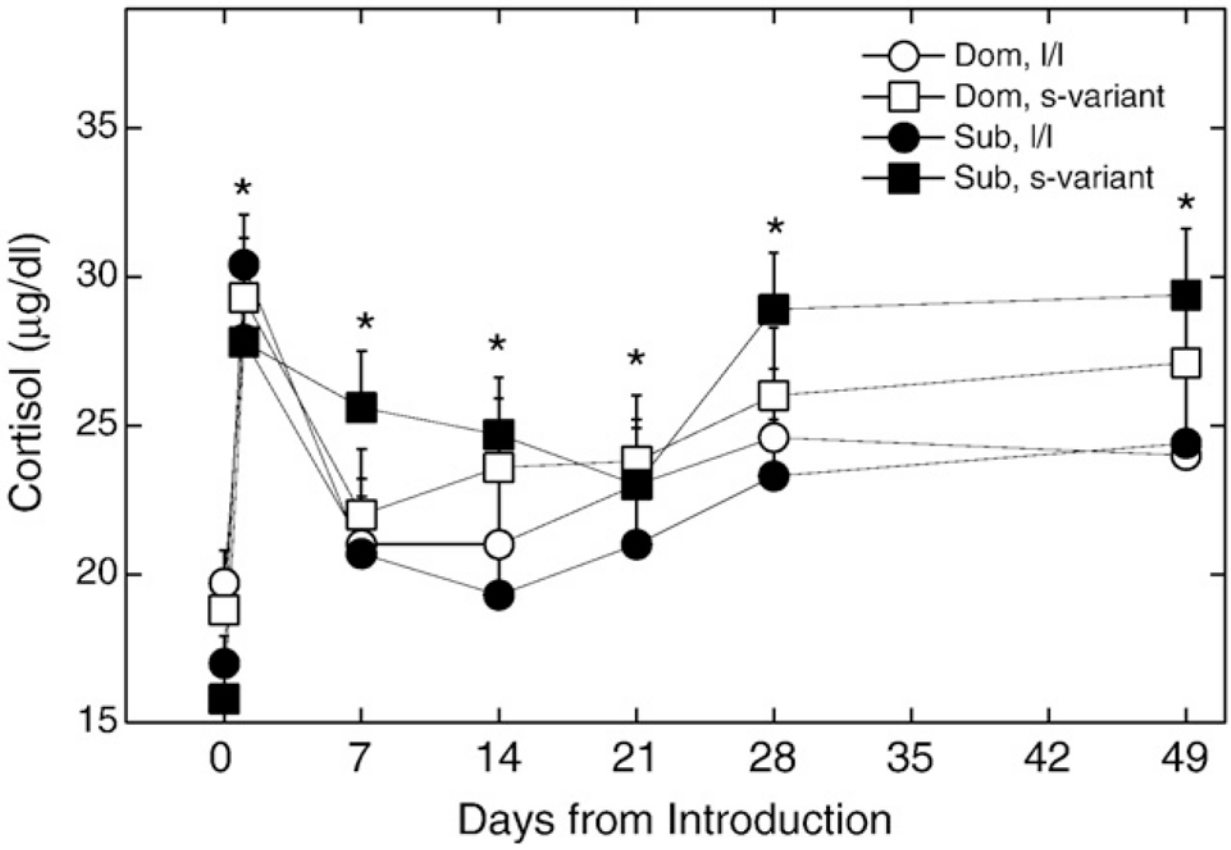

Fig. 1.

Mean±SEM measures of morning serum cortisol at baseline and through the group formation for dominant (Dom) and subordinate (Sub) females at each SERT genotype (l/l and s-variant). Asterisks indicated time points for all groups are significantly different from baseline values.

