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Journal Title: Hormones and Behavior
Volume: Volume 62, Number 5
Publisher: Elsevier: 12 months | 2012-11, Pages 612-620
Type of Work: Article | Post-print: After Peer Review
Publisher DOI: 10.1016/j.yhbeh.2012.09.010
Permanent URL: http://pid.emory.edu/ark:/25593/g27p3

Final published version: http://dx.doi.org/10.1016/j.yhbeh.2012.09.010

Copyright information:

Published in final edited form as:
Horm Behav. 2012 November ; 62(5): 612–620. doi:10.1016/j.yhbeh.2012.09.010.

Social status modifies estradiol activation of sociosexual behavior in female rhesus monkeys

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Abstract

Estrogen (E2) has activational effects on sexual motivation and mitigating effects on anxiety-like behaviors that can be attenuated with chronic exposure to psychosocial stress. Some studies suggest that this attenuation can be overcome by higher doses of E2, while others show that chronic psychosocial stress may alter the mechanisms of E2 function, thus reducing any positive benefit from higher doses of E2. To determine the interaction between psychosocial stress and E2 dose on behavior, we examined the scope of attenuation across a suite of socioemotional behaviors, including reproduction, affiliation, aggression, submission, and anxiety-like behaviors on 36 ovariectomized female rhesus monkeys. Females were exposed to graded psychosocial stress, established by an intrinsic female dominance hierarchy, where subordinate animals receive high amounts of harassment. Our data show that E2 dose-dependently increased sexual motivation and male-affiliation in dominant (e.g. low-stress) females, while subordinate females showed no positive effects of E2, even at higher doses. In addition, contact aggression was attenuated in dominant females, while non-contact aggression was attenuated in both dominant and middle-ranking females. These results suggest that the stress-induced attenuation of E2's activational effects on sexual behavior and affiliation with males may not be overcome with higher doses of E2. Furthermore, the observed behavioral consequences of psychosocial stress and E2 dose may be dependent on the behaviors of all the females in the social-group, and better resolution on these effects depends on isolating treatment to individuals within the group to minimize alterations in social-group interactions.

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Keywords

estradiol; sexual behavior; affiliation; social subordination; rhesus monkeys

Introduction

While the activational effects of estradiol (E2) on the expression of sociosexual behavior are well established (Beach, 1976; Wallen, 1990; Wallen and Goy, 1977; Zehr et al., 1998) and the observed magnitude of these behaviors is disrupted by exposure to chronic and acute stress (Pierce et al., 2008; White and Uphouse, 2004), the mechanisms underlying the synergistic effects of the limbic-hypothalamic-pituitary-adrenal (LHPA) axis and the limbic-hypothalamic-pituitary-gonadal (LHPG) axis on behavior are unknown. In response to a stressor, the LHPA axis mounts a systemic response to increase adrenal glucocorticoid secretion, and under conditions of chronic stress, a sustained LHPA axis response can lead to subsequent hypo- or hypercortisolemia and a dysregulation of glucocorticoid negative feedback (Chrousos, 2009; Chrousos and Gold, 1992). Consequently, chronic exposure to stress or stress hormones can potentiate E2 negative-feedback inhibition of LHPG axis function (Ronnekleiv et al., 2010) and result in reduced hypothalamic release of gonadotropin releasing hormone (GnRH) (Oakley et al., 2009), pituitary release of luteinizing hormone (LH) (Berga and Loucks, 2007; Chrousos et al., 1998; Michopoulos et al., 2009), or impair normal ovarian function and subsequent ovulation (Adams et al., 1985). The mechanism for this inhibition, however, is unclear, as administration of exogenous glucocorticoids do not consistently interfere with reproductive physiology in women (Saketos et al., 1993; Samuels et al., 1994) or monkeys (Lovejoy and Wallen, 1990).

In addition to the potentiation of E2 negative-feedback of the LHPG axis, stress can concomitantly attenuate the expression of E2's activational effects on female sexually motivated behavior in ovariectomized rats (White and Uphouse, 2004), ewes (Pierce et al., 2008), and intact rhesus monkeys (Wallen, 1990). In ovariectomized females rats receiving E2 and progesterone (P4) replacement, over-expression of corticotropin-releasing factor (CRF) in the central nucleus of the amygdala inhibits expression of sexual behaviors (Keen-Rhinehart et al., 2009). However, supraphysiological concentrations of E2 can rescue lordosis behavior in rodents following acute exposure to restraint stress (Uphouse et al., 2005; White and Uphouse, 2004), suggesting a balance between the LHPA and LHPG axes. Nevertheless, E2 reinstatement of sexual behavior following stress has only been shown following acute physical restraint, and may not generalize to sexual behavior following chronic exposure to stress. Support for the latter comes from behavioral observations of intact rhesus monkeys exposed to increasing amounts of chronic stress due to social subordination (Wallen, 1990). Subordinate females expressed sexual behavior mainly during peak periovulatory E2 concentrations, whereas more dominant females expressed both proceptive and receptive behaviors significantly earlier in the cycle when E2 levels were lower, suggesting higher sensitivity to E2's activational effects. Despite the implications of these data, little is know about the interaction between graded exposure to chronic social subordination stress and increasing doses of E2 on the expression of female sociosexual behavior.

In conjunction with sexual motivation, data from human clinical research and animal models demonstrate a role for E2 in altering anxious, affiliative, and aggressive behavior in females, although the directionality of the effect may be context dependent. In human clinical studies, major hormonal shifts in LHPG activity precipitate onset of mood and anxiety disorders (Halbreich and Kahn, 2001), and E2 replacement therapy (ERT) is anxiolytic and anti-depressive during peri-menopause (Cagnacci et al., 1997; Schmidt et al., 2000) and postpartum (Moses-Kolko et al., 2009). Rodent studies, however, suggest that the effects of E2 are often inconsistent, as E2 can act to increase or decrease anxiety depending on its interaction with E2 receptors (ERs) (Lund et al., 2005; Walf and Frye, 2005), and it can be anxiolytic in familiar environments such as a female's home cage while anxiogenic in novel environments (Morgan and Pfaff, 2001). Data on the effects of E2 on either affiliation or aggression are more limited. The majority of rodent research focuses on E2's role in augmenting maternal bonding and increasing post-partum maternal aggression, and not on it's influence on prosocial or agonistic behavior (Bos et al., In Press). Studies observing gonadally intact rhesus monkeys suggest that affiliation toward males and aggression toward females increases with peak estrus behavior (Carpenter, 1942) and in conjunction with peak ovarian E2 (Walker et al., 1983; Wallen et al., 1984). Ovariectomized rhesus monkeys given E2 replacement, without access to male partners, also show an amplification of aggression toward females along with an attenuation of anxiety-like behaviors (Michopoulos et al., 2011; Pope et al., 1987). Increased female-female aggression, however, was seen in conjunction with increased affiliative behavior between females, suggesting that higher rates of proximity and interaction may be linked to higher rates of aggression or vice versa. Taken together, these data suggest that chronic stress can moderate E2's effects on anxious, aggressive, and affiliative behavior, similar to that seen in sexual behavior.

