

Effects of affiliation arousal (hope of closeness) and affiliation stress (fear of rejection) on progesterone and cortisol

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Abstract

Our prior research has suggested a connection between progesterone (PROG) and implicit affiliation motivation, the non-conscious drive for positive social contact. In particular, experimental arousal of affiliation motivation led to relative PROG increase in women and men [Schultheiss, O.C., Wirth, M.M., Stanton, S.J., 2004. Effects of affiliation and power motivation arousal on salivary progesterone and testosterone. *Horm. Behav.* 46(5), 592–599]. The present study aimed to (1) replicate this effect, (2) simultaneously assess cortisol (CORT) levels in this paradigm in order to rule out non-specific adrenal effects induced by affiliation arousal, and (3) examine effects on PROG and CORT of approach (hope for closeness, HOC) versus avoidance (fear of rejection, FOR) affiliation arousal. These motivational states were experimentally aroused in participants using film segments containing approach- or avoidance-oriented affiliation-related themes; a neutral film segment was used as a control condition. The film segments affected participants' implicit affiliation motivation and self-reported mood, demonstrating effectiveness of the manipulation. In the FOR condition, participants' CORT and PROG were increased post-film, consistent with the idea that fear of rejection is stressful. We did not replicate our prior finding of PROG increase following the HOC manipulation. However, relationships between PROG and implicit affiliation motivation were apparent across conditions. In particular, PROG co-varied positively with affiliation motivation, and baseline affiliation motivation positively predicted PROG increase in the FOR condition. As prior research implicates PROG in down-regulation of stress, we speculate that PROG release during stress may encourage affiliation for stress reduction purposes.

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Introduction

Steroid hormones play important roles in social motivation. For example, it is well established in human and non-human animals that testosterone is involved in dominance and intra-species aggression (e.g., Monaghan and Glickman, 2002) and that reproductive hormones are necessary in non-human animals for proper expression of sexual and parental behavior (Baum, 2002; Rosenblatt, 2002). Steroid hormones also are important factors in emotional processes. In particular, the HPA axis and cortisol (CORT), its major output in humans, are key components of the stress response and are dysregulated in a number of affective disorders (e.g., Plotsky et al., 1998). Other steroid hormones also play roles in stress and affect. For

example, progesterone (PROG) exerts anxiolytic and sedative effects, apparently via actions of its metabolites, such as allopregnanolone (3 alpha-hydroxy-5 alpha-pregnane-20-one, or 3 α ,5 α -tetrahydroPROG) on GABA-A receptors (Bitran et al., 1995; Majewska et al., 1986; Paul and Purdy, 1992; Reddy and Apanites, 2005; Soderpalm et al., 2004; Wieland et al., 1991). PROG and allopregnanolone are released during stress (Barbaccia et al., 1996a; Klatzkin et al., 2006; Romeo et al., 2005) and suppress HPA axis activity and CRH-induced anxiety (Guo et al., 1995; Patchev et al., 1994, 1996). Decreased allopregnanolone levels are found in depressed patients (Eser et al., 2006) and women with premenstrual syndrome (Bicikova et al., 1998), and increased PROG and allopregnanolone have been seen in anxiety and panic disorder, possibly as a compensatory response to panic attacks or anxiety (Brambilla et al., 2003, 2004, 2005; Eser et al., 2006). Administration of progestins decreased panic and anxiety in response to a

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challenge in women with panic disorder (Le Melleo et al., 2001).

Compared to testosterone, little attention has been devoted to involvement of PROG in social motivation, beyond the fact that PROG is among many hormones of pregnancy implicated in maternal behavior in non-human animals and possibly humans (Fleming et al., 1997a; Pryce et al., 1993; Rosenblatt, 2002) and that progestins have been used to decrease sexual motivation (Cooper, 1986). Several indirect lines of evidence warrant closer examination of the involvement of PROG in the motivation to affiliate. Affiliation and attachment are known to be associated with other anxiolytic neuroactive substances, such as opioids (Depue and Morrone-Strupinsky, 2005; Keverne et al., 1989; Panksepp et al., 1994) and oxytocin (Carter et al., 1992; Insel, 1997; Uvnas-Moberg, 1998b). PROG and oxytocin also exhibit complex interrelationships; for example, though PROG down-regulates oxytocin receptors in uterine tissue, it up-regulates them in the hypothalamus (Schumacher et al., 1999); and oxytocin stimulates release of PROG from the ovary independent of luteinizing hormone (Miyamoto and Schams, 1991). It is also of interest that separation distress, which can be thought of as the avoidance aspect of affiliation motivation, has been linked to brain systems underlying panic (Panksepp, 1998), and PROG metabolites reduce separation distress in young rodents (Zimmerberg et al., 1994).

In general, it is important to characterize the physiological profile of affiliation motivation since the latter has been tied to health outcomes (McAdams and Vaillant, 1982; McClelland, 1989; McClelland and Kirshnit, 1988). We have gathered evidence for a connection between PROG and implicit affiliation motivation, defined as the non-conscious aspect of the drive for warm, close interactions with others. First, correlational evidence for such a connection was obtained (Schultheiss et al., 2003): women taking oral contraceptives containing progestins had higher trait affiliation motivation than cycling women, and among cycling women, salivary PROG positively correlated with affiliation motivation in the luteal (high-PROG) phase of the menstrual cycle. In a subsequent study, we experimentally aroused affiliation motivation using film segments. This manipulation successfully increased affiliation motivation and also led to increase in PROG, as compared to arousal of power motivation or a motivationally neutral control condition (Schultheiss et al., 2004). The effect on PROG was un-moderated by sex or by menstrual cycle or hormonal contraceptive status in women, suggesting that the change in PROG was adrenal in origin.