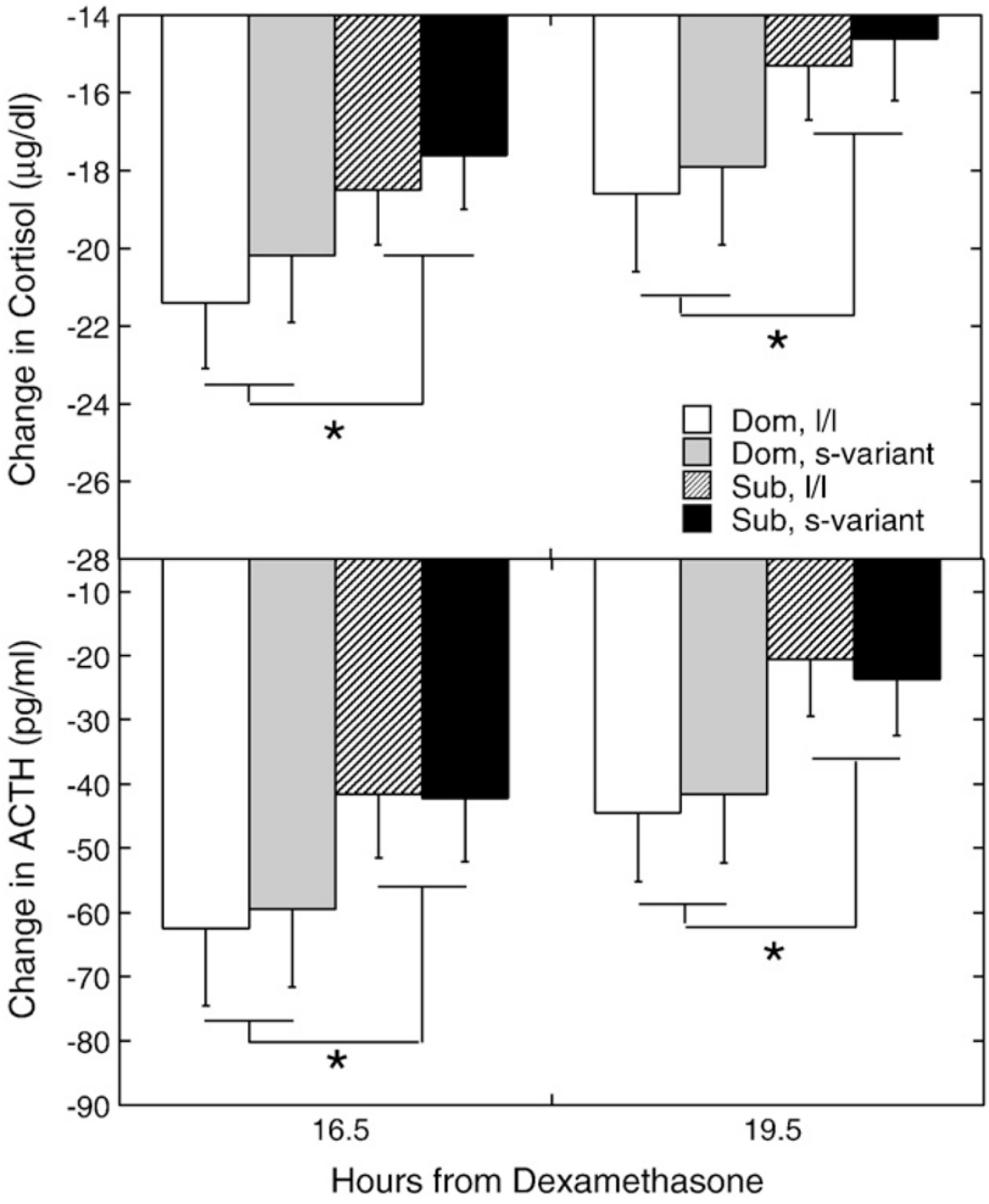

Fig. 2.

Mean±SEM decrease in plasma cortisol (upper panel) and ACTH (lower panel) at two time points from dexamethasone for dominant (Dom) and subordinate (Sub) females at each SERT genotype (l/l and s-variant). Values were calculated as the change in hormone concentrations at 0900 and 1200 h to those observed 24 h earlier prior to dexamethasone. The asterisk indicates hormone values were suppressed significantly more in dominant compared to subordinate animals ($p<0.05$).