The present study tested the hypothesis that stress from chronic subordination diminishes the behavioral efficacy of E2 on a range of socio-emotional behaviors in female rhesus monkeys. We hypothesize that females exposed to chronic stress develop a physiological state that blunts E2's dose dependant beneficial effect on attenuating anxiety and increasing motivation to engage in sexual behavior, prosocial male-directed behaviors, and aggressive female-directed behaviors. Furthermore, we hypothesize that socially subordinate females will require higher levels of E2 in order to stimulate these behaviors.

Material and Methods

Subjects

Subjects were 50 ovariectomized adult female rhesus monkeys (*Macaca mulatta*) socially housed and maintained at the Yerkes National Primate Research Center (YNPRC). Ten groups of five females, each with one resident adult male, were housed in run-type enclosures that measured 20 × 15 × 8 feet each and included both indoor and outdoor areas. Animals were between the ages of 11-14, and were fed a standard commercial low-fat high-fiber diet (Ralston Purina Company, St. Louis MO) *ad libitum* supplemented daily with seasonal fruits and vegetables. All procedures were approved by the Emory University Institutional Animal Care and Use Committee (IACUC) 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."

Social groups were comprised of previously multiparous adult females between 7 and 10 years of age, selected from larger breeding group as described previously (Jarrell et al., 2008). Females were ovariectomized six months prior to small group formation and then placed in one of ten groups of five unfamiliar female conspecifics, and remained in these groups for at least four years prior to the onset of the present study. An individual resident male was introduced into each group approximately three years following group formation. Since social group formation, females participated in several protocols in which they received replacement therapy with physiological concentrations of gonadal steroids (Collura et al., 2009; Michopoulos et al., 2009; Michopoulos et al., 2011; Michopoulos and Wilson, 2011). All females had at least three months of E2-washout between the completion of any previous study and the beginning of the current study. During the course of data collection, ten females were unable to complete all treatment conditions due to intermittent health concerns, and were therefore excluded from the analysis, reducing the sample size to 40 rhesus monkey females. Four additional females were excluded based on changes in social rank bringing the final sample size to 36.

Social Subordination Stress

The use of dominance social hierarchy to model adverse health outcomes associated with chronic psychosocial stress is well-established in the literature (Adams et al., 1985; Kaplan et al., 1996; Michopoulos et al., 2011; Michopoulos et al., In Press; Paiardini et al., 2009). Rhesus monkey social groups are organized by a linear dominance hierarchy that maintains group stability (Bernstein, 1970) at the expense of lower-ranking females, which receive disproportionally greater amounts of aggression from higher-ranking females and engage in higher levels of submissive behavior (Bernstein and Gordon, 1974; Bernstein et al., 1974; Shively and Kaplan, 1984). As a consequence, subordinate females are exposed to unstable and stressful environments, which can result in LHPA-axis dysregulation (Michopoulos et al., 2012) evinced through increased adrenal gland size (Kaplan et al., 1984) and hypercortisolemia operationally defined as elevated cortisol response to social challenge (Cohen et al., 1997) and impaired physiological responses to dexamethasone suppression (Jarrell et al., 2008; Kaplan et al., 2010; Shively et al., 1997; Wilson et al., 2008) or ACTH challenge (Shively, 1998).

In the wild, social groups have a matrilineal structure in which social rank is determined by birth (de Waal and Luttrell, 1985), and familial, or kin support acts as a natural buffer to rank-related stress (Kikusui et al., 2006). In the present study, all females were taken from middle ranking social groups within larger compounds, and placed in small non-kin groups to maximize rank-related social stress (Jarrell et al., 2008). New social status rankings were determined based on observations of dyadic agonistic interactions (e.g. submissive behaviors) between females within a group. Females were then classified into one of three rank categories; Alpha – the highest ranking female (N=8), Middle – the second and third ranking female (N=16), and Subordinate – the fourth and the fifth ranking female (N=12). This division reflects the uniqueness of the alpha female, who receives little to no aggression

and does not express submissive behaviors during dyadic interaction (Table 2), and therefore is not exposed to the chronic stress of social subordination (Michopoulos et al., 2011). A total of 4 females changed ranks midway through the 12 month study, such that they could no longer be classified as one of the three ranking categories, and were therefore excluded from the current analysis (N=36, Supplemental Table S1).

Experimental Design

Rhesus monkeys exhibit a seasonal breeding cycle with ovulatory cycles and fertility occurring from August to April followed by anovulation, secondary to reduced gonadotropin secretion during the May to July nonbreeding season (Walker et al., 1984). Therefore, all animals were studied during months defined as the breeding season for gonadally intact females, notably from January to April, 2010 followed by August to December, 2010. As data collection was spread across two consecutive breeding seasons, all doses were counterbalanced across time. The conditions consisted of one placebo treatment (0μg/kg/day) and three E2 replacement treatments at low (2μg/kg/day), medium (4μg/kg/day), and high (8μg/kg/day) doses delivered using 21-day sustained release pellets (Innovative Research of America) containing either 17β-estradiol or cholesterol (placebo). Pellets were implanted subcutaneously between the scapula following anesthesia with ketamine (10mg/kg intramuscular injection), antiseptic preparation of the site, and sterile procedures as described previously (Mook et al., 2005). Each pellet was designed to continuously release E2 at one of the four treatment doses over the course of three weeks, and was followed by a minimum of a three-week washout period of no treatment. Members of each small group received identical treatments, and order of treatment conditions was counterbalanced across groups. Pellets were implanted three days prior to the initiation of behavioral observations.

Behavioral Outcome Measures

Behavioral group observations, consisting of simultaneous focal group observations on the members of each small group, were conducted live for 30 min, at 14:00 hr on three separate days during both week one and week three of each treatment. Thus, a total of six observations were obtained ranging from day 3 to day 20 of each treatment. Data were recorded in an actor – behavior – recipient format using the “HandObs” program developed by the Center for Behavioral Neuroscience (Graves and Wallen, 2006). Inter-observer reliability exceeded 90% in each of three live reliability trials. Behavior was coded using a well-established ethogram (Jarrell et al., 2008; Pope et al., 1987) and was analyzed both individually and in the behavioral categories of (1) proceptive sexual behavior directed toward males (e.g. female hindquarter presentation, handslap, standup, threataway, or crouch behavior), (2) receptive sexual behavior toward males (e.g. received hiptouch or mount from male), (3) affiliative behavior toward males (initiates proximity or grooming), (4) affiliative behavior toward females (initiates proximity or grooming), (5) anxiety-like behavior (yawns, body-shakes, pacing, or self-directed scratching and bodily exploration), (6) agonistic behavior toward females (attacks, chases, or threats with and without contact), and (7) submissive behavior toward females (withdraws or grimaces). Additionally, duration (seconds) of affiliative behavior directed and received from both males and females was calculated.