The first goal of the present study was to replicate the Schultheiss et al. (2004) effect of affiliation motivation arousal on PROG. Furthermore, we have observed that PROG and CORT correlate highly in men and positively co-vary in both sexes (Wirth et al., *in press*). As mentioned above, stress leads to PROG release; it has been found that adrenocorticotropin hormone (ACTH) causes release of both PROG and allopregnanolone in addition to CORT in humans (Genazzani et al., 1998). Thus, it is necessary to determine whether the increase in PROG seen previously was not merely a side effect of a stress

response. Therefore, we investigated whether the effect of affiliation motivation arousal on PROG was due to general adrenal activation by assessing CORT in the present study. Thirdly, we sought to examine effects on PROG and CORT of two aspects of the motivation to affiliate: desire to be close to others (approach motivation) and fear of rejection/separation (avoidance motivation) (Boyatzis, 1973).

We expected arousal of hope of closeness to lead to PROG increase in both sexes, as in our prior study. No specific hypotheses were made regarding PROG response to fear of rejection arousal as this aspect of the study was exploratory; we did, however, suspect that it would increase, especially since PROG is released during stress (e.g., Barbaccia et al., 1996a; Klatzkin et al., 2006). We also hypothesized that fear of rejection arousal would lead to increase in CORT and that this effect might be more pronounced in those high in baseline affiliation motivation. CORT was not expected to increase following hope of affiliation arousal as we suspected that the effect on PROG in our earlier study was specific to PROG and not merely a result of general adrenal activation. Finally, we hypothesized that other, more complex relationships between PROG and affiliation might emerge. In order to test these hypotheses, we used a design similar to that used in Schultheiss et al. (2004), in which participants completed an instrument designed to measure implicit motives and provided saliva samples for hormonal analysis before and after viewing film segments chosen to arouse hope of closeness and fear of rejection. As the film segments presumably can affect mood as well as motivation, we also assessed self-reported mood in this study. However, we did not expect self-reported mood to relate to hormonal responses; implicit measures generally are better predictors of biological and behavioral outcomes than explicit (i.e., self-report) measures (Mazur and Booth, 1998; McClelland et al., 1989; Schultheiss et al., 2005).

Methods

Participants

Ninety participants (50 men: average age 19.7 ± 2.3 years; 40 women: average age 19.6 ± 1.8 years) were recruited through the University of Michigan introductory psychology subject pool, as well as through flyers advertising a paid research study open to undergraduate and graduate students between the ages of 18 and 35 and not majoring in psychology. Subject pool participants received 2 h of subject pool credit; participants recruited via flyers received \$20 for their participation. Sixteen of the forty women reported using hormone-based contraceptives. All participants provided informed consent and were debriefed at the end of the session; the study had received prior approval by the University of Michigan Institutional Review Board. One woman was excluded from statistical analyses on PROG due to PROG levels in excess of 600 pg/ml, possibly indicating pregnancy. One man was excluded from all hormonal analyses due to a missing saliva sample. One woman was excluded from analyses on self-reported mood as her computer-entered data were missing. Thus, the complete data set included 87 participants (49 men).

Procedure

Sessions lasted approximately 100 min and were run by a female experimenter. Sessions took place between 12:00 and 18:00 from November 2004 to May 2005. Participants were randomly assigned to one of three motivational arousal conditions: hope of closeness (HOC), fear of rejection

(FOR), and control (CON) (30 participants per condition). One to six participants were present in a given session. All questionnaire data were collected using computers; experimental programming was done using Experimental Run Time System software (ERTS; Berisoft Corp., Frankfurt, Germany). At the start of the session (0 min), participants completed a picture-story exercise (pre-film PSE) for assessment of implicit motives. At 20 min (T1), they completed a mood questionnaire (see below) and provided a saliva sample followed by two short questionnaires concerning emotion regulation. At 35 min, the film segment was shown (duration 30 min; see descriptions below). Following the film segment (at 65 min), participants completed a second PSE and mood questionnaire (post-film PSE; mood at T2). The order of the post-film PSE and mood questionnaire was counterbalanced across participants; a second saliva sample (T2) at 70 min (10 min post-film) accompanied the first task. Participants then completed a personality inventory as a filler task, provided biographical information including menstrual and hormonal contraceptive status, indicated whether they had seen the film previously, and then completed a final mood questionnaire and provided a third saliva sample (T3) at 95 min (35 min post-film).

Film segments

Each film segment was 30 min long and represented a contiguous portion of the original movie. Film excerpts were presented on a 32-inch (~81 cm) color TV in a semi-dark room with a viewing distance of 2 to 3 m. Film segments used in HOC and CON conditions are identical to those used in Schultheiss et al. (2004).

In the HOC condition, participants viewed a segment of *The Bridges of Madison County* (director: Clint Eastwood) in order to arouse approach-based (hope for closeness) affiliation motivation. The excerpt shown depicts how a farmer's wife, after her family departs for an exhibition, makes the acquaintance of a photographer who stops at her farm to ask for directions. The two feel attracted to each other; the farmer's wife invites the photographer for lunch, and they spend time together in the outdoors. Although the excerpt does not reveal whether the two protagonists actually become lovers, it is rich with cues related to affiliation and intimacy, while featuring no cues related to fear of rejection or separation anxiety.

In the FOR condition, participants viewed a segment of *A.I.* (director: Steven Spielberg) in order to arouse avoidance-based (fear of rejection) affiliation motivation. The segment begins with a mother programming her robotic child to love her, after which he calls her "Mommy" for the first time and melts into her arms. Later scenes show the robotic child interacting with other children, and the mother's arrival at the decision that she must get rid of the robot. The final scene depicts the mother bringing the robotic child into the forest and abandoning him there; when he realizes what is happening, he exhibits a powerful panic response and begs his "mother" not to leave him.

In the CON (control) condition, participants viewed a segment of *Amazon: Land of the Flooded Forest* (a National Geographic documentary written and produced by Barbara Jampel). Although interesting and engaging to watch, this film does not contain cues related to affiliation or other social motives or emotions. The excerpt presents the fauna and flora in and around the *Amazon* as well as some of the daily activities (e.g., fishing) of the people who live at the river.

Mood adjective checklist

Participants' affect was assessed with a modified version of the University of Wales Mood Adjective Check List (MACL; Matthews et al., 1990). Four items each (two positively and two negatively scored) were taken from the Hedonic Tone, Energetic Activation and Tense Activation Scales of the MACL. We added to these 6–7 items each related to positive and negative "approach" affiliation (i.e., hope for closeness) and to positive and negative "avoidance" affiliation (i.e., fear of rejection/separation anxiety). We also added 9 items to capture other emotional states thought to possibly be aroused by the film segments, including some items assessing sexual motivation (see Table 1 for all items). Items were presented in random order with the primer "Right now I feel..." and participants could endorse each item on a 4-point scale featuring the labels definitely not, slightly not, slightly, and definitely. Items were presented to participants in randomized order. After recoding of negatively keyed items, sum scores were calculated for hedonic tone, energetic activation, tense activation, fear of rejection, and hope for closeness. Factor-analysis clearly differentiated fear of rejection from hope for closeness; at each time point, these scales showed a significant negative correlation (R for T1, T2, T3: -0.40, -0.63, -0.69; all $P < 0.001$). Internal consistencies of all 5 scales were assessed using Cronbach's alpha and were satisfactory for each assessment (see Table 1). Ratings on the items Sexual, Desiring, and Flirtatious correlated strongly and were combined into a measure of self-reported sexual motivation (Cronbach's alphas 0.76, 0.78, and 0.69 for T1, T2, and T3, respectively).

Picture-story exercise

In the PSE, participants are shown a picture cue typically depicting multiple actors in ambiguous interpersonal situations, and they are instructed to write whatever story comes to mind describing what is happening in the picture (Schultheiss and Pang, in press; Smith, 1992). In the present study, participants were given a maximum of 5 min to write and could not move on to the next picture before 4 min have passed in order to encourage them to generate sufficient text for scoring of motive imagery. Two PSEs were used, each consisting of four picture cues, for assessment of implicit affiliation motivation before and after the film segment. Picture set A consisted of "trapeze artists" (a man and woman about to catch each other on trapeze swings), "park bench" (a couple sitting together on a bench by a river), Thematic Apperception Test

Table 1
Mood adjective checklist items (Cronbach's alpha for entire scale at T1, T2, and T3)

	Hedonic tone	Energetic activation	Tense activation	Fear of rejection	Hope for closeness
Positively keyed items	Happy Satisfied	Energetic Active	Nervous Tense	Lost Forlorn Isolated Abandoned Panicky Lonely Rejected	Sociable Affectionate Gregarious Warm Loving Compassionate
Negatively keyed items	Sad Depressed (0.75, 0.75, 0.59)	Sluggish Tired (0.82, 0.77, 0.50)	Relaxed Calm (0.73, 0.79, 0.51)	Safe Secure Protected Accepted Attached Loved Trusting (0.86, 0.90, 0.87)	Cold Aloof Distant Remote Detached Unfriendly Independent (0.84, 0.86, 0.85)

Other items: Frustrated, Hopeful, Confident, Self-assured, Angry, Annoyed, Afraid, Sexual, Desiring, Flirtatious (the last three items composed the sexual motivation scale).

(TAT) card 3BM (Murray, 1943) (depicting from behind a person with head on arms, appearing distraught), and “excluded boy” (depicting schoolgirls talking while an unhappy-looking boy stands apart). Picture set B consisted of “mountain” (a woman helping a young girl mountain climbing), “horses” (an older man and younger woman leading horses and talking), TAT card 13B (depicting a small boy sitting alone in a doorway), and “excluded girl” (college-aged girls talking while a fourth girl stands apart with arms crossed). Three of these pictures have been repeatedly used and validated in implicit motive research (“trapeze artists”, “park bench”, “horses”), two were taken from the original TAT picture set, and the other three were used here for the first time. Pictures were selected to pull for themes of hope for closeness and fear of rejection and were matched by motivational content between the two sets (i.e., person helping another person do something dangerous; two people spending time together; isolation; exclusion from peers). Order of presentation of the two picture sets was counterbalanced across participants, and within each set, order of picture presentation was randomized.

Stories were analyzed using two different motive scoring systems. Winter’s (1994) Manual for Scoring Motive Imagery in Running Text was used to score implicit affiliation motivation (need for affiliation, or nAff), power (nPow) and achievement (nAch) motivation. This system scores affiliation imagery when text describes one character’s expression of positive, friendly feelings towards another; sadness or other negative feelings about separation from another or disruption of a relationship; characters engaging in affiliative activities together; and empathetic nurturing or helping (Winter, 1994). Stories were also scored for implicit affiliation motivation using the original need for Affiliation scoring system (Heyns et al., 1958), which places affiliation themes into the following categories: stated need for affiliation, instrumental activity performed to achieve affiliation, anticipation of affiliation, obstacles or blocks to affiliation, affective states resulting from gain or loss of affiliation, and general affiliation themes. Most categories in the Heyns et al. (1958) system distinguish between approach and avoidance aspects of the affiliation motive (e.g., instrumental activity performed to gain friendship *versus* to prevent abandonment). From these categories, two variables were created for pre-film and post-film stories: total number of positive/approach affiliation images (pos-Aff) and total number of negative/avoidance affiliation images (neg-Aff).