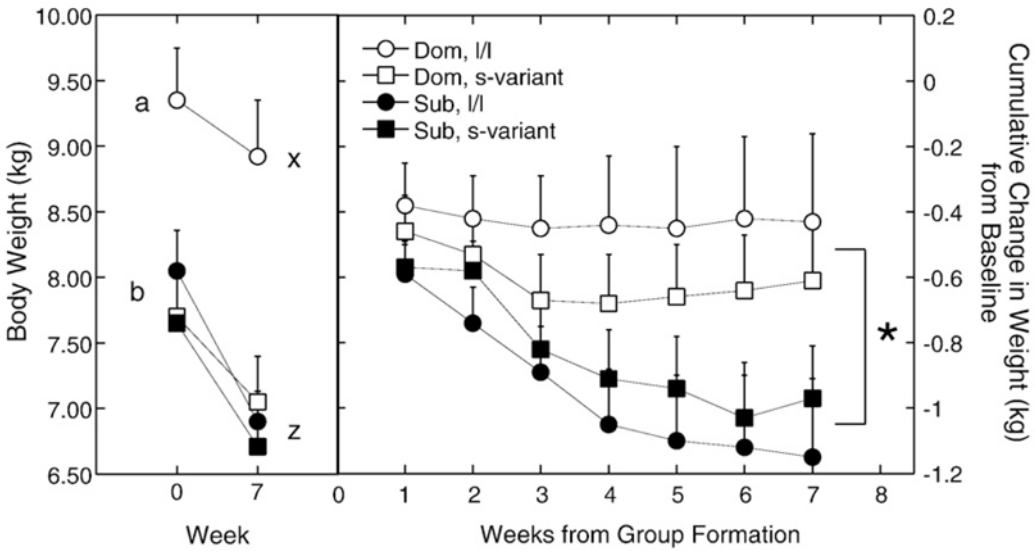

Fig. 3.

Mean±SEM body weights at baseline and week 7 (left panel) and cumulative weight changes from 1 through week 7 of the group formation (right panel) for dominant (Dom) and subordinate (Sub) females at each SERT genotype (l/l and s-variant). Different letters for initial and week 7 weights indicate groups differed significantly ($p<0.05$).

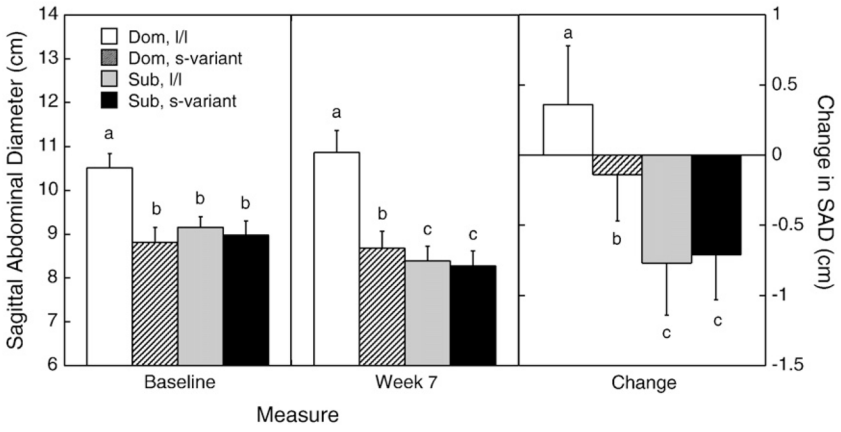

Fig. 4.

Mean±SEM measures of sagittal abdominal diameter (SAD) at baseline (left panel), week 7 (center panel), and the change from baseline to week 7 (right panel) of the group formation for dominant (Dom) and subordinate (Sub) females of each SERT genotype (l/l and s-variant). Different letters for each measure indicate groups are significantly different ($p<0.05$).