Hormonal Assay

To confirm E2 concentrations, 3mL of serum were collected from the saphenous vein located on the back of the leg directly following observations. Animals were trained to present their hind legs for conscious venipuncture, which allows for the collection of blood in awake unanesthetized monkeys. Serum was collected at five time points, two during week one, one during week two (no behavioral observations), and two during week three. All assays were done in the Biomarkers Core Laboratory at the YNPRC using a modification of a previously validated commercial assay (Siemens/Diagnostic Products Corporation, Los Angeles, CA (Pazol et al., 2004). The assay had a sensitivity of 5 pg/mL and intraassay and interassay coefficients of variation (CVs) of 7.95% and 11.3% respectively.

Statistical Assessment

Data were analyzed using repeated measures analysis of variance (rmANOVA) with rank categorization (Alpha, Middle, Subordinate) and genotype (serotonin transporter, long and short variant: see Jarrell et al., 2008) as between subject factors, and dose (0, 2, 4, 8 μg/kg/day), week (1^{st}, 3^{rd}), and observation (1, 2, 3) as with-in subjects factors. Main and interaction effects of genotype and observation are not reported. All analyses were conducted using IBM SPSS 19, a statistical software package. Variance was not normally distributed across all variables, and data were log transformed using the formula $Log_{10}(X+1)$ to account for values of zero. Variance and sphericity were reduced with this transformation, but not all values were normally distributed following the transformation. These data are reported with a Greenhouse-Geisser correction. All results where $p \leq .05$ were considered significant, and post-hoc tests were conducted, if necessary, using a Bonferroni correction for multiple comparisons. All non-significant main and interaction effects with a $p<.10$ were reported with an effect size (Cohen's d), and a $d>.50$ was considered a non-significant trend. Results are summarized as mean ± standard error of the mean (SEM) of untransformed data.

Results

Hormonal Assays

Serum samples collected during E2 treatment (Figure 1) show a significant main effect of dose ($F_{2,50}$ =35.67, $p<0.001$; low dose: 36.8±4.8pg/mL, medium dose: 80.3±7.4pg/mL, high dose: 128.2±11.5 pg/mL) and sample time point ($F_{2,50}$ =41.60, $p<0.001$) on serum E2, but no other main effects or interaction effects were significant. Serum samples collected during placebo treatment were not assayed for E2, because serum concentrations in ovariectomized females are below assay detectability (Zehr et al., 1998). As serum levels of E2 were significantly different across time, we further investigated the data to assess the differences in serum E2 between observational weeks 1 and 3 in a second rmANOVA model using rank as a between subject variable, and dose (2,4,8 μg/kg/day), week (1,3), and sample number (1,2) as with-in subject variables. Results showed a significant main effect of dose ($F_{2,54}$ =39.75, $p<0.001$;) and week ($F_{2,54}$ =93.25, $p<0.001$) on E2, but no effect of rank ($F_{2,27}$ =0.54, p=0.588). Additionally, there was a non-significant trend for a dose by week interaction ($F_{2,54}$ =3.36, p=0.060, d=0.51). Post hoc tests were significant for all dose x week interactions, such that the 8μg/kg/day dose resulted in a higher serum E2 concentration

than the 4ug/kg/day dose which was greater than the 2μg/kg/day dose and week one was greater than week three (Table 1).

Sexual and Social Behavior

Rank had a significant effect on proceptive ($F_{2,30}$ =4.11, p=0.026) and receptive sexual behaviors ($F_{2,30}$ =12.57, p<0.001), as well as on female initiation of affiliation towards males ($F_{2,30}$ =5.47, p=0.009) and affiliation toward females ($F_{2,30}$ =4.41, p=0.021). Post hoc analysis determined that alpha females had a higher frequency (per 30 minutes) of proceptive sexual behavior (1.03 ± .19) than subordinate females (.21 ± .15, p=0.024) and a higher frequency of receptive sexual behavior (1.89 ± .30) compared to both middle-ranking (.28 ± .21, p<0.001) and subordinate females (.30 ± .24, p<0.001). Alpha females also showed a higher frequency of initiating male-directed affiliation (1.78 ± .43) compared to subordinate females (.40 ± .34, p=0.007), while female-directed affiliation was highest in middle-ranking females (3.0 ± .44) and greater than subordinate females (1.34 ± .50, p=0.024).

There was a main effect of E2 dose and an interaction between dose and rank for both proceptive sexual behavior (dose: $F_{3,90}$=3.41, p=0.021; interaction: $F_{6,90}$=2.30, p=0.041; Figure 2a) and receptive sexual behavior (dose: $F_{3,90}$=4.84, p=0.008; interaction; $F_{6,90}$=2.45, p=0.031; Figure 2b), but not male-directed affiliation (dose: $F_{3,90}$=1.74, p=0.182; interaction: $F_{6,90}$=.77, p=0.592; Figure 3) or female-directed affiliation (dose: $F_{3,90}$ =0.17, p=0.914; interaction: $F_{6,90}$=0.28, p=0.945, Table 2). In comparison to the lowest dose, proceptive sexual behavior was maximally enhanced at a medium dose of 4μg/kg/day (p=0.046), while receptive sexual behavior was enhanced at both a medium dose (4μg/kg/day, p=0.026) and a high dose (8μg/kg/day, p=0.011) of E2. Post hoc analysis of the interaction between dose and rank revealed that at the medium dose, alpha females showed significantly more proceptive sexual behavior than middle-ranking females (p=0.008) and subordinate females (p=0.001) and significantly more receptive behavior than middle-ranking females (p=0.001) and subordinate females (p=0.001). There were no significant differences between middle-ranking and subordinate females for either proceptive (p=0.612) or receptive (p>0.999) behavior. At the highest dose, alpha females showed greater frequency of receptive behavior only when compared to subordinate females (p=0.048). Within ranks, alpha females showed a higher frequency of proceptive behavior following the medium dose compared to both the placebo (p=0.019) and low dose (p=0.024), as well as a higher frequency of receptive sexual behavior following both the medium (p=0.002) and the high (p=0.034) dose as compared to the low dose. There was no significant difference between the medium and high dose in proceptive sexual behavior (p=0.058) or receptive sexual behavior (p=0.585). Middle ranking and subordinate females did not show any significant alterations in proceptive or receptive sexual behavior as a result of E2 dose.

There was no main effect of observation week when looking at proceptive ($F_{1,30}$=1.06, p=0.313) and receptive ($F_{1,30}$=3.01, p=0.093, d=39) sexual behavior. However, proceptive behavior did show a dose by week interaction effect ($F_{3,90}$=2.96, p=0.036) while receptive behavior did not ($F_{3,90}$=1.53, p=0.222; Table 1). Post hoc analysis showed that the only significant difference in behavior between week one and week three of treatment was seen

in the placebo dose. There was no change in behavior as a result of treatment week for the 2, 4, or 8 μg/kg/day doses. The behavioral data shows that the difference in the placebo dose was due to reduced sexual activity during week three as compared to week one (p=0.012).

