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Salience of emotional displays of danger and contagion in faces is enhanced when progesterone levels are raised

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Abstract

Findings from previous studies of hormone-mediated behavior in women suggest that raised progesterone level increases the probability of behaviors that will reduce the likelihood of disruption to fetal development during pregnancy (e.g. increased avoidance of sources of contagion). Here, we tested women's ($N = 52$) sensitivity to potential cues to nearby sources of contagion (disgusted facial expressions with averted gaze) and nearby physical threat (fearful facial expressions with averted gaze) at two points in the menstrual cycle differing in progesterone level. Women demonstrated a greater tendency to perceive fearful and disgusted expressions with averted gaze as more intense than those with direct gaze when their progesterone level was relatively high. By contrast, change in progesterone level was not associated with any change in perceptions of happy expressions with direct and averted gaze, indicating that our findings for disgusted and fearful expressions were not due to a general response bias. Collectively, our findings suggest women are more sensitive to facial cues signalling nearby contagion and physical threat when raised progesterone level prepares the body for pregnancy.

Keywords: Emotion; Face perception; Salivary progesterone; Menstrual cycle

Introduction

Previous studies have shown that changes in women's progesterone level are associated with changes in the strength of their aversions to possible sources of contagion (Jones et al., 2005a,b; Fessler, 2002; Flaxman and Sherman, 2000). Raised progesterone levels during the luteal phase of the menstrual cycle, pregnancy and following oral contraceptive use are associated with increased aversions to facial cues of illness such as pallor (Jones et al., 2005a,b). These changes in the strength of aversions to facial cues of illness occur independently of changes in preferences for masculine faces (Jones et al., 2005b) and complement findings for increased aversions to possible sources of contagion in food preferences during pregnancy (Fessler, 2002; Flaxman and Sherman, 2000; see also Fessler et al., 2005; Pepper and Roberts, in press). As pregnancy is characterized by raised progesterone level (Gilbert, 2000), these progesterone-mediated changes in contagion avoidance are thought to reflect a mechanism that reduces the likelihood of pregnant women contracting illnesses that could disrupt fetal development (Jones et al., 2005a,b; Fessler, 2002; Flaxman and Sherman, 2000; Fessler et al., 2005).

While the studies of hormone-mediated face perception described above investigated changes in preferences for physical cues in faces that change only with a slow time course of days or years (e.g. apparent health), faces also contain changeable social signals that indicate the direction of others' attention (e.g. gaze direction) and their emotional state (e.g. expressions; Haxby et al., 2000). In a recent study of the effects of menstrual cycle phase on emotion perception, Pearson and Lewis (2005) found that recognition accuracy for fearful facial expressions (but not other expressions) was better during the

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preovulatory phase of the menstrual cycle than during menstruation. While all of the face images in Pearson and Lewis's (2005) study were shown with direct gaze, other recent studies have demonstrated that the meaning of facial expressions can change depending on the direction of gaze displayed (Adams and Kleck, 2005, 2003; Adams et al., 2003; Frigerio et al., 2002; Jones et al., 2006).

The emotions fear and disgust may have evolved, at least in part, because of the importance of avoiding threats to physical safety and sources of contagion, respectively (e.g. Calder et al., 2001; Sprengelmeyer et al., 1997; Curtis et al., 2004). Several recent studies of fear perception have demonstrated that people tend to perceive fearful faces with averted gaze as more afraid than fearful faces with direct gaze, suggesting people are more sensitive to signals of nearby threat than signals that someone is afraid of them (Adams and Kleck, 2005, 2003). Similarly, one could argue that disgust expressions with averted gaze indicate the presence of a nearby source of contagion. Disgust expressions with direct gaze may indicate disgust with the perceiver, which does not place the perceiver in as much danger of contagion. Hence, similar to fear, disgust with averted gaze could be found more 'disgusting' or dangerous to individuals who wish to avoid contagion than disgust expressions with direct gaze. Thus, the extent to which people perceive fearful or disgusted faces as looking more fearful or disgusted with averted gaze than direct gaze may indicate their sensitivity to facial cues signalling the presence of nearby physical threat and nearby sources of contagion, respectively. While recognition accuracy for fearful expressions has been shown to vary during the menstrual cycle (Pearson and Lewis, 2005), we know of no studies that have tested if changes in women's hormone levels are associated with changes in the effect of gaze direction on the perceived emotional intensity of facial expressions.