Motive scoring was conducted by two trained scorers who had previously achieved over 85% agreement with training materials pre-scored by experts. Motive scorers were blind to participants’ experimental condition. Motive scores on pre-film and post-film stories were added together to create pre-film and post-film motive scores. Participants wrote, on average, 341 ± 64 words containing 4.7 ± 2.1 nAff images (Winter, 1994 system) in the four pre-film stories, and 319 ± 61 words containing 4.3 ± 1.8 nAff images in the four post-film stories. By the Heyns et al. (1958) system, pre-film stories contained 5.9 ± 3.1 pos-Aff images and 1.5 ± 1.4 neg-Aff images; post-film stories contained 5.4 ± 3.0 pos-Aff and 1.5 ± 1.5 neg-Aff images. Word counts did not correlate with motive scores, so motive scores were not corrected for word count. Motive scores were converted to z-scores before statistical analysis.

Saliva collection and hormone assays

Salivary measurements are an excellent non-invasive way to assess the unbound (i.e., bioactive) portion of steroid hormones in humans (Riad-Fahmy et al., 1983). At each sampling point, participants used a fresh sugar-free chewing gum to collect 7.5 ml saliva in a sterile polypropylene vial. Vials were closed and frozen immediately at the end of each data collection session. Samples were freed from mucopolysaccharides and other residuals by three freeze–thaw cycles with subsequent centrifugation. Salivary CORT and PROG levels were determined by solid-phase 125I radioimmunoassays (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA). Hormones were measured using 400 μ l saliva samples in combination with water-diluted standards (analytical range: CORT, 0.5 to 50 ng/ml; PROG, 5 to 400 pg/ml) and overnight incubation at room temperature. Mean CORT and PROG assay sensitivities ($B0-3 \times SD$) were 0.015 ng/ml and 1.88 pg/ml, respectively. Mean intra-assay coefficients of variation (CV), calculated using participant samples, were 4.33% for CORT and 10.33% for PROG. Pools of saliva collected from a number of volunteers at 8:00 and 20:00 showed expected circadian variation in CORT (averages 4.28 and 0.89 ng/ml). Inter-assay CVs for these pools averaged 5.38% for CORT and 8.93% for PROG.

Statistical analyses

All analyses were conducted with SYSTAT 10 and involved regression analysis, repeated-measures and simple ANCOVA, ANOVA, correlation and bi-partial correlation analyses, and *t*-tests. Descriptive statistics are given as mean \pm SD. An alpha level of 0.05 (two-tailed) was employed in all analyses.

Unless otherwise specified in the Results section, repeated-measures ANCOVA are performed on post-film mood or hormone levels at both T2 and T3, using film condition as the independent variable and mood or hormone level at T1 as a covariate. This approach is used to examine post-film effects on mood or hormones while controlling for possible differences by condition at baseline. Even when an ANCOVA approach is used, for ease of viewing, figures display data at all time points rather than residuals of post-film measures after regressing out pre-film (baseline) measures.

Results

Self-reported mood

ANOVA revealed that, at baseline (T1), participants did not differ by condition in self-reported hedonic tone, energetic activation, tense activation, fear of rejection, hope for closeness, or sexual motivation.

ANCOVA and ANOVA revealed post-film differences by condition in self-reported hedonic tone, tense activation, fear of rejection, and sexual motivation (Fig. 1). Specifically, hedonic tone decreased and tense activation and fear of rejection increased post-film following the *A.I.* film segment (FOR condition), and sexual motivation increased post-film following the *Bridges* segment (HOC condition). Results of statistical tests used to obtain these conclusions are detailed below.

Repeated-measures ANCOVA on post-film hedonic tone (at T2 and T3) by condition, with hedonic tone at T1 as a covariate, showed a significant time \times condition interaction on post-film hedonic tone, $F(2,85)=3.38$, $P=0.039$, as well as an overall effect of condition on post-film hedonic tone, $F(2,85)=5.32$, $P=0.007$. Follow-up ANOVA by time point traced these effects to participants reporting a decrease in hedonic tone immediately post-film (T2) in the FOR condition which partially recovered at T3.

A parallel repeated-measures ANCOVA on tense activation also showed a significant time \times condition interaction, $F(2,85)=3.75$, $P=0.028$, and an overall effect of condition on tense activation, $F(2,85)=6.32$, $P=0.003$. Again, self-reported tense activation increased after the film segment in the FOR condition, but partially recovered in the third mood measurement.

ANCOVA on self-reported fear of rejection showed that fear of rejection increased in the FOR condition at T2 and remained increased compared to the other groups at T3, with an overall effect of condition on post-film fear of rejection, $F(2,85)=4.87$, $P=0.010$ (the repeated-measures \times condition interaction was not significant).