Since dose did not have an effect on either male or female affiliation, data were collapsed across all nonplacebo doses. Results showed that there was a main effect of E2 treatment on male-directed affiliation ($F_{1,30}$ =9.45, p=0.004, Figure 3), but not female-directed affiliation ($F_{1,30}$ =0.85, p=0.363). Affiliation directed toward males and females both showed a main effect of Rank (male: $F_{2,?0}$ =5.50, p=0.009; female: $F_{2,30}$ =4.09, p=0.027) but no interaction effect of Treatment and Rank (male: $F_{1,30}$ =0.04, p=0.840; female: $F_{1,30}$ =0.01, p=0.915). Post Hoc analysis showed that following E2, females initiated significantly more affiliative behaviors toward males (Placebo: 0.75±0.17, E2: 1.15±0.24, p=0.031), and alpha females increased male-directed affiliation (Placebo: 1.25±0.34, E2: 2.77±0.63, p=0.020), while middle-ranking (Placebo: 0.77±0.24, E2: 1.04±0.34, p=0.075) and subordinate females (Placebo: 0.24±0.27, E2: 0.45±0.39, p=0.365) did not.

Further analysis was done to identify E2's effects on time spent in affiliation, as opposed to frequency of initiating affiliation. Total affiliation time was analyzed using rmANOVA with sex of partner as a with-in subject measure. Sex of partner significantly affected duration of affiliation ($F_{1,30}$=10.80, p=0.003), being longer with female (4.06±0.42s) than male partners (2.11±.61s), but durations were unaffected by E2 dose ($F_{3,90}$=1.17, p=0.325) or rank ($F_{2,30}$=1.54, p=0.231). An interaction with sex-of-partner and rank ($F_{2,30}$=7.19, p=0.003) emerged, such that middle-ranking females (female: 5.40±0.54s; male: 2.96±0.78s, p<0.001) and subordinate females (female: 4.0±0.56s; male: 0.81±0.82s, p=0.001) were found to spend more time with females compared to males, while alpha females distributed affiliation time equally between males and females (female: 3.0±0.79s; male: 3.04±1.15s, p=0.234).

Agonistic Behavior

There was a main effect of rank on aggression directed toward other females ($F_{2,30}$=5.02, p=0.013), aggression received from other female ($F_{2,30}$=15.35, p<0.001), and submission toward females ($F_{2,30}$=18.50, p<0.001; Table 2). However, there was no main effect of dose, or an interaction between dose and rank on directed aggression (dose: $F_{3,30}$=0.97, p=0.399; interaction: $F_{6,90}$=0.77, p=0.596) or aggression received (dose: $F_{3,30}$=1.51, p=0.225; interaction: $F_{6,90}$=0.92, p=0.484). There was a non-significant trend for a main effect of dose on submission ($F_{3,30}$=2.42, p=0.071, d=.59) but not a dose by rank interaction ($F_{6,90}$=1.23, p=0.299). Additionally, there were no significant main effect of observation week on the frequency (per 30 minutes) of aggression directed toward other females (week one: 2.24±0.56; week three: 2.56±0.59, $F_{1,30}$=3.21, p=0.084, d=.41), aggression received from other female (week one: 1.77±0.51, week three: 2.18±0.55, $F_{1,30}$=2.72, p=0.110), and submission toward females (week one: 4.17±0.74, week three: 5.21±1.01, $F_{1,30}$=18.50, p=.056, d=.49).

As the aggression category included both contact and non-contact aggressive behaviors that may reflect different levels of motivation, each was analyzed independently using the same rmANOVA model. There was a significant main effect of dose and a main effect of rank, but no interaction effect between dose and rank for both contact aggression (dose,

$F_{3,90}$=3.80, p=0.022; rank, $F_{2,3}$0=11.89, p<0.001; interaction, $F_{6,90}$=1.48, p=0.212) and non-contact aggression (dose, $F_{3,90}$=7.40, p=0.001; rank, $F_{2,3}$0=4.57, p=0.019; interaction, $F_{6,90}$=1.46, p=0.215). To compare the frequencies of these two aggression types, another rmANOVA model was run with type of aggression (contact versus non-contact) as a within subjects factor. There was a main effect of aggression type ($F_{1,30}$ =32.38, p<0.001), rank ($F_{2,30}$=6.36, p=0.005), and dose ($F_{3,90}$=7.72, p<0.001), and an interaction between dose and aggression type ($F_{3,90}$=5.92, p=0.001, Figure 4). Post hoc analysis illustrated that frequency of non-contact threat (2.73±0.67) during 30 minute observations was significantly greater than that of contact threat (0.16±0.03, p<0.001), and that E2 uniquely attenuated the expression of contact and non-contact aggression. Contact aggression was significantly attenuated in alpha females with a medium dose of 4μg/kg/day, when compared with either the placebo (0μg/kg/day, p=0.039) or the high dose (8μg/kg/day, p=0.007). However, E2 attenuated non-contact aggression in both alpha and middle-ranking females. Alpha females showed reduced non-contact aggression with either a dose of 2μg/kg/day (p=0.017) and 4μg/kg/day (p=0.039), while E2 attenuated non-contact aggression in middle-ranking females at all doses (2μg/kg/day, p=0.002; 4μg/kg/day, p=0.050; 8μg/kg/day, p=0.004).

Sociosexual versus Aggressive Behavior

In order to better compare expression of sexual motivation, male-directed affiliation, and female-aggression across all doses and rank categories, we added the frequency of sexual behaviors and male-directed affiliation for each female across all six observations (180 minutes) during each of the four E2 treatment conditions to create a new ‘sociosexual’ behavior variable for each female at each dose. In parallel, we added contact and non-contact aggressive behaviors given across all six observations to create an ‘aggressive’ behavior variable. These two behavioral categories were analyzed using a new rmANOVA, including both rank categorization (Alpha, Middle, Subordinate) and genotype (not reported) as between subject factors, and dose (0, 2, 4, 8 μg/kg/day) and behavior (sociosexual, aggressive) as within subject factors to statistically test if sociosexual and aggressive behavior were similarly influenced by social rank and E2 dose. We found that, as expected, there was a main effect of dose ($F_{3,90}$=3.50, p=0.033), a main effect of rank ($F_{2,30}$=13.59, p<0.001) on both behaviors combined, but no main effect of behavior sub-type ($F_{1,30}$=1.90, p=0.178; Figure 5). Additionally, the data show a significant interaction between dose and behavior type ($F_{3,90}$=4.56, p=0.005) and a non-significant trend for an interaction between dose, behavior, and rank ($F_{6,90}$=1.99, p=0.075, d=0.70), indicating no significant difference in the occurrence of sociosexual and aggressive behavior and that E2 doses do not uniformly alter the occurrence of sociosexual and aggressive behaviors. Post hoc analysis show that at a dose of 4μg/kg/day, total sociosexual behavior observed (22.79±4.95) is greater than aggressive behavior (12.10±3.21; p=0.016) while the occurrence of these behaviors was not significantly different at other doses (p's>0.05). Furthermore, this difference is driven by the alpha female, who alone shows significantly greater sociosexual behavior compared to aggressive behavior following only the medium 4μg/kg/day dose (p=0.002).