In light of the above, we tested if the extent to which fearful and disgusted faces were perceived as more afraid and disgusted with averted gaze than with direct gaze was greater when progesterone level was high than when progesterone level was relatively low. In addition, we compared the effects of change in progesterone level on perceptions of fearful and disgusted expressions with direct and averted gaze with the effect of change in progesterone level on perceptions of happy faces in which gaze direction had also been manipulated. We undertook this latter comparison in an effort to establish if raised progesterone level is associated with increased sensitivity to social signals of nearby threat and contagion or if raised progesterone level increases the perceived intensity of any emotional expression with averted gaze. While we hypothesized that raised progesterone level would be associated with an increased tendency to perceive fearful and disgusted expressions with averted gaze as more intense than those with direct gaze, we did not predict an association between change in progesterone level and the effect of gaze direction on perceptions of happy faces.

Methods

Stimuli

First we manufactured male and female composite (i.e. averaged) faces with fearful, disgusted and happy expressions. Using methods for manufacturing composite faces that have been used in many previous studies of face perception (e.g. Perrett et al., 2002; DeBruine et al., 2005; Jones et al., 2005a,b), the shape, color and texture information from full face photographs of 6 males and 6 females randomly selected from the Karolinska Directed Emotional Faces (KDEF) image set (Lundqvist and Litton, 1998) were averaged to produce male and female composites (see Tiddeman et al., 2001 for technical details of the computer graphic methods that were used to manufacture composite faces). This was done separately for each expression (fearful, disgusted and happy) using the same 12 identities. All images used to manufacture these expression composites had direct gaze.

Following Jones et al. (2006), versions of each of the expression composites with averted gaze were manufactured by transforming the position of the irises by 100% of the differences in shape between an average of 20 other faces with direct gaze and an average of the same faces with gaze averted to the left. This method for manipulating gaze direction in face images ensures that the magnitude of the change in gaze direction is identical for each expression and that versions of the same expression with direct and averted gaze differ only in the position of the irises (Jones et al., 2006). Examples of face images used in the study are shown in Fig. 1. Six pairs of composite faces were manufactured in total (each pair consisting of 2 face images differing only in gaze direction). These six pairs of composites represented the following conditions: male happy, female happy, male fearful, female fearful, male disgust, female disgust.

Participants

Fifty-two female participants (age: M = 19.30 years, SD = 1.63; 82.7% White European), reporting no hormonal contraceptive use or pregnancy and with normal or corrected-to-normal vision, took part in the study. All participants were undergraduate students at the University of Aberdeen and were participating in the study in return for course credit. All participants reported normal menstrual cycles.

Procedure

Participants were tested on four occasions, at weekly intervals, and at approximately the same time of day. The interval between test sessions for each participant was the same (i.e. 1 week). In each test session, participants viewed pairs of composite faces that were matched in terms of identity, sex and expression and differed only in gaze direction. For pairs of composites with fearful expressions, participants were instructed to indicate which face looked more afraid. For pairs of composites with disgusted expressions, participants were instructed to indicate which face looked more disgusted. For pairs of composites with happy expressions, participants were instructed to indicate which face looked happier. In addition to indicating which face looked more afraid, disgusted or happy, participants also indicated the magnitude of this perceived difference by choosing from the options 'much more', 'more', 'somewhat more' or 'slightly more'. Trial order and the side of the screen on which any particular image was shown were fully randomized. This paradigm has been used to assess face preferences in previous studies of the effects of menstrual cycle phase on attraction (e.g. Jones et al., 2005a,b).

Participants deposited between 3 and 5 mL of saliva by spitting directly into plastic pharmaceutical vials upon arrival to the laboratory each testing session. The vials were sealed and frozen at −20°C until analysis. Participants also reported the date of the onset of their last period of menstrual bleeding and the expected date for the onset of their next period of menstrual bleeding.