ANCOVA on self-reported sexual motivation revealed a significant time \times condition effect, $F(2,85)=3.57$, $P=0.033$, and an overall condition effect, $F(2,85)=5.19$, $P=0.007$, such that sexual motivation was increased post-film in the HOC condition (*Bridges* segment). This effect was not moderated by participant sex, although men reported greater sexual motiva-

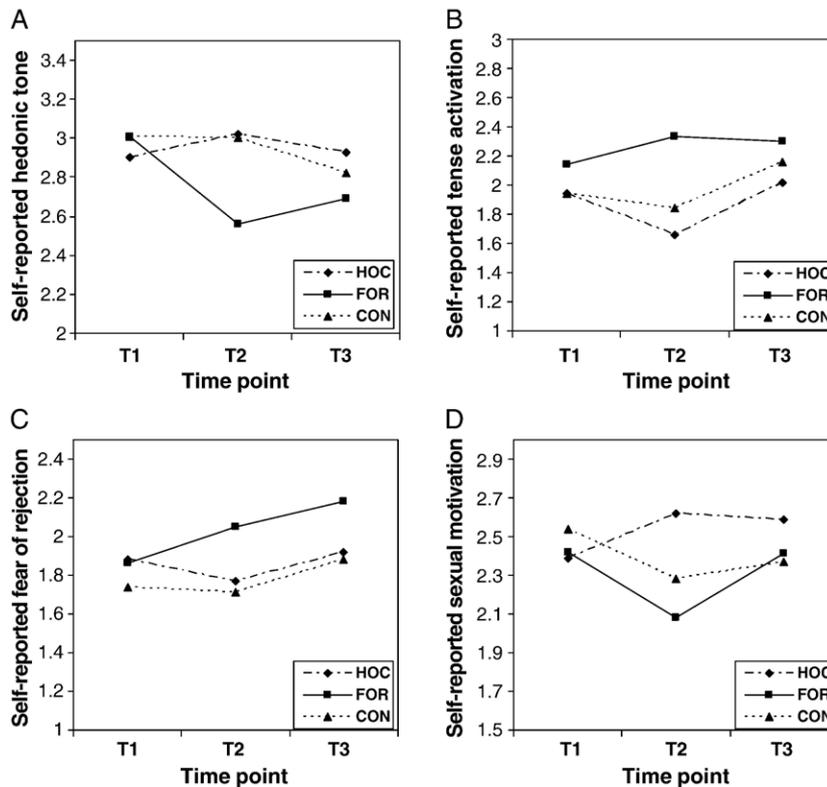


Fig. 1. Effects of condition on self-reported mood: (A) hedonic tone, (B) tense activation, (C) fear of rejection, (D) sexual motivation. Scales contained 4, 4, 14, and 3 items, respectively. All items were rated on a 1–4 scale.

tion than women at baseline (men: 2.61 ± 0.73 ; women: 2.23 ± 0.88 ; $T(1,87) = 2.20$, $P = 0.03$).

Of the 89 participants with complete mood data, 17 reported having seen the film before. Of those, 11 were in the FOR condition (*A.I.*) and 6 were in the HOC condition (*Bridges*). An additional 3 participants in the control condition (*Amazon*) reported not being sure if they had seen the film previously. All effects of condition on self-reported mood remained the same or became stronger when participants who reported having seen the film were excluded.

Hormones

Raw CORT and PROG data by condition and sex are shown in Table 2. CORT data were skewed and thus were log-transformed prior to all statistical analysis. PROG data were skewed in women but not in men. For consistency, they were log-transformed in both sexes. Two women and one man who were outliers in terms of change in PROG were excluded from analyses on PROG only (leaving 37 women and 48 men).

CORT stayed relatively steady post-film in the FOR condition, in contrast to the other two conditions, in which it dropped (Fig. 2). This is reflected in a repeated-measures ANCOVA by condition on post-film CORT in which there is a significant interaction of time \times condition, $F(2,85) = 6.07$, $P = 0.003$. When sex was added as a factor, the time \times condition \times sex interaction was significant, $F(2,82) = 3.14$, $P = 0.049$. Follow-up analyses revealed that the time \times condition effect was significant in male ($F(2,45) = 7.41$, $P = 0.002$) but not in female participants, in whom CORT tended to drop over time in all three conditions. This analysis remained non-significant when the 16 women using hormonal contraceptives were excluded (leaving 22 women in the analysis).

PROG, like CORT, tended to increase between T2 and T3 in the FOR condition but drop in the other conditions, particularly HOC (Fig. 3). This is shown in a repeated-measures ANCOVA on PROG, revealing a significant interaction of time \times condition, $F(2,81) = 4.74$, $P = 0.011$. When sex was added as a factor, it did not moderate the time \times condition interaction on post-film PROG. Neither did exclusion of women using hormonal contraceptives change the results.

Table 2
Raw salivary cortisol (CORT) and progesterone (PROG) concentrations by participant sex and condition: mean (SD)

Sex/Condition	FOR (<i>A.I.</i> film)			HOC (<i>Bridges</i> film)			CON (<i>Amazon</i> /control film)		
Male, PROG (pg/ml)	24.7 (11.2)	17.4 (6.3)	18.6 (7.3)	21.8 (7.9)	16.5 (5.2)	14.4 (5.6)	18.5 (8.2)	16.0 (5.9)	14.7 (4.7)
Male, CORT (ng/ml)	3.38 (2.67)	2.41 (2.57)	2.35 (1.91)	3.29 (1.48)	2.15 (0.98)	1.50 (0.58)	2.69 (1.90)	1.59 (0.93)	1.43 (0.99)
Female, PROG	16.7 (15.2)	15.9 (17.5)	15.4 (14.7)	24.7 (21.1)	18.7 (18.3)	17.9 (22.0)	29.5 (28.8)	23.5 (22.8)	29.8 (33.7)
Female, CORT	1.82 (0.79)	1.30 (0.46)	1.15 (0.34)	2.03 (0.97)	1.75 (1.24)	1.51 (1.05)	2.84 (2.25)	1.76 (1.20)	1.54 (0.82)