Anxiety-Like Behavior

Grouping all anxiety-like behaviors together, there were no rank, dose, or interaction effect on anxiety-like behavior (rank: $F_{2,30}$=2.17, p=0.132; dose: $F_{3,90}$=0.05, p=0.986; interaction: $F_{6,90}$=1.65, p=0.142; Table 1). There was no effect of week on frequency of anxiety behavior ($F_{6,90}$=0.05, p=0.828). Only when looking at individual anxious behaviors collapsed across all E2 doses, did E2 significantly increased self-scratch (Placebo: 2.84±0.49, E2: 2.90±0.31, $F_{1,30}$=5.33, p=0.028) and self-explore behavior (Placebo: 0.71±0.14, E2: 0.80±0.12, $F_{1,30}$=7.40, p=0.011). Similarly, across all doses, an interaction effect emerged between treatment and rank on yawning behaviors ($F_{2,30}$=3.77, p=0.035). Post hoc analysis showed that E2 increased yawning in middle-ranking (Placebo: 0.38±0.19, E2: 0.52±0.14, p=0.011) females. We found no main effect of rank on any individual anxiety-like behaviors (all p<0.10).

Discussion

The current findings demonstrate that exogenous administration of E2 increases sexual and male-directed social behavior in female rhesus monkeys, thus adding support for the activational effects of E2 on rhesus monkey behavior as suggested by the correlation of endogenous E2 with increased sexual behavior (Michael and Zumpe, 1993; Wallen et al., 1984; Wilson et al., 1982). Importantly, social subordination attenuated E2's activational effects on sociosexual behavior, and higher doses of E2 were not sufficient to increase the frequency or duration of these behaviors in subordinate females. E2's main effect on behavior was seen in addition to the influence of rank on sexual and male-affiliative behaviors, suggesting a synergistic effect of both treatment and social status on sociosexual behavior. These findings are novel in light of the current literature which suggests that concentrations of endogenous (Michael and Zumpe, 1993; Wallen et al., 1984; Wilson et al., 1982) or exogenous (Zehr et al., 1998) E2, higher than those observed in the current study, continue to increase sexual behavior in rhesus monkeys. The current study additionally provides data on the effects of E2 dose and chronic social subordination on non-mating behaviors, showing that E2 replacement attenuated female agonistic behaviors in dominant and middle-ranking females. In contrast, E2 had no effect on either female-directed affiliation or self-directed anxiety-like behavior, suggesting that E2's putative effects on alleviating anxiety-like behavior are not seen in rhesus social group interactions. These findings further suggest that the dose-dependent increase in sexually motivated behaviors by E2 is attenuated in lower ranking female rhesus monkeys, despite the alleviation of aggressive behaviors by alpha and middle-ranking females and no detectable change in anxiety-like behavior.

Why the largest dose employed, which produced blood E2 levels substantially below the preovulatory peak seen in intact cycling rhesus monkeys, did not increase sexual activity above and beyond the medium dose is puzzling. An inverted U-shaped dose response curve was seen for sexual behaviors in the alpha females, with the medium dose of E2 creating the greatest increases in behavior and the low and high doses being inadequate to do so. We hypothesize that this dose-response curve is a product of the pellets used for E2 administration, and may be related to the subsequent fluctuation of E2. Females received

subcutaneous E2 pellet implants following a minimum three-week period of E2 washout, and the resulting spikes in circulating E2 may not mimic the gradual increases observed during a natural ovarian cycle (Wallen et al., 1984). Additionally, studies looking at the exogenous administration of E2 mainly used Silastic capsules, which allow for a chronic release of E2 so long as it remains implanted subcutaneously (Zehr et al., 1998). The administration of E2 via subcutaneous pellets with their rapid release profile may have diminished E2's efficacy to induce behavioral changes. Future studies may benefit from using alternative methods producing a more consistent release profile. However, despite the significant decline in E2 serum concentrations between observation week one and week three across all doses, there were no significant differences in either proceptive or receptive sexual behavior or male-directed affiliation. Taken together, we see that E2 has a significantly different effect on sociosexual behavior following the medium dose of E2 that is absent in middle and subordinate ranking females. We suggest that the lack of a U-shaped curve in lower ranking female behavior may result from exposure to chronic subordination stress dampening the physiological and behavioral responses to E2.

Our data support the hypothesis that exposure to social subordination, a model of psychosocial stress that induces stress-related changes in physiology (Sapolsky, 1995), alters the activational effect of E2 on neural systems that mediate sociosexual behavior in rhesus monkeys. The effects of stress on LHPG axis function have been shown at the hypothalamic-pituitary level, whereby chronic stress amplifies LHPG axis sensitivity to E2 negative-feedback, resulting in the suppression of pituitary LH secretion (Michopoulos et al., 2009). The mechanism by which this interaction takes place is still unknown. In rhesus monkeys, glucocorticoids are one possible mediator, as chronic administration (2-3 months) of hydrocortisone acetate was able to elevate circulating cortisol and reduce serum concentrations of LH and FSH in gonadectomized males (Dubey and Plant, 1985). Additionally, in intact female cynomolgus monkeys, serum concentrations of E2, FSH, and LH were significantly reduced following administration of the adrenal androgen dehydroepiandrosterone sulfate (DHEAS) (Kowalski and Chatterton, 1992a), but not adrenocorticotropin-(1-24) (ACTH) (Kowalski and Chatterton, 1992b) over the course of one menstrual cycle. Furthermore, following the administration of metyrapone, an adrenal steroid synthesis inhibitor, subsequent elevations in CRH and ACTH were insufficient to decrease LH (Van Vugt et al., 1997). However, the majority of studies suggest that glucocorticoid suppression of the LHPG axis may be specific to chronic administration, and CRH is the mediator of short-term suppression of the LHPG axis (Tilbrook et al., 2000).

Furthermore, the mechanism by which chronic stress alters the effects of E2 on sexual behavior remains unclear. The current study suggests that exposure to chronic stress desensitizes brain systems that mediate the sociosexual effects of E2 on behavior, independent of LHPG and ovarian function, and further suggests that this desensitization is not overcome by increased administration of E2. So far, two mechanisms have recently garnered support from research on the effects of corticosterone on adrenalectomized female rats, although it is important to keep in mind that species differences exist in LHPA function (Rivier and Rivest, 1991; Tilbrook et al., 2000). In one study, adrenalectomized gonadally intact female rats showed a dose-response increase in ERs (specifically the β-subtype) mRNA expression in the hypothalamic paraventricular nucleus after implantation

subcutaneous corticosterone pellets (Isgor et al., 2003). These data suggest a mechanism by which chronic activation of the LHPA axis and rising levels of CRF and corticosterone alter the expression of ERs to then affect behavior. In another study, ovariectomized and adrenalectomized female rats given E2 were found to have no change in the expression of ERs in the pituitary following dexamethasone injection, however there was an attenuation of the downstream effects of ERs on progesterone receptor expression and pituitary weight (Naoki et al., 1985). Thus, a second mechanism might utilize the disruption of ER function as a transcription factor to reduce or alter the expression of genes and proteins normally regulated by E2 that in turn regulate socio-sexual behavior.