Hormone assay data

Progesterone data were assayed by the Biological Sciences Laboratory at Queen Margaret University College (Edinburgh, UK), using an enzyme linked immunosorbent assay (ELISA) kit purchased from Salimetrics (PA, USA). The assay procedure was based on the competitive binding technique, whereby progesterone in the standards and unknowns competes with progesterone linked to horseradish peroxidase for the antibody binding site. Briefly, 50 μL of standards, controls and unknowns in duplicates were pipetted into a micro-titre plate that was previously coated with rabbit anti-progesterone antibody followed by 150 μL of assay diluent containing progesterone–horseradish peroxidase conjugate. After incubation at room temperature for 1 h and 4 successive washes, 200 μL of the

Fig. 1. Examples of face stimuli used in the study. The top row shows composites of female faces with fearful, disgusted and happy expressions with direct gaze. The bottom row shows the corresponding composites with averted gaze.

substrate solution (tetra-methyl benzidine) was added. The plate was then incubated at room temperature for 30 min, 50 μL stop solution added and the colour intensity read in the ELISA plate reader (Dynex MRX) at 450 nm.

The sensitivity of the assay, defined as lower limit of detection that can be distinguished from the zero standard, was 5 pg/mL (interpolating the mean absorbance of 20 zero standards plus 2 SDs). Cross reactivity with related compounds was minimal. Mean inter- and intra-assay coefficient of variation, obtained over a concentration range of 28–884 pg/mL (12 assay runs), were 7.6% and 6.2%, respectively. Recovery of the assay, as determined by spiking saliva samples with known quantities of progesterone, ranged from 93.0% to 108.8%. Linearity of the assay after diluting saliva samples up to 16 times ranged from 90.8% to 103.8% recovery.

Saliva samples were also analyzed for cortisol so that we could establish whether or not test sessions that were characterized by high and low levels of salivary progesterone also differed in salivary cortisol. Salivary cortisol levels were determined by an in-house ELISA method by the Biological Sciences Laboratory at Queen Margaret University College (Edinburgh, UK). Briefly, saliva samples were first extracted with diethylether and the re-constituted samples were then pipetted into the 96-well pre-coated ELISA plate. Increasing doses of cortisol standards and quality controls were included and the plate incubated with anti-cortisol antibody for 2 h at room temperature. Following the addition of the enzyme tracer and further incubation for 1 h, the plate was developed and read using MRX ELISA reader. Cross-reactivity of the assay with most interfering steroids was minimal except for deoxy-cortisol (1%) and cortisone (0.75%). Assay sensitivity determined by the signal generated from 2 SD plus the signal at zero point was 0.05 ng/mL. Intra- and inter-assay imprecision data were 4.6% and 6.8%, respectively. Recovery results for cortisol levels of 2.4–40.8 ng/mL were 94.8–106.7%.

Initial processing of data

Responses on the face perception test were coded using a 0–7 scale with the following endpoints: 0 = composite with averted gaze looked *much more* fearful, disgusted, or happy and 7 = composite with direct gaze looked *much more* fearful, disgusted, or happy.

For each participant, the test sessions with the highest and lowest progesterone levels were identified from the hormone analysis of the saliva samples. 'High progesterone' (M = 176.33 pg/mL, SE = 17.67) and 'low progesterone' (M = 47.35 pg/mL, SE = 4.93) test sessions differed significantly in salivary progesterone level [$t(51) = 8.31$, $p < .001$], but not in salivary cortisol level [$t(51) = -0.50$, $p = .62$]. The mean increase in progesterone level from the test session with the lowest progesterone level to the test session with the highest progesterone level was 128.98 pg/mL (SE = 15.51). Allocation of test sessions to 'high progesterone' and 'low progesterone' groups did not confound order of test sessions and progesterone level ($p = .89$).

For each participant, the number of days until the onset of menses for the high and low progesterone test sessions were calculated from the expected or reported date for the onset of their next period of menstrual bleeding. Diary data was incomplete for three participants. For the 49 participants for whom we had complete diary data, the number of days until the next period of menstrual bleeding was significantly different for high and low progesterone test sessions [$t(49) = 2.844$, $p = .007$]. The low progesterone test sessions tended to correspond to the late follicular phase of the menstrual cycle (mean number of days until onset of menses = 17.45 days, SE = 1.35) and the high progesterone test sessions tended to correspond to the luteal phase of the menstrual cycle (mean number of days until onset of menses = 11.92 days, SE = 1.30).