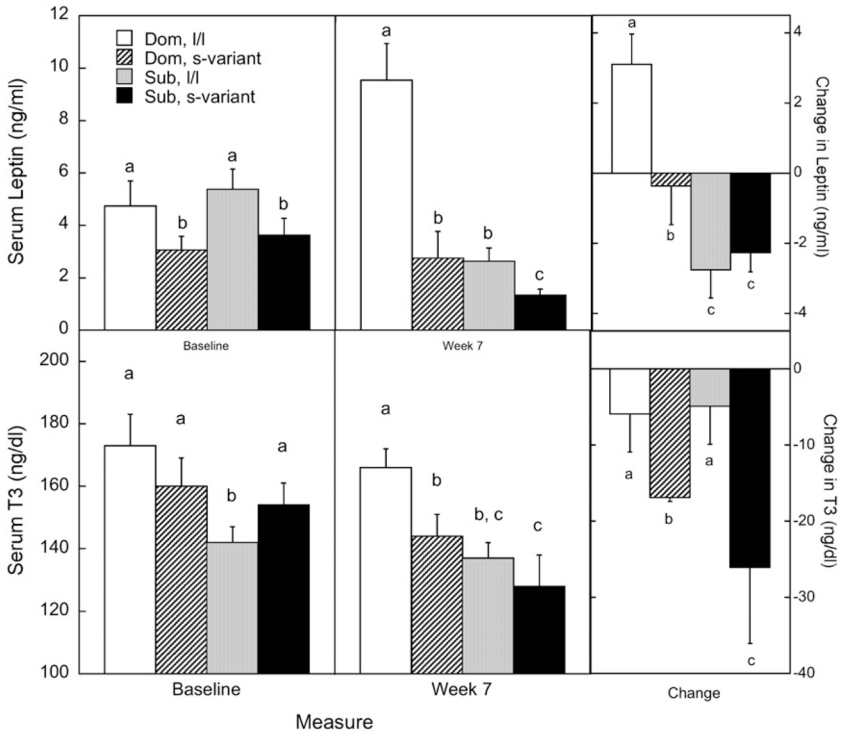

Fig. 5.

Mean±SEM measures of serum leptin (upper panels) and T3 (lower panels) at baseline (left panel), week 7 (center panel), and the change from baseline to week 7 (right panel) of the group formation for dominant (Dom) and subordinate (Sub) females at each SERT genotype (l/l and s-variant). Different letters for each measure indicate groups are significantly different ($p<0.05$).

Table 1

Schematic representation of the formation of eight-5 member groups

Natal groups all middle ranking females					Formation of new groups	New groups					
Gp1	**Gp2**	**Gp3**	**Gp4**	**Gp5**		**Gp**	**New ranks**				
M-*l*	M-l	M-l	M-l	M-l	»»	#1	1-l	2-l	3-l	4-l	5-l
M-*l*	M-l	M-l	M-l	M-l	»»	#2	1-l	2-l	3-l	4-l	5-l
M-*l*	M-l	M-l	M-l	M-l	»»	#3	1-l	2-l	3-l	4-l	5-l
M-*l*	M-l	M-l	M-l	M-l	»»	#4	1-l	2-l	3-l	4-l	5-l
M-*s*	M-s	M-s	M-s	M-s	»»	#5	1-s	2-s	3-s	4-s	5-s
M-*s*	M-s	M-s	M-s	M-s	»»	#6	1-s	2-s	3-s	4-s	5-s
M-*s*	M-s	M-s	M-s	M-s	»»	#7	1-s	2-s	3-s	4-s	5-s
M-*s*	M-s	M-s	M-s	M-s	»»	#8	1-s	2-s	3-s	4-s	5-s

l=homozygous for long SERT allele; *s*=heterozygous or homozygous for short SERT allele.

New groups were formed so each was homogenous to either the *l/l ("l") or l/s-s/s ("s")* SERT polymorphism. Furthermore, none of the females in the new group came from the same original group. Each female was considered middle ranking ("M") in terms of her dominance status within her original group. As new groups formed, females attained specific dominance positions from 1 through 5. For analysis, females ranked 1 and 2 were considered dominant and females ranked 3–5 were considered subordinate [50].

Table 2

Mean±SEM for rates (number per hour) for the initiation of affiliative, aggressive, submissive, or anxiety-like behavior and for the duration of affiliative behavior during each week following the formation of the new groups for dominant l/l; dominant s-variant; subordinate l/l; and subordinate s-variant females