The current study additionally demonstrates that administration of E2 decreases both non-contact and contact aggression in parallel to the potentiation of sexually motivated behavior. However, we suggest that these data represent a byproduct of E2's activational effects on a female's motivation to engage in socio-sexual behavior combined with ready access to a male, and not a direct effect of E2 on aggressive behavior. Greater access and proximity to males increases rates of sexual behavior uncoupled from hormonal state (Wallen, 1990) and may serve to decrease aggression in females (Walker et al., 1983). Male-female pairs of rhesus monkeys observed in large compounds showed tighter coupling between the female's ovarian cycle and fluctuations in sexual activity than did pairs of rhesus monkeys observed in small cages (Wallen, 1982). Multi-female groups, when given restricted access to a single male, also showed higher correlations between sexual behavior and hormonal condition than when observed in male-female pairs (Wallen and Winston, 1984). The decreased coupling of sexual behavior and E2, as a result of small group enclosures and unrestricted access to males in the current study therefore provides greater opportunities for sexual behavior. This, however, may only be true of alpha females, and social subordination stress may elevate threshold levels of E2 needed to elicit sexual behavior (Wallen, 1990). Regardless, the increase in reproductively motivated behavior in alpha females, and the environmental opportunity to access males may be the cause of the decreased aggressive behavior seen following the medium dose of E2. In support of this hypothesis, ovariectomized females pair-housed with a male do not show any increase in aggression following treatment with E2 (Zumpe and Michael, 1970), and intact multi-female groups with unrestricted access to a single-male showed a corresponding decrease in aggressive behavior (Walker et al., 1983). In contrast, intact multi-female groups with restricted access to males showed an increases in less severe non-contact aggression with higher levels of E2 during periovulation (Walker et al., 1983), as did E2 treated ovariectomized females with no access to males (Michopoulos et al., 2011; Wallen and Tannenbaum, 1997). The trend for aggression to increase in alpha females following the highest dose of E2, without a corresponding increase in proceptive sexual behavior, requires further investigation, and suggests that the decreased aggression may be due to increased motivation for sexual behavior and not increased receipt of sexual male sexual behavior.

E2 has also been implicated in the reduction of anxiety-like behavior in animals and in elevating positive emotion and affect in human beings (Halbreich and Kahn, 2001; Toufexis et al., 2006). Hence, it is somewhat surprising that we did not observe very many changes in anxiety-like behavior related to E2 in this study. In fact, E2 tended to increase self-directed anxiety behavior in our subjects irrespective of rank. This is in contrast to what has been

observed in female rodents. For example, proestrous females, in which E2 levels are peaking, show reduced periods of immobility in the Porsolt forced swim tests, increased latencies in burying an electrified prod, and more time spent in the open arms of the elevated plus maze than do diestrous females or males (Marcondes et al., 2001). In other studies looking at the effect of long periods of anovulation in female macaques, it was found that these hypoestrogenic females exhibited behavior analogous to that seen in women with clinical depression (Shively et al., 2002). The discrepancies between these reports and the present study may be related to the type of anxiety-like behavior being scored and stress level present when the behavioral observations were undertaken. For example, rodent studies concentrate on changes in motor activity (i.e. freezing) and exploratory behavior, while in this present study we looked at self-directed anxietylike behaviors. In addition, our behavioral observations were not undertaken during experimental conditions that have been shown to enhance stress reactivity for primates wherein changes in anxiety-like behavior due to E2 may be easier to determine. Thus, we are currently examining the interaction of E2 and social rank on approach/avoidance behavior, a paradigm that has been shown to measure anxiety as indexed by a more complete range of behaviors, including exploratory behavior, in macaques (Coleman et al., 2011).

Conclusion

These data show that social subordination inhibits the expression of E2-mediated increases in sexual and male-affiliative behaviors, and that these changes may not be overcome by higher doses of E2. Furthermore, female-female aggression was seen to decrease following E2 treatment, suggesting that E2's influence on aggressive behavior is influenced by social and environmental factor more so than on hormonally mediated changes in neural systems underlying aggression. Finally, the current study does not support the hypothesis that E2 alters the expression of anxiety-like behavior, as measured in our study. Future studies using paradigms that enhance stress reactivity are necessary to elucidating the complex interaction between stress, E2 and anxiety-like behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

1. Social status attenuates estradiol's activational effects on sexual behavior and affiliation in female rhesus monkeys.
2. Stress induced attenuation of sociosexual behavior may not be overcome with higher doses of E2.
3. Estradiol's effects on female-female aggression may be context dependent on social-group environment.

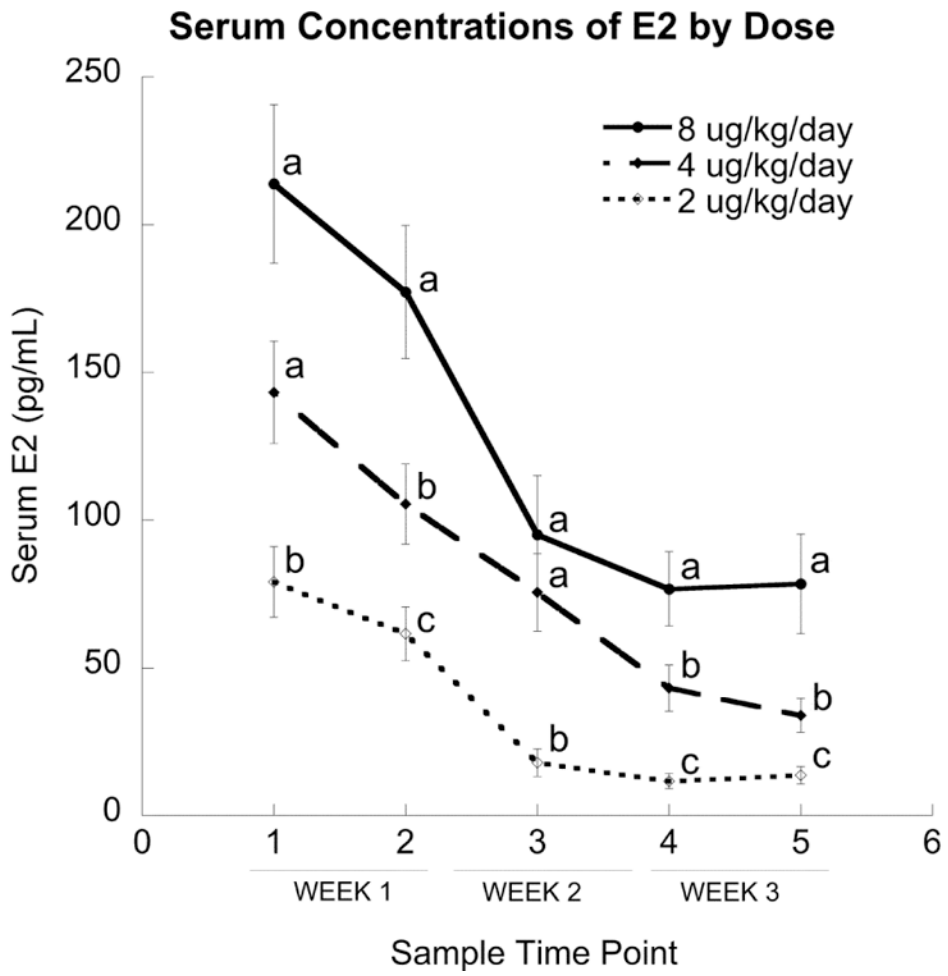

Figure 1.