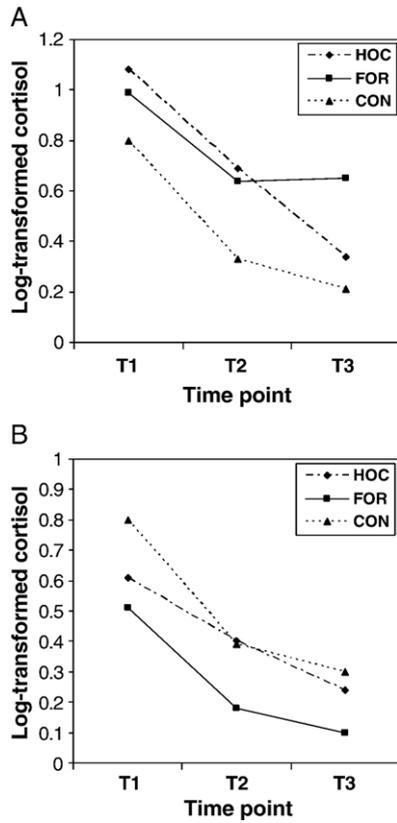


Fig. 2. Effects of condition on cortisol in (A) men; (B) women. Hormone levels are log-transformed due to skew.

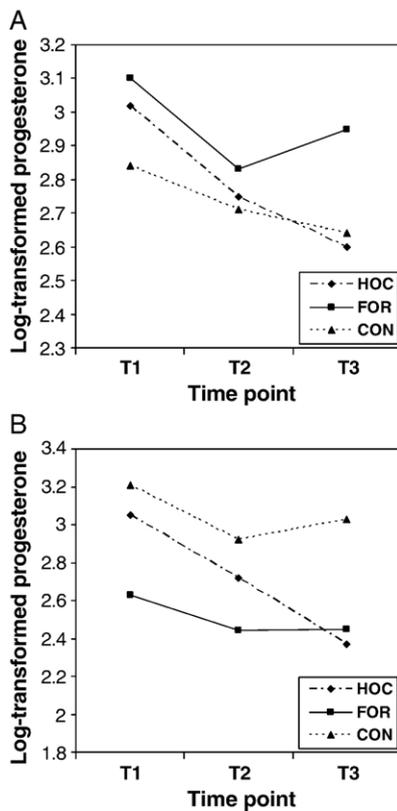


Fig. 3. Effects of condition on progesterone in (A) men; (B) women. Hormone levels are log-transformed due to skew.

Excluding the 17 participants who had seen the films previously strengthened effects of condition on PROG and CORT (PROG analysis, a main effect of condition emerged, $F(2,60)=3.97, P=0.024$; CORT analysis, time \times condition effect, $F(2,63)=6.59, P=0.003$).

PROG and CORT were strongly positively correlated at all three time points in men and also co-varied positively together in both sexes (i.e., showed significant bi-partial correlations); for details, see Wirth et al. (in press).

Implicit motives

As sometimes found in previous studies (e.g., Schultheiss et al., 2003), baseline (pre-film) implicit affiliation motivation as obtained via the Winter (1994) scoring system (need for Affiliation; nAff) was higher in women ($0.22 \pm 0.94, z$ -scores) than in men ($-0.26 \pm 0.98; T(1,84)=2.27, P=0.03$). Post-film nAff did not differ between the sexes.

Overall, implicit affiliation motivation measured by the different scoring systems tended to increase in the HOC and decrease in the FOR condition (Fig. 4). Statistics supporting this statement are detailed below.

ANCOVA on post-film nAff by condition, controlling for pre-film nAff, did not reveal a significant effect of condition.

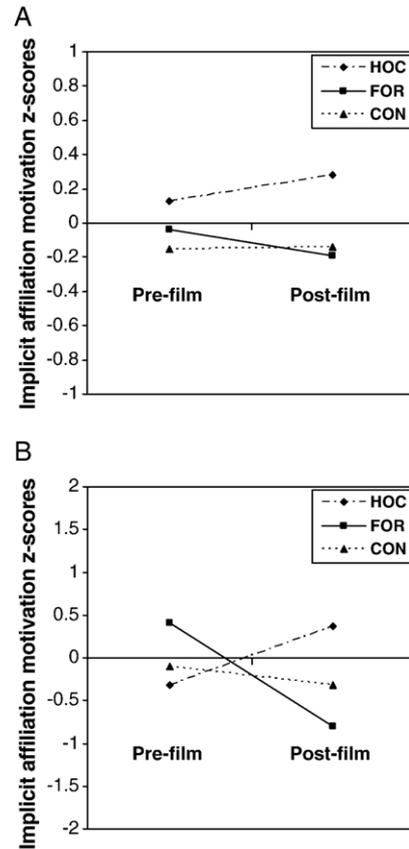


Fig. 4. Effects of condition on (A) implicit affiliation motivation scored using the Winter (1994) system (nAff), and (B) sum of positive and negative implicit affiliation motivation scored using the Heyns et al. (1958) system (total-Aff). Occurrences of motive imagery were converted to z-scores prior to analysis/graphical presentation.

However, when analysis was restricted to the HOC and FOR conditions (CON condition excluded from analysis), the ANCOVA revealed a trend, $F(1,59)=2.73$, $P=0.103$, reflecting the fact that nAff tended to decrease post-film in the FOR condition and increase in the HOC condition (Fig. 4a). This trend was still present when participant sex was added as a covariate. Implicit achievement and power motivation did not show any effects by condition.