Results

Responses from each woman's highest and lowest progesterone test sessions were compared using a repeated measures ANOVA [within-subjects factors: progesterone level (high, low), sex of face judged (male, female), and expression (fear,

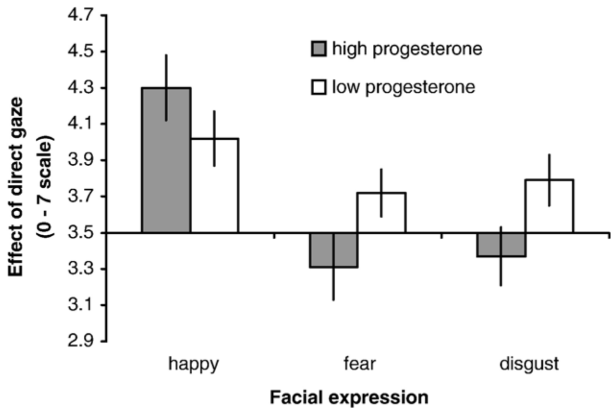

Fig. 2. The significant interaction between progesterone level and the effect of gaze direction on perceptions of the intensity of fearful, disgusted and happy facial expressions. Bars show means and SE of the means. Scores on the *Y*-axis that are greater than 3.5 indicate a tendency to perceive expressions with direct gaze as more intense while scores that are less than 3.5 indicate a tendency to perceive expressions with averted gaze as more intense. Chance (i.e. no effect of gaze direction on perceived intensity of emotion) is 3.5.

disgust, happy)]. This analysis revealed a significant main effect of type of expression [$F(2,51)=12.26$, $p<.001$], whereby direct gaze increased the perceived intensity of happy expressions more than for disgusted or fearful expressions. As we predicted, however, this main effect of expression was qualified by a significant interaction with progesterone level [$F(2,51)=3.14$, $p=.048$; Fig. 2]. There were no other significant effects (all $F<2.18$, all $p>.146$).

A planned Helmert contrast (Hays, 1994, pp. 448–449) demonstrated that progesterone level had a significantly different effect on our control condition (i.e. perceptions of happy expressions) than on perceptions of fearful and disgusted expressions [$F(1,51)=5.740$, $p=.020$]. A repeated-measures ANOVA was therefore carried out to establish the effect of progesterone level on perceptions of fearful and disgusted expressions [within-subjects factors: progesterone level (high, low), and expression (fear, disgust)]. This latter analysis revealed a significant main effect of progesterone level [$F(1,51)=8.928$, $p=.004$], no significant effect of expression [$F(1,51)=0.194$, $p=.661$] and no significant interaction between expression and progesterone level [$F(1,51)=0.001$, $p=.975$]. Paired-samples *t*-tests showed that the effect of progesterone level on perceptions of fearful expressions was significant [$t(51)=-2.175$, $p=.034$] and that the effect of progesterone level on perceptions of disgusted expressions approached significance [$t(51)=-1.895$, $p=.064$]. A paired-samples *t*-test indicated there was no significant effect of progesterone level on perceptions of happy expressions [$t(51)=1.09$, $p=.278$].

Discussion

Consistent with our hypotheses, women demonstrated a greater tendency to perceive fearful and disgusted facial expressions with averted gaze as more intense than those with direct gaze when their progesterone level was relatively high. Also supporting our hypotheses, progesterone level did not have a significant effect on perceptions of happy faces. This latter finding demonstrates that the observed association between raised progesterone level and change in perceptions of disgusted and fearful expressions is not simply a by-product of a possible general response bias whereby raised progesterone level increased the perceived intensity of all emotional expressions with averted gaze.