Serum concentrations of E2 following low (2μg/kg/day), medium (4μg/kg/day), and high (8μg/kg/day) doses delivered using E2 sustained pellets low. Post-Hoc tests show significant dose differences in serum samples across all doses. Data are presented as average frequencies ± standard error. (a) > (b) > (c), $p<.05$. For sample time point 1, the high dose was greater than the medium dose only at the trend level ($p=.066$).

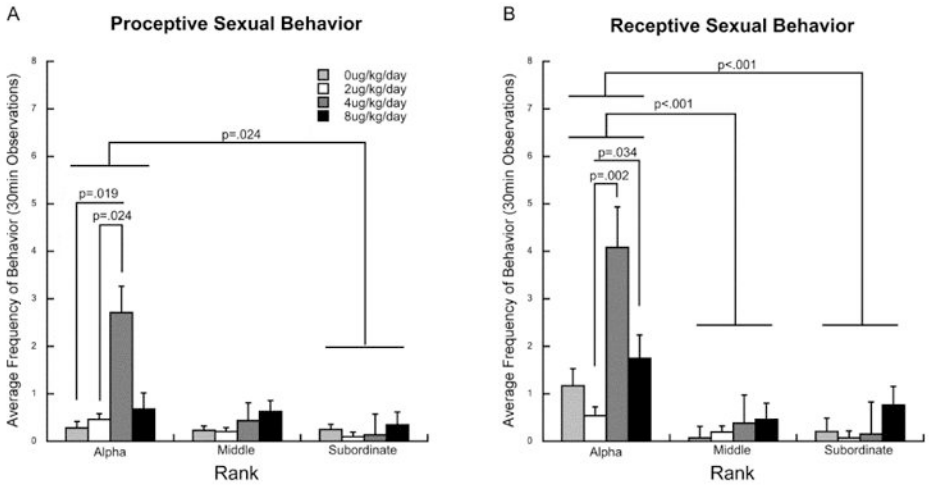

Figure 2.

Interaction between Rank and Dose on sexual behavior and toward males. Both (A) proceptive and (B) receptive behavior showed a main effect of Rank, a main effect of Dose, and an interaction effect between Rank and Dose. Post-hoc analysis showed activational effects only in Alpha females. Data are presented as average frequencies ± standard error.

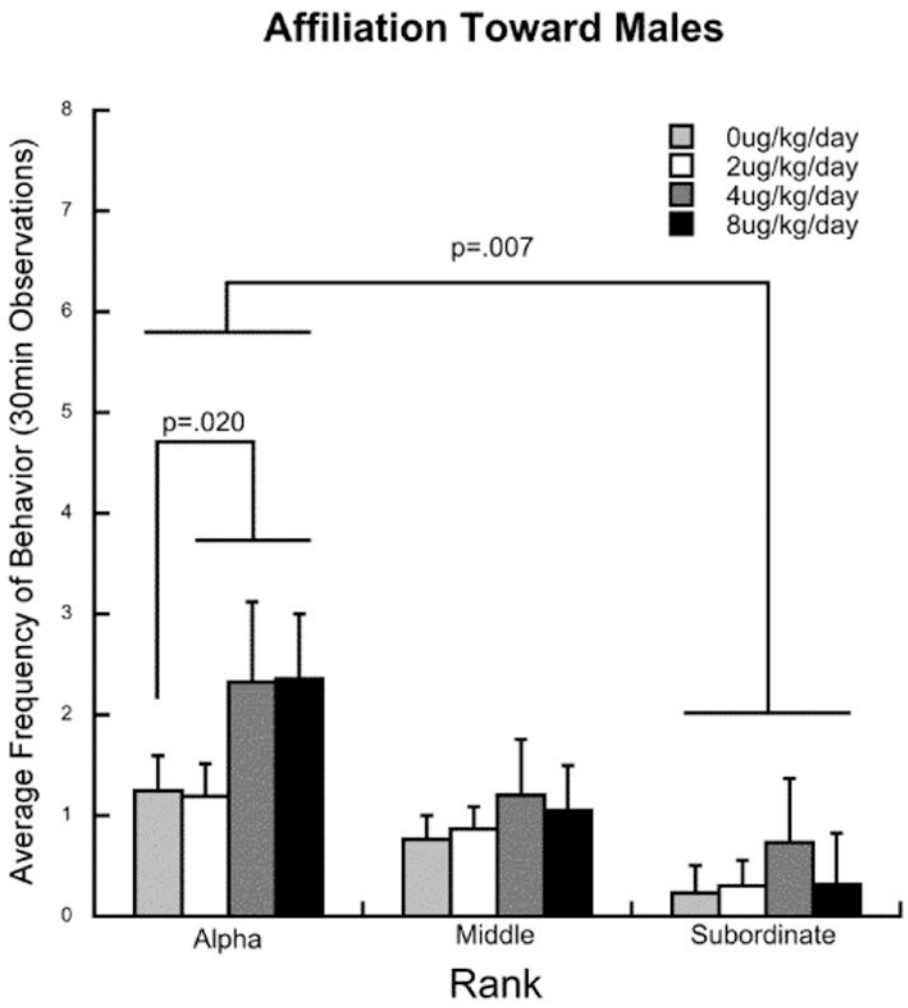

Figure 3.

Initiation of male-directed affiliation showed a main effect of Rank, but no main effect of Dose or interaction effect between Rank and Dose. Post-hoc analysis showed activational effects of only in Alpha females. Data are presented as average frequencies ± standard error.

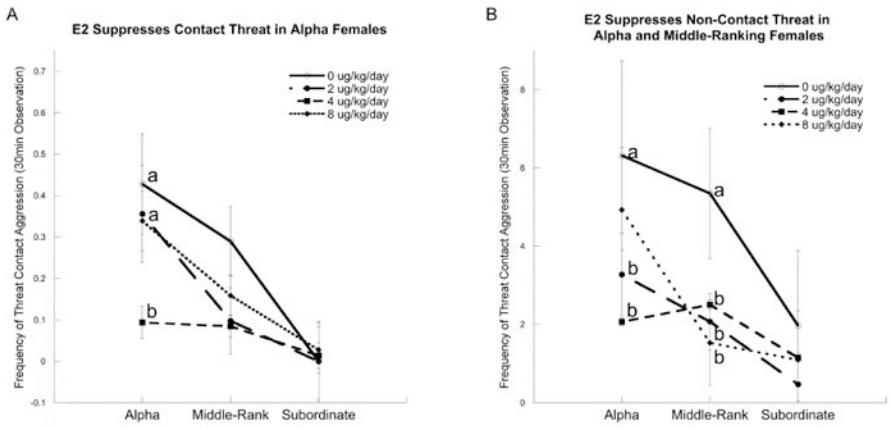

Figure 4.

Aggressive behavior, separated into Contact Aggression and Non-Contact Aggression, show significantly different responses to E2 Dose. Post-Hoc tests show significant dose differences in (A) the Alpha Females, and (B) Alpha and Middle-Ranking Females. Data are presented as average frequencies ± standard error. (a) > (b), $p<.05$.

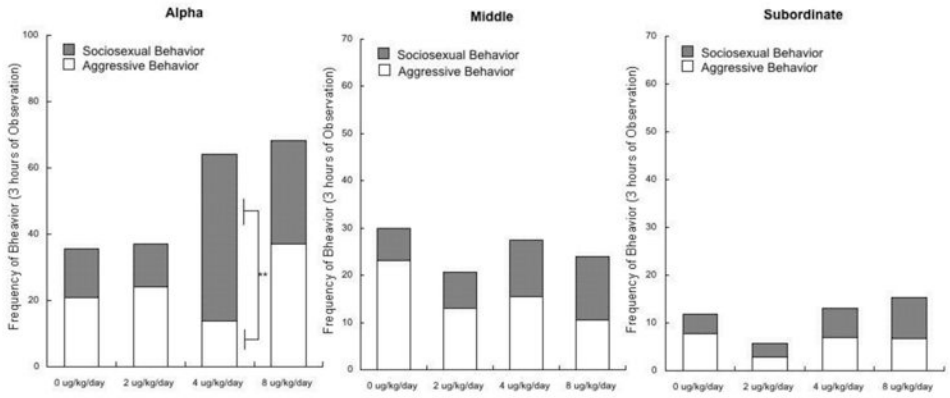

Figure 5.

Sociosexual behavior, defined as the combination of Proceptive, Receptive and Male-Affiliation behavior was compared to total Aggression in a rmANOVA using dose (0,2,4,8μg/kg/day) as a within subject variable and rank as a between subject variable. We found a significant main effect of dose ($F_{3,90}$ =3.50, p=0.033) and rank ($F_{2,30}$ =13.59, p<.001) but no main effect of behavior type ($F_{1,30}$ =1.90, p=0.178). However, there was an interaction between dose and behavior ($F_{3,90}$ =4.56, p=0.005). ** = p<0.01.

Table 1

Effects of Treatment Week on Female Rhesus Monkey Serum E2 and Behavior. Significant main and interaction effects of E2 Dose ([1]), Observation Week([2]), Dose by Week([3]), on serum E2 concentration, proceptive and receptive sexual behavior are listed in the table below. Significant changes in values based on post hoc analysis are shown between Observation Week (* = difference from Week one $p < 0.05$) and E2 Dose ([a] = difference from *0µg/kg/day;* [b] = difference from *2µg/kg/day* $p < 0.05$; [c] = difference from *4µg/kg/day* $p < 0.05$.) All data are raw averages of behavior per 30 minute observation session or serum E2 concentration taken during observational week 1 and week 3.

	***Serum E2 (pg/mL)*[1,2]**		***Proceptive Behavior (/30min)*[1,3]**		***Receptive Behavior (/30min)*[1]**	
E2 Dose (µg/kg/day)	***Week 1***	***Week 3***	***Week 1***	***Week 3***	***Week 1***	***Week 3***
0	-----	-----	0.35 ± 0.10	0.16 ± 0.50 *	0.44 ± 0.17	0.53 ± 0.20
2	73.97 ± 9.66	13.84 ± 2.98 *	0.16 ± 0.04	0.35 ± 0.10	0.35 ± 0.14	0.19 ± 0.12
4	127.79 ± 13.46 [b]	38.86 ± 6.15 * [b]	0.57 ± 0.13	1.62 ± 0.51 [a]	2.03 ± 0.57	1.05 ± 0.30 *
8	202.38 ± 19.72 [b,c]	78.15 ± 12.68 * [b,c]	0.52 ± 0.25	0.58 ± 0.16	1.07 ± 0.36	0.91 ± 0.20

Horm Behav. Author manuscript; available in PMC 2014 May 05.

Table 2

Effects of E2 on Female Rhesus Monkey Behavior. Main effect of Rank ([1]) on female-affiliation given, female-aggression received and given, female-submission given, and self-directed anxious behaviors are listed in the table below. Significant changes in valued based on post hoc analysis are shown between Rank categories (* = difference from Alpha $p < 0.05$; # = difference from Middle-Ranking Female $p < 0.05$) and E2 Dose ([a] = difference from *0μg/kg/day* $p < 0.05$; [b] = difference from *2μg/kg/day* $p < 0.05$; [c] = difference from *4μg/kg/day* $p < 0.05$.) All data are raw averages of behavior frequency during six 30-minute behavioral observations.

BEHAVIOR	*E2 Dose (μg/kg/day)*	*Alpha*	*Middle*	*Subordinate*
Affiliation Toward Females[1]	0	3.44 ± 0.88	3.24 ± 0.61	1.36 ± 0.70
	2	3.09 ± 0.78	2.72 ± 0.54	1.31 ± 0.62
	4	2.72 ± 0.88	3.12 ± 0.61	1.46 ± 0.70
	8	2.52 ± 0.67	2.93 ± 0.47	1.25 ± 0.53
Aggression Toward Females[1]	0	3.41 ± 1.90	3.78 ± 1.31	1.29 ± 1.50
	2	3.63 ± 1.04	2.17 ± 0.72	0.47 ± 0.82 *
	4	2.17 ± 1.15	2.59 ± 0.79	1.17 ± 0.91
	8	5.27 ± 1.62	1.69 ± 1.11	1.13 ± 1.28 *
Aggression From Females[1]	0	0.02 ± 1.45	1.51 ± 1.00	5.68 ± 1.15 * #
	2	0.00 ± 0.79	1.30 ± 0.54	3.01 ± 0.62 * #
	4	0.02 ± 1.24	1.01 ± 0.86	4.50 ± 0.98 * #
	8	0.00 ± 1.37	2.29 ± 0.95 * c	4.38 ± 1.09 *
Submission Toward Females[1]	0	0.02 ± 2.44	6.84 ± 1.68 *	8.75 ± 1.93 *
	2	0.00 ± 1.59	4.11 ± 1.10 * a	7.39 ± 1.26 *
	4	0.00 ± 2.02	4.30 ± 1.40 *	8.99 ± 1.60 * #
	8	0.02 ± 2.27	6.32 ± 1.57 * b c	9.54 ± 1.80 *
Anxiety-like Behavior	0	5.14 ± 1.29	5.76 ± 0.89	3.74 ± 1.02
	2	4.12 ± 1.50	7.00 ± 1.04	3.92 ± 1.19
	4	3.28 ± 1.01	6.53 ± 0.69 *	4.22 ± 0.80
	8	4.97 ± 1.11	4.79 ± 0.77	4.04 ± 0.88