Affiliation motivation scores as obtained via the Heyns et al. (1958) scoring system were aggregated into “positive” and “negative” affiliation sub-scores, according to whether imagery reflected approach (e.g., striving for closeness or to be with others) or avoidance (e.g., striving not to lose closeness or be alone) affiliation motivation. Against expectations, however, both sub-scores were increased post-film in the HOC condition and decreased in the FOR condition. This is seen in a significant time \times condition effect in a repeated-measures ANOVA by condition on negative affiliation (neg-Aff), $F(2,87)=4.16$, $P=0.019$. This effect was not moderated by sex. Though the time \times condition effect failed to reach significance in a parallel analysis on positive affiliation (pos-Aff) motivation, this variable similarly appeared to decrease in FOR and increase in HOC. Pos-Aff and neg-Aff were also found to positively correlate both pre- ($R=0.57$, $P<0.001$) and post-film ($R=0.50$, $P<0.001$). Therefore, these scores were collapsed into a total affiliation score for the Heyns system (total-Aff). Repeated-measures ANOVA by condition on total-Aff revealed a significant time \times condition effect, $F(2,87)=3.29$, $P=0.042$, reflecting the fact that total-Aff decreased in the FOR and increased in the HOC conditions (Fig. 4b).

Excluding the 17 participants who had seen the films previously left all effects of condition on implicit motives unchanged.

The total-Aff scores obtained from the Heyns et al. (1958) system were strongly positively correlated with nAff scores obtained from the Winter (1994) scoring system both pre-film ($R=0.655$, $P<0.001$) and post-film ($R=0.556$, $P<0.001$).

Hormone—implicit motive relationships

Again, the two women and one man who were outliers in terms of change in PROG were excluded from analyses on PROG (leaving 37 women and 48 men). Analyses were performed on motive z-scores and log-transformed hormone levels.

Correlation analysis revealed strong relationships in women between baseline Winter system affiliation motivation (nAff) and CORT (for T1, T2, T3, $R=0.36$, 0.41 , 0.33 ; $P=0.02$, 0.01 , 0.04), as well as between post-film nAff and PROG (for T1, T2, T3, $R=0.33$, 0.44 , 0.31 ; $P=0.049$, 0.007 , 0.064). No correlations were found between hormones and achievement or power motivation, or between hormones and any motive scores in men.

Bi-partial correlation analysis was used to estimate the relationship between change in PROG or CORT and change in affiliation motivation from before to after the film. These analyses revealed a positive relationship between change in

PROG from T1 to T2 and change in nAff, $R=0.229$, $P=0.035$. (The same analysis performed with total-Aff from the Heyns et al. (1958) system revealed a trend, $R=0.195$, $P=0.074$.) Excluding women on hormonal contraceptives left the results unchanged. Change in CORT bore no relationship to change in affiliation motivation as scored by either system. Neither did changes in PROG or CORT relate to changes in implicit power or achievement motivation.

In the FOR condition, baseline affiliation motivation predicted post-film increase in PROG. This is shown by regression analysis on post-film PROG (log-transformed PROG at T2 and T3 averaged together) by condition and baseline pos-Aff z-scores, controlling for PROG at T1, revealing a significant interaction of condition and baseline pos-Aff on post-film PROG, $F(2,80)=3.93$, $P=0.024$. (The same analysis performed with nAff also revealed a trend, $F(2,80)=2.84$, $P=0.064$.) Sex did not moderate this effect, and exclusion of women on hormonal contraceptives or participants who had seen the film left the effect unchanged. Follow-up correlation analysis was conducted on the averaged residualized PROG-2 and PROG-3 (i.e., with PROG-1 was regressed out). In the FOR condition only, baseline pos-Aff correlated positively with averaged residualized post-film PROG ($R=0.450$, $P=0.014$; Fig. 5). In contrast, these effects were not present when using post-film affiliation motivation, power or achievement motivation as the independent variable. Furthermore, contrary to hypothesis, there was no baseline pos-Aff \times condition interaction on CORT, nor did baseline pos-Aff correlate with residualized CORT in any condition.

Hormone—self-reported mood relationships

No relationships between hormones and self-reported mood were expected as implicit motives tend to predict changes in behavior and biological variables better than self-reported measures (see Introduction). For completeness, however,

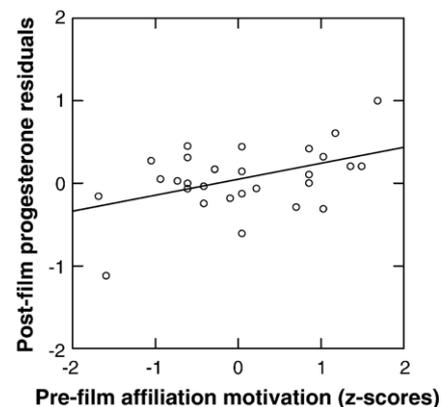


Fig. 5. In the FOR condition (*A.I.* film segment), relationship between baseline (pre-film) positive (approach-related) implicit affiliation motivation as scored using the Heyns et al. (1958) system (*x*-axis), and post-film residualized progesterone (*y*-axis). Affiliation motivation data are shown as z-scores. *y*-axis represents the residuals of averaged log-transformed progesterone at both post-film time points T2 and T3 after log-transformed progesterone at T1 (baseline) has been regressed out.

repeated-measures ANCOVA controlling for a given self-reported mood score at T1 was used to examine if baseline (T1) PROG or CORT levels affected change in self-reported hedonic tone, tense activation, fear of rejection, or sexual motivation. No relationships were found. Similarly, baseline self-reported mood did not affect change in hormone levels.

Bi-partial correlation analysis was used to examine if PROG or CORT co-varied along with any of the above four mood variables. No consistent relationships were found between change in either hormone and change in any mood measure.