The extent to which people perceive fearful or disgusted facial expressions as more fearful or disgusted with averted than direct gaze is an indication of their sensitivity to facial cues signalling the presence of nearby physical threat and nearby sources of contagion (e.g. Adams and Kleck, 2003, 2005). Thus, our findings suggest that raised progesterone level is associated with increased sensitivity to facial cues to the presence of nearby physical threat and nearby sources of contagion. Raised progesterone level is a characteristic of pregnancy (Gilbert, 2000) and both physical trauma and illness pose significant risks to fetal development (Goldenberg and Thompson, 2003; Shah et al., 1998). Therefore, increased sensitivity to cues signalling nearby threat or sources of contagion when progesterone level is raised may reflect a mechanism that reduces the likelihood of physical trauma or illness disrupting fetal development. In this way, our findings complement those from previous studies showing that raised progesterone level is associated with increased contagion avoidance in the domains of face and food preferences and support the proposal that raised progesterone level triggers behaviours that reduce disruptions to fetal development (Jones et al., 2005a,b; Fessler, 2002; Flaxman and Sherman, 2000; Fessler et al., 2005; Pepper and Roberts, in press). While previous studies demonstrating associations between raised progesterone level and behaviors that would help protect the developing fetus have focused on contagion avoidance, our findings for change in perceptions of fearful expressions suggest that raised progesterone level is also associated with increased sensitivity to facial cues of physical danger.

Although our findings demonstrate that change in progesterone level is associated with a change in women's perceptions of fearful and disgusted expressions in which gaze direction had been manipulated, it remains unclear whether these effects reflect an increase in the perceived emotional intensity of one face (e.g. face images with averted gaze) or a decrease in the perceived intensity of the other face (e.g. face images with direct gaze). Similarly, although our findings for change in perceptions of fear and disgust cannot be attributed to possible effects of change in salivary cortisol level (because there was no significant difference in salivary cortisol between high and low progesterone test sessions), these findings may not necessarily reflect a direct effect of change in progesterone level but could be due to changes in other hormones (or ratios of hormones) that are correlated with change in progesterone. These issues remain important topics to be investigated in future studies.

While other studies have demonstrated effects of menstrual cycle phase on face preferences (Penton-Voak et al., 1999; Johnston et al., 2001; Jones et al., 2005a,b; DeBruine et al., 2005) and that raised estrogen level is associated with improved recognition of fearful expressions (but not other emotional expressions; Pearson and Lewis, 2005), here we show that the manner in which gaze direction alters expression perception is associated with changes in progesterone level. Thus, our findings demonstrate that changes in hormone levels can affect the integration of different facial cues in person perception and add to a growing literature suggesting that raised progesterone level increases the probability of behaviors, attitudes and perceptions that enhance the mother's self protection and reduce risk of harm to a developing fetus during pregnancy.

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References

Adams Jr., R.B., Kleck, R.E., 2003. Perceived gaze direction and the processing of facial displays of emotion. Psychol. Sci. 141, 644–647.

Adams Jr., R.B., Kleck, R.E., 2005. Effects of direct and averted gaze on the perception of facially communicated emotion. Emotion 5, 3–11.

Adams Jr., R.B., Gordon, H.L., Baird, A.A., Ambady, N., Kleck, R.E., 2003. Effects of gaze on amygdala sensitivity to anger and fear faces. Science 300, 1536–1537.

Calder, A.J., Lawrence, A.D., Young, A.W., 2001. Neuropsychology of fear and loathing. Nat. Rev., Neurosci. 2, 352–363.

Curtis, V., Aunger, R., Rabie, T., 2004. Evidence that disgust evolved to protect from risk of disease. Proc. R. Soc. London, B 271, S131–S133.

DeBruine, L.M., Jones, B.C., Perrett, D.I., 2005. Women's attractiveness judgments of self-resembling faces change across the menstrual cycle. Horm. Behav. 47, 379–383.

Fessler, D.M., 2002. Reproductive immunosuppression and diet. Curr. Anthropol. 43, 19–61.

Fessler, D.M.T., Eng, S.J., Navarrete, C.D., 2005. Elevated disgust sensitivity in the first trimester of pregnancy: evidence supporting the compensatory prophylaxis hypothesis. Evol. Hum. Behav. 26, 344–351.

Flaxman, S.M., Sherman, P.W., 2000. Morning sickness: a mechanism for protecting mother and embryo. Q. Rev. Biol. 75, 113–148.

Frigerio, E., Burt, D.M., Montagne, B., Murray, L.K., Perrett, D.I., 2002. Facial affect perception in alcoholics. Psychiatry Res. 113, 161–171.

Gilbert, S.F., 2000. Developmental Biology. Sinauer, USA.

Goldenberg, R.L., Thompson, C., 2003. The infectious origins of stillbirth. Am. J. Obstet. Gynecol. 189, 861–873.

Haxby, J.V., Hoffman, E.A., Gobbini, M.I., 2000. The distributed human neural system for face perception. Trends Cogn. Sci. 6, 223–233.

Hays, W.L., 1994. Statistics, 5th edition. Harcourt Brace, Fort Worth, TX, USA.

Johnston, V.S., Hagel, R., Franklin, M., Fink, B., Grammer, K., 2001. Male facial attractiveness: evidence for a hormone-mediated adaptive design. Evol. Hum. Behav. 22, 251–267.

Jones, B.C., Perrett, D.I., Little, A.C., Boothroyd, L., Cornwell, R.E., Feinberg, D.R., Tiddeman, B.P., Whiten, S., Pitman, R.M., Hillier, S.G., Burt, D.M., Stirrat, M.R., Law Smith, M.J., Moore, F.R., 2005a. Menstrual cycle, pregnancy and oral contraceptive use alter attraction to apparent health in faces. Proc. R. Soc. London, B 272, 347–354.

Jones, B.C., Little, A.C., Boothroyd, L., DeBruine, L.M., Feinberg, D.R., Law Smith, M.J., Cornwell, R.E., Moore, F.R., Perrett, D.I., 2005b. Commitment to relationships and preferences for femininity and apparent health in faces are strongest on days of the menstrual cycle when progesterone level is high. Horm. Behav. 48, 283–290.

Jones, B.C., DeBruine, L.M., Little, A.C., Conway, C., Feinberg, D.R., 2006. Integrating gaze direction and expression in preferences for attractive faces. Psychol. Sci. 17, 588–591.

Lundqvist, D., Litton, J.-E., 1998. "The averaged Karolinska directed emotional faces—AKDEF". *AKDEF CDROM* Stockholm: Psychology section, Karolinska Institute.

Pearson, R., Lewis, M.B., 2005. Fear recognition across the menstrual cycle. Horm. Behav. 47, 267–271.

Penton-Voak, I.S., Perrett, D.I., Castles, D.L., Kobayashi, T., Burt, D.M., Murray, L.K., Minamisawa, R., 1999. Menstrual cycle alters face preference. Nature 399, 741–742.

Pepper, G.V., Roberts, S.C., in press. Rates of nausea and vomiting in pregnancy and dietary characteristics across populations. Proc. R. Soc. London, B 273, 2675–2679 [Electronic publication ahead of print; doi:10.1098/rspb.2006.3633).

Perrett, D.I., Penton-Voak, I.S., Little, A.C, Tiddeman, B.P., Burt, D.M, Schmidt, N., Oxley, R., Barrett, L., 2002. Facial attractiveness judgements reflect learning of parental age characteristics. Proc. R. Soc. London, B 269, 873–880.

Shah, K.H., Simons, R.K., Holbrook, T., Fortlage, D., Winchell, R.J., Hoyt, D.B., 1998. Trauma in pregnancy: maternal and fetal outcomes. J. Trauma Inj. Infect. Crit. Care 45, 83–86.

Sprengelmeyer, R., Young, A.W., Pundt, I., Sprengelmeyer, A., Calder, A.J., Berrios, G., Winkel, R., Vollmoeller, W., Kuhn, W., Sartory, G., Przuntek, H., 1997. Disgust implicated in obsessive–compulsive disorder. Proc. R. Soc. London, B 264, 1767–1773.

Tiddeman, B.P., Perrett, D.I., Burt, D.M., 2001. Prototyping and transforming facial textures for perception research. IEEE Comput. Graph. Appl., Res. 21, 42–50.