Group	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Affiliation (number per h) [4, 7]						
Dom, l/l	8.6±1.4	6.7±1.9[b]	4.9±1.2[b]	5.5±1.2	5.1±1.3[b]	4.3±1.1
Dom, s-variant	7.5±0.8	11.3±2.5[a]	8.3±1.7[a]	7.8±1.1	5.9±0.7[b]	5.7±1.1
Sub, l/l	6.6±1.3	5.9±0.9[b]	6.5±0.9[a,b]	6.7±1.4	8.1±1.2[a]	5.6±1.2
Sub, s-variant	9.3±1.3	5.8 0.7[b]	5.4±0.7[b]	6.7±1.5	5.5±0.8[b]	4.1±0.6
Affiliation duration (min/h) [4]						
Dom, l/l	20.8±5.9	19.6±6.0	15.9±2.9	15.7±3.3	19.8±2.6	14.1±1.9
Dom, s-variant	28.1±8.5	35.2±11.1	21.3±4.8	25.6±8.1	17.6±3.2	12.9±2.6
Sub, l/l	15.2±4.6	19.4±5.3	15.6±4.6	20.9±5.3	19.6±2.4	15.5±2.9
Sub, s-variant	25.8±5.4	22.1±3.9	17.7±5.9	19.8±5.2	22.1±2.6	18.9±2.9
Aggression (number per h) [1, 2, 6]						
Dom, l/l	0.2±0.1[a]	0.9±0.7	0.3±0.2[a]	0.5±0.2	0.5±0.3[a]	0.6±0.1
Dom, s-variant	1.2±1.19[b]	0.3±0.1	0.9±0.3[b]	1.0±0.2	1.4±0.3[b]	0.8±0.2
Sub, l/l	0.2±0.1[a]	0.4±0.1	0[a]	0.3±0.2	0.3±0.2[a]	0.1±0.1
Sub, s-variant	1.4±1.1[b]	0.1±0.1	0.5±0.2[b]	0.3±0.2	0.8±0.5[b]	0.1±0.1
Submission (number per h) [1, 4, 6]						
Dom, l/l	0.1±0.1[a]	0.2±0.2	1.0±0.1[a]	0.2±0.1	1.4±0.1[a]	0
Dom, s-variant	0.6±0.3[b]	0.1±0.1	1.5±0.1[b]	0.2±0.1	1.9±0.1[b]	0.2±0.1
Sub, l/l	1.7±0.5[a]	2.3±0.8	1.25±0.1[a]	2.7±0.9	1.5±0.1[a]	1.8±0.6
Sub, s-variant	3.5±1.3[b]	1.1±0.3	1.41±0.1[b]	2.2±0.9	3.1±0.3[b]	1.7±0.4
Anxiety behavior (number per h) [4]						
Dom, l/l	4.9±1.1	5.8±1.3	6.8±1.6	4.1±1.4	6.3±1.2	5.9±1.1
Dom, s-variant	4.5±0.9	5.1±1.0	4.6±0.9	2.5±0.7	3.7±1.0	4.8±0.8
Sub, l/l	4.3±0.9	5.5±1.1	5.3±1.1	6±1.0	3.9±0.7	6.0±1.4
Sub, s-variant	4.6±1.1	6.8±1.4	6.5±1.9	3.4±0.8	4.0±0.7	7.4±1.3

Numbered superscripts for each behavioral category indicate the following main and interaction effects were significant ($p<0.05$): Status=1, SERT=2, Status by SERT=3, Weeks=4, Weeks by Status=5, Weeks by SERT=6, and Weeks by Status by SERT=7. Lettered superscripts within a behavioral category represent significant pair-wise comparisons if the interaction of status and/or genotype with weeks was significant. See text for further explanation of post hoc analyses.

Table 3

Metabolic hormone concentrations (mean±sem) obtained at baseline and week 7 after the formation of the new groups

Parameter	Time	Dominant, l/l	Dominant, s-variant	Subordinate, l/l	Subordinate, s-variant
Insulin (μU/ml)	Baseline	76.7±19.4	45.7±11.2	44.5±6.8	39.7±8.0
	Week 7	62.7±10.5[a]	58.4±13.7[a]	37.2±4.6[b]	44.2[b]±11.3
Glucose (mg/dl)	Baseline[a]	93.5±7.4	83.2±4.2	84.2±2.9	79.1±3.1
	Week 7[b]	73.6±3.5	79.1±4.4	73.3±7.0	70.6±2.9
Ghrelin (pg/ml)	Baseline[a]	14.9±4.4	21.3±6.3	19.0±5.4	22.1±3.1
	Week 7[b]	10.5±3.3	21.2±6.9	17.9±4.6	14.4±2.0
T4 (ng/ml)	Baseline	5.72±0.58	4.87±0.33	5.27±0.26	4.99±0.35
	Week 7	5.42±0.38	5.37±0.50	5.53±0.37	6.63±1.00
T3:T4	Baseline[a]	0.31±0.01	0.34±0.02	0.27±0.01	0.32±0.02
	Week 7[b]	0.32±0.02[a]	0.28±0.03[a]	0.25±0.02[b]	0.22±0.02[b]

Different superscripts indicate groups were significantly different.