Discussion

In this study, as in Schultheiss et al. (2004), we demonstrated effective manipulation of both explicit (self-reported) mood and implicit motivation using film segments. In the fear of rejection (FOR) condition (*A.I.* film segment), participants reported increased tense activation and fear of rejection feelings and reduced hedonic tone post-film. In the hope of closeness (HOC) condition (*Bridges* segment), participants reported increased feelings related to sexual motivation. Furthermore, different measures of implicit affiliation motivation revealed that this motive increased in the HOC and decreased in the FOR condition. The latter finding was unexpected as the negative subscale of the Heyns et al. (1958) system was expected to capture implicit fear of rejection motivation presumably aroused by the *A.I.* film segment. However, the strong positive correlation between this negative subscale and the positive subscale, as well as with affiliation motivation as scored by the Winter (1994) system, indicates that the negative subscale may not effectively capture the avoidance side of the affiliation motive (to our knowledge, this is the first attempt to validate these subscales in a positive- and negative-affiliation arousal paradigm). Nonetheless, the film segments had effects on implicit affiliation motivation, with the HOC condition replicating effects on this motive seen in Schultheiss et al. (2004).

The manipulation also exerted effects on hormone levels. In the FOR condition, participants' CORT stayed level post-film, going against the trend of declining CORT levels in the other conditions (an expected trend, given diurnal variation in CORT). PROG followed a similar pattern; as noted, CORT and PROG positively co-vary together (Wirth et al., *in press*), and ACTH leads to release of PROG as well as CORT (Genazzani et al., 1998). Together with effects on self-reported mood, we can conclude that, as predicted, viewing the *A.I.* film segment was stressful for participants. This finding is in line with the fact that maternal separation is stressful for young humans as well as non-human animals (Christensson et al., 1995); what is interesting is that this stress response could be experienced vicariously in adults watching a fictional film depicting maternal separation. The effect of this film segment on CORT and on mood reinforces both the ideas that vicarious experience can alter hormone responses (Bernhardt et al., 1998) and that films are excellent tools to engage motivational and emotional systems in human subjects without the complications of face-to-face interaction (Schultheiss et al., 2004). Besides

suggesting a stress response, the effect of the FOR condition on CORT is interesting in light of evidence that CORT plays an important role in affiliation and attachment; for example, postpartum CORT levels predict attraction to infant-related cues in new mothers (Fleming et al., 1997b).

PROG did not increase post-film in the HOC condition, failing to replicate our prior finding. One potential explanation for this discrepancy is that, in Schultheiss et al. (2004), the PROG effect emerged when the HOC condition (*Bridges* segment) was compared to the control condition and a condition in which participants viewed a film clip filled with themes of power and dominance. The power condition led to a robust decrease in affiliation motivation and an increase in power motivation, in sharp contrast to the HOC condition. Another possibility, given the relationships between CORT and PROG, is that, for idiosyncratic reasons, participants displayed a stress response in the HOC condition in the 2004 study, and this drove the increase in PROG levels. However, we analyzed the saliva samples from the 2004 study for CORT *post hoc* and found no effects of condition (unpublished data). Furthermore, self-reported tense activation was highest in the power condition in that study (unpublished data). Self-reported sexual motivation data were not collected in the prior study, so it is possible that participants in the present study had a different response regarding this variable to the *Bridges* segment; this in turn could impact hormonal responses.

On the other hand, relationships did exist between PROG and affiliation motivation in the present study. First, in women, affiliation motivation displayed positive correlations with both PROG and CORT; notably, PROG related to affiliation post-manipulation only. Second, in all participants regardless of condition, affiliation motivation and PROG co-varied together from before to after the film (i.e., an increase in affiliation was accompanied by an increase in PROG, and similarly for decreases). This relationship was not present for implicit power or achievement motivation, or for CORT, despite the close relationships between CORT and PROG in general. Third, baseline affiliation motivation positively predicted change in PROG following the fear of rejection-themed film segment. Again, this relationship was not present in other implicit motives, nor did baseline affiliation motivation affect CORT response to the *A.I.* film segment, contrary to our hypothesis. Together with our past research (Schultheiss et al., 2003, 2004), these effects point to a nuanced, bi-directional relationship between PROG and affiliation motivation. This relationship may be particularly important in conditions of affiliation deprivation (i.e., separation/rejection). Interestingly, elevated plasma oxytocin levels have been linked to relationship distress in humans (Taylor et al., 2006; Turner et al., 1999), paralleling our findings of increased PROG and stronger affiliation–PROG relationships in the fear of rejection condition. These effects could be explained by increases in hormones that promote affiliation-seeking in response to withdrawal of affiliation.

As stated in the Introduction section, PROG is released during stress (e.g., Barbaccia et al., 1996b) and has anxiolytic effects via its metabolites (Paul and Purdy, 1992; Soderpalm et al., 2004). We have demonstrated that PROG has specific

relationships to affiliation. In these respects, PROG appears to follow a similar pattern to the hormone oxytocin, which is also released during stress (Gimpl and Fahrenholz, 2001; Hashimoto et al., 1989; Jorgensen et al., 2002; Onaka, 2004; Taylor et al., 2000), exerts an anti-stress response (Heinrichs et al., 2003; Uvnas-Moberg, 1998a; Uvnas-Moberg et al., 1994), and has been linked to affiliation and attachment (Carter et al., 1992; Insel, 1997). Taylor and colleagues have suggested that oxytocin's release during stress promotes a "tend-and-befriend" rather than a "fight-or-flight" response (Taylor et al., 2000). We believe that PROG may be acting in a similar manner. In other words, along with a more immediate effect to shut down the stress response, PROG release during stress may encourage a social animal to seek friendly contact with conspecifics in order to promote longer-term coping. Further examination of behavioral effects of PROG release following stress, as well as examination of effects on behavior and affiliation motivation of experimental manipulation of PROG, is necessary to confirm this hypothesis. Since PROG varies greatly across the menstrual cycle and reproductive status in women, further study is also warranted of sex differences in the impact of PROG functioning on stress, affiliation motivation, and behavior.

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