Emotional perception and neuroendocrine changes

MAURIZIO CODISPOTI, a GILBERTO GERRA, b ORNELLA MONTEBAROCCI, a AMIR ZAIMOVIC, b MARIA AUGUSTA RAGGI, c AND BRUNO BALDARO a

aDepartment of Psychology, University of Bologna, Bologna, Italy
bCentro Studi Farmacotossicodipendenze, Azienda Unità Sanitaria Locale, Parma, Italy
cDepartment of Pharmaceutical Sciences, University of Bologna, Bologna, Italy

Abstract

The present study was designed to investigate the neuroendocrine modifications during affective states. In particular, we investigate if the pleasantness of the stimuli has a different effect on neuroendocrine responses. To address this issue, we compared the effects of pleasant, neutral, and unpleasant pictures on catecholamine, adrenocorticotrophic hormone (ACTH), cortisol, and prolactin plasma levels. Ten male participants were submitted to three experimental sessions, each on one of the three experimental days, a week apart in a counterbalanced order. Although in the subjective arousal rating, pleasant (erotic pictures) and unpleasant stimuli (pictures of mutilated bodies) receive the same high score, a different neuroendocrine pattern was obtained: unpleasant stimuli elicited a decrease in prolactin concentration and increases in noradrenaline, cortisol, and ACTH levels, whereas pleasant slide set viewing induced an increase in prolactin levels. The results suggest that the neuroendocrine system responds selectively to affective motivationally relevant pictures.

Descriptors: Emotions, Prolactin, Arousal, Hormones, Natural scenes

The present study was designed to investigate how processing of emotional visual stimuli affects the neuroendocrine response.

Several theorists have advocated a biphasic approach to emotion, which posits that emotion fundamentally stems from varying activation in centrally organized appetitive and defensive motivational systems, which have evolved to mediate the wide range of adaptive behaviors necessary for an organism struggling to survive in the physical world (Konorski, 1967; Lang, Bradley, & Cuthbert, 1990; Wundt, 1896).

According to this conception, emotional behavior can be defined by a first dimension of affective valence, which controls the direction of emotional engagement (approach or withdrawal), and a second dimension of arousal, which dictates the intensity or amount of activity involved in the behavior.

Recently, Lang and coworkers collected a set of pictures depicting a wide variety of objects, events, and situations into the International Affective Pictures System (IAPS; Lang, Bradley, & Cuthbert, 1995) with the aim of providing a set of standardized materials for use in studies of emotion. When subjects are asked to judge the pleasure and arousal of these pictures, an affective space results, in which proceeding vertically in each direction from the center of the space (where neutral events cluster) stimuli are rated as progressively more pleasant or more unpleasant, and in which ratings of arousal tend to increase as ratings of hedonic valence change in either direction.

Previous studies (Bradley, 2000) have demonstrated that physiological and behavioral responses to pictures covary either with system engagement (valence) or with motive intensity (arousal). In addition, a recent study has shown, consistent with the motivational hypothesis, that reports of the strongest emotional arousal, largest skin conductance responses, and greatest modulation of the startle reflex occurred when participants viewed pictures depicting threats, mutilated bodies, and erotica (Bradley, Codispoti, Cuthbert, & Lang, 2001).

These affective response patterns were obtained when pictures were presented in an intermixed series of brief (e.g., 6 s) presentation with relatively long (e.g., 10–20 s) interpicture intervals. Clear phasic emotional reactions to individual stimuli are reliably evoked. Earlier, Bradley, Cuthbert, and Lang (1996) had shown that presentation of a continuous series (5 min long) of affective pictures of the same valence (pleasant, neutral, or unpleasant) produced similar emotional modifications. Repetitive processing of pictures of the same affective valence continued to elicit emotional reactions during stimuli presentation.

During the past 50 years, many investigators, particularly Levi, Mason, Frankenhaeuser, Levine, Henry, L ovallo, and their colleagues, have established that the neuroendocrine system considerably reflects emotional reactions (Frankenhaeuser, 1971; Henry, 1992; Levi, 1965; Levine, Coe, & Wiener, 1984; L ovallo & Thomas, 2000; Mason, 1968).

Several studies have found an increase in adrenocortico-trophic hormone (ACTH), cortisol, and catecholamine secretion after stress exposure (Brown, Sirota, Niaura, & Engebretson, 1993; Buchanan, al’Absi, & L ovallo, 1999; Gerra et al., 1996;
The effects of pleasant stimuli on neuroendocrine responses were not consistent across investigations. Brown et al. (1993) have found an increase in the cortisol response, whereas others research has shown a decrease in the cortisol response (Berk et al., 1989; Buchanan et al., 1999) or no change (Gerra et al., 1996; Hubert & De Jong-Meyer, 1991) compared to baseline values. Recently, Kruger and coworkers (Exton et al., 2001; Kruger et al., 1998) reported a prolactin rise after orgasm, but not after sexual arousal. Kruger and colleagues speculated that prolactin increase after orgasm may regulate a negative feedback sexual-satiation mechanism (Kruger, Haake, Hartmann, Schedlowski, & Exton, 2002).

Although all previous studies indicated that cortisol secretion is increased during unpleasant states, little is known about the effect of pleasant states on other neuroendocrine parameters such as ACTH and catecholamine. Additionally, studies used different stimuli to elicit pleasant and unpleasant emotional states (e.g., speech test, films, Velten Mood Induction Procedure, music) and different methodologies (e.g., urine, saliva, blood), making it difficult to compare the studies. Moreover, different potential confounds are present within each study: Most of the pleasant stimuli used are not as arousing as the unpleasant ones (e.g., several pleasant movies were Disney cartoons).

The present study was designed to investigate the neuroendocrine modifications during affective states, using the same elicitation procedure for high arousing pleasant and unpleasant stimuli. In particular, we investigate if the valence of the stimuli affects the neuroendocrine responses differentially. Given that the release of corticosteroids such as cortisol is the end stage of a complex process that includes pituitary stimulation of the adrenal glands, we investigated ACTH modifications, in addition to mere changes in cortisol level, to evaluate the sensitivity of the two hormones to emotional stimuli.

Prolactin level was measured to examine the modifications of this hormone during two specific pleasant and unpleasant states such as the ones elicited during viewing of erotic pictures and pictures of mutilated bodies. To address these issues, we compared high arousing pleasant and unpleasant stimuli on catecholamine, adrenocorticotropic hormone, cortisol, and prolactin plasma levels. Since previous studies have shown that cortisol plays an important role in regulating prolactin release (Dinan, Scott, Thakore, Naesdal, & Keeling, 2001), the relationship between cortisol and prolactin levels was also examined.

**Method**

**Participants**

Ten healthy male volunteers, recruited from the hospital staff and university college (21–30 years; $M \pm SE$: 24.9±3.9) participated in the study. After completely describing the study to the participants, written informed consent was obtained. All the participants were Caucasians; they were university students (5), hospital workers (3), and nurses (2); none had financial difficulties or other stressful life events at the time of the study, following the criteria of the Holmes and Rahe scale, giving a weight (score) to the single events (Holmes & Rahe, 1967); 6 out of 10 were living with their parents; 3 participants were married; no psychiatric symptoms were found in the history of the participants.

Exclusion criteria included severe chronic liver or renal diseases or other chronic physical disorders, recent weight loss or obesity, endocrinopathies, neurological disorders, immunopathies, and, in particular, HIV disease. All the participants were submitted to a clinical evaluation of internal medicine. Transaminases (GOT, GPT), liver function indexes, creatinine clearance, basal hormonal levels, white cells subpopulations, and blood pressure measurements were found to be in the normal range in all participants. Positive family history for cardiovascular diseases and hypertension was an exclusion criterion. Neurological objective evaluation did not find signs of central nervous system diseases. Most of the subjects included in the study practiced sport activities, but not competitively, and attended university or job activities regularly. Blood phobia was evaluated and excluded in all participants utilizing the Mutilation Questionnaire (Klorman, Weerts, Hastingus, Melamed, & Lang, 1974).

All participants presented a negative history of psychiatric disorders.

Volunteers were controlled by urinary drug screening for 4 weeks before the study and immediately before (2 hr) the experimental days, to exclude substance abuse. They also abstained from smoking or drinking beverages containing caffeine in the 7 days leading up to the biochemical investigations.

Physical training and other stressful events (e.g., school exams) were carefully avoided in the 7 days preceding the tests and during the experimental weeks. Food and drink, except for water, were avoided from midday to 6.00 p.m. of the day of the tests, which started at 4.00 p.m. and lasted until 6.00 p.m. The subjects had a light lunch at 11.00 a.m., including a ham sandwich and orange juice.

**Materials and Procedure**

Fifty-four pictures (18 pleasant, 18 neutral, and 18 unpleasant) were selected from the International Affective Picture System (Center for the Study of Emotion and Attention, 1995). The pictures were arranged in three sets: a pleasant set of pictures (erotic stimuli), a neutral set of pictures (household objects), and an unpleasant set of pictures (mutilated bodies). The average valence and arousal normative ratings expressed as an ordered pair was 7.4 and 6.6 for the pleasant slide set, 3.2 and 2.5 for the neutral slide set, and 2.1 and 6.7 for the unpleasant slide set. Within each set, the 18 pictures were presented three times in three different blocks. Pictures were presented in different orders in the three blocks. Each picture was presented for 30 s followed by a 3-s interpicture interval, so each set lasted for 3 min.

The participants were submitted to three experimental sessions, each on one of the 3 experimental days, a week apart in a counterbalanced order across subjects, according to a Latin square (Group A ($N=3$): day 1 pleasant pictures, day 2 neutral pictures, day 3 unpleasant pictures; Group B ($N=3$): day 1 neutral pictures, day 2 pleasant pictures, day 3 unpleasant pictures; Group C ($N=4$): day 1 unpleasant pictures, day 2 pleasant pictures, day 3 neutral pictures).

In each experimental session, participants sat in a recliner in a small room (room A). The participants filled out a consent form and completed the Mutilation Questionnaire (Klorman et al., 1974).

Then, a catheter was inserted into an antecubital vein kept patent by saline infusion. A first basal blood sample was drawn for hormone assays (baseline 0). After 30 min, a second basal
blood sample was drawn (baseline 1). These two samples were obtained to evaluate emotional reaction to venipuncture and will be reported elsewhere. Previous studies (Gerra et al., 1998; Kirschbaum, Pirke, & Hellhammer, 1993) have shown that the baseline hormonal values, sampled 30 min after the i.v. insertion, were not influenced by the emotional reaction to venipuncture.

Then, the participants were transferred into room B. Participants sat in a recliner. Pictures were presented on a 17-in. radius monitor, situated approximately 1 m from the participant. They were then instructed that a series of pictures would be displayed and that they should look at each picture for the entire time it was exposed on the screen. Following this, each participant was familiarized with the Self-Assessment Manikin procedure (SAM; Lang, 1980), which involves pleasure and arousal ratings. After one of the three slide sets was presented, participants were told to evaluate the slide show, using the SAM. After slide viewing, a third blood sample for hormonal assays was collected. A video camera in room B permitted the research assistant to observe the participants during the experimental session.

Neuroendocrine Measures

Prolactin (PRL) and cortisol (CORT) plasma concentrations were measured utilizing a competitive enzyme immuno-assay by commercial kits (AIA-PACK, Eurogenetics Italy; Turin, Italy). ACTH was measured by commercial kits (Medical System DPC—Immulite; Los Angeles, CA). The determination of adrenaline (EPI) and noradrenaline (NE) was carried out by means of high-performance liquid chromatography with electrochemical detection (Raggi et al., 1999). The mobile phase was composed of methanol (2.5%) and an aqueous solution of citric acid, EDTA, and sodium 1-octanesulfonate at pH 2.9 (97.5%); the stationary phase was a reversed phase C8 column (150 × 4.6 mm i.d., 5 μm). An accurate solid-phase extraction procedure of the catecholamines from human plasma was carried out on Oasis HLB cartridge, after catecholamine complexation with diphenylborate.

The intraassay and interassay coefficients of variation were 3.7% and 7.5% for Cort, 4% and 7% for PRL, 6% and 10% for ACTH, 4% and 10% for NE, and 5% and 12% for EPI. Assay sensitivities were 0.3 nmol/L for CORT and PRL, 15 pg/mL for ACTH, 1 pg/mL for NE and EPI.

Statistical Analysis

Subjective ratings (valence and arousal) were analyzed in a one-way repeated-measure multivariate analysis of variance (MANOVA), using picture content (pleasant, unpleasant, neutral) as the independent variable.

For each experimental session, CORT, ACTH, PRL, EPI, and NE plasma levels were sampled 30 min after i.v. insertion (baseline 1) and after viewing the slides (postpicture values). Neuroendocrine responses were analyzed in a two-way repeated-measures analysis of variance (ANOVA), using picture content (pleasant, unpleasant, neutral) and time (baseline, poststimulus) as the independent variables. To safeguard against violations of sphericity, in all cases where repeated-measure analysis was used, the multivariate statistic Wilks’ Lambda was used to test the significance of the main effects and interactions. A Tukey post hoc test was used to clarify significant interactions.

Results

Subjective Ratings

Ratings of pleasure and arousal reflected the original selection criteria based on these variables. There was a main effect for valence, $F(2,8) = 24.69, p < .001$, and all pairwise comparisons were significant, $p < .05$, as expected. Arousal ratings also differed, $F(2,8) = 18.29, p < .001$, with pleasant and unpleasant slide sets not differing in rated arousal and both rating as more arousing than neutral, $p < .05$.

Neuroendocrine Measures

Baseline levels for all the dependent variables did not differ as a function of picture content.

Figure 1a shows noradrenaline levels before and after pleasant, neutral, and unpleasant slide sets. Analysis of noradrenaline levels showed a significant affective content by time interaction, $F(2,8) = 6.56, p < .05$. A Tukey post hoc test indicated that this interaction occurred due to an increase in noradrenaline levels after the unpleasant slide set compared to the baseline level, $p < .01$, and a significant decrease after the neutral slide set compared to the baseline level, $p < .05$. After slide set viewing, noradrenaline levels were higher for the unpleasant condition compared to the neutral, $p < .01$, and pleasant, $p < .05$, conditions. In addition, after slide set viewing, noradrenaline levels were higher for the pleasant condition compared to the neutral one, $p < .05$.

Analysis of the adrenaline level showed no affective content by time interaction and no main effect of time, $F < 1$ (see Figure 1b).

As shown in Figure 1c, the serum cortisol level increased after unpleasant slide set viewing. Multivariate analysis of variance indicated a significant affective content by time interaction $F(2,8) = 6.35, p < .05$, and the post hoc test (Tukey) showed that this is due to an increase in cortisol levels after the unpleasant slide set in comparison with baseline levels, $p < .05$. After slide set viewing, cortisol levels were higher for the unpleasant condition compared to the neutral, $p < .05$, and pleasant, $p < .01$, conditions.

ACTH data were consistent with cortisol results (see Figure 1d), showing a significant affective content by time interaction, $F(2,8) = 13.90, p < .01$, and an increase in ACTH levels after the unpleasant slide set compared to baseline levels, $p < .001$. After slide set viewing, ACTH levels were higher for the unpleasant condition compared to the neutral, $p < .005$, and pleasant, $p < .005$ conditions.

Differentially, the prolactin level was increased by the pleasant slide set and decreased by the unpleasant one. Multivariate analysis of variance indicated a significant affective Content × Time interaction, $F(2,8) = 13.95, p < .01$, and post hoc test (Tukey) showed that this is due to an increase in prolactin levels after the pleasant slide set compared to baseline levels, $p < .01$, and to a decrease after the unpleasant slide set in comparison with baseline levels, $p < .01$. Prolactin levels sampled after picture viewing indicated a larger increase for the pleasant set compared to the neutral, $p < .05$, and the unpleasant set, $p < .01$; in addition, prolactin levels were lower for the unpleasant condition compared to the neutral one, $p < .05$.

No significant correlations were found among baseline cortisol levels and prolactin levels or prolactin change scores in the pleasant, neutral, and unpleasant conditions, $p > .05$.

Discussion

The present study clearly shows that the neuroendocrine system responds selectively to affective motivationally relevant pictures. Although in the subjective arousal rating, pleasant and unpleasant stimuli receive the same high score, a different neuroendocrine pattern was obtained: Unpleasant stimuli elicited a decrease in prolactin concentration and
increases in noradrenaline, cortisol, and ACTH levels, whereas pleasant slide set viewing induced an increase in prolactin level.

Taken as a whole, the biological responses to unpleasant pictures obtained in the present study are consistent with previous reports that showed neuroendocrine changes associated to stressful and unpleasant emotions (Biondi & Picardi, 1999; Carrasco & Van de Kar; 2003; Lovallo & Thomas, 2000). Nevertheless, since commonly used psychological stressors are mental arithmetic, speech task, the Stroop test, videogame playing, films, and interviews, it is extremely important to keep in mind the heterogeneity of neuroendocrine responses obtained by these stimuli that are quantitatively and qualitatively different. Although previous studies suggested that adrenaline is influenced mainly by mental stress and noradrenaline by physical demands (Frankenhaeuser, 1971), in the present study adrenaline was not affected by picture content. This might be due to the nature of the stimuli used in the present research compared to previous works (i.e., acute vs. chronic), and further investigation is needed to evaluate the sensitivity of adrenaline and noradrenaline to emotional stimuli.

The present data revealed that catecholamine, ACTH, and cortisol were not involved in the activation process induced by the view of pleasant erotic pictures. Similar results were also reported by others in male subjects: Sexual arousal was found to be unable to affect adrenaline, noradrenaline and cortisol (Carani et al., 1990; Exton et al., 2000). The lack of stress hormone activation in response to the emotional arousal induced by erotic slides may be due to a specific neuroendocrine response including the release of oxytocin: Oxytocin has been hypothesized as part of a complex response profile related to the perception of the environment as safe (Porges, 1998). In this way, Uvnas-Moberg (1997) and Carter and Altemus (1997) propose that oxytocin promotes states resistant to stress. Oxytocin, which has been associated with orgasm (Blaicher et al., 1999) and positive states such as physical proximity and prosocial behavior (Carter, Lederhendler, & Kirkpatrick, 1997), could have been released during the pleasant session in our experiment, exerting a stimulating action on prolactin secretion (Sobrinho, 1993). On the other hand, we did not find a relationship between baseline cortisol level and prolactin response. This result may be due to the low statistic power in the present study or may suggest that
cortisol plays an important role in prolactin release when such release is stimulated by specific conditions (i.e., buspirone).

The present study revealed that pleasant arousing pictures prompt higher prolactin levels compared to neutral and unpleasant conditions. Although a low libido level is currently considered the most critical behavioral change in hyperprolactinaemic subjects, short-term hyperprolactinaemia and acute low doses of this hormone may facilitate sexual behavior in male rats (Cruz-Casallas, Nasello, Hucke, & Felicio, 1999; Drago, 1984; Drago & Lissandrello, 2000). In fact, previous studies have shown increased levels of plasma prolactin in male rats exposed to a receptive female before mating (Kamel, Wright, Mock, & Franckel, 1977). Recently, it was found that sexual motivation, induced by an encounter with a sexually proceptive female, promotes oxytocin and prolactin secretion in sexually naive male rats (Hillegaart, Alster, Uvnas-Moberg, & Ahlenius, 1998).

On the other hand, the reasons for the decrease in prolactin levels after unpleasant and stressful emotions are not entirely clear. Stress has been found to increase the neurochemical activity of dopaminergic neurons and dopamine release in humans (Finlay & Zigmond, 1997; Pan, Porcella, & Gessa, 2000) and the rise in noradrenaline, observed in our study, may include an increase in dopamine, which could be responsible for prolactin suppression (Arbogast & Voogt, 1996; Franklin et al., 1999; Kojima, Arita, Kuwana, & Kimura, 1995).

Theorell (1992) found that serum prolactin reacts in a bimodal fashion in conjunction with stress; it increases in subjects that experience passive helplessness, whereas it decreases in conjunction with increased anxiety and active coping. However, it should be noted that Theorell focused his research on chronic (tonic) stress condition while the present results are obtained during acute (phasic) emotional states.

The present findings need to be interpreted with great caution because of the small sample size (even though the results are very consistent across participants and the fact that, given the standard error of the neuroendocrine measures, most of the psychoneuroendocrine studies used a similar number of participants). Moreover, further studies on a mixed sample may investigate the possible gender differences in the responses to pleasant and unpleasant emotional states. Finally, since it is well established that physiological responses vary not only as a function of the pleasantness of the stimuli but vary within the pleasant and unpleasant categories of visual stimuli as well (Bradley et al., 2001), and given that we used only erotic pictures in the pleasant slide set and mutilated bodies in the unpleasant one, future studies should clarify if the affective neuroendocrine patterns found in the present study can be extended to other pleasant and unpleasant semantic categories of stimuli.

To summarize, the present findings seem to indicate that while pictures of erotic and mutilated bodies were rated as similarly intense in terms of arousal using a continuous picture presentation paradigm, the pattern of neuroendocrine changes for each picture category was quite different. Using a similar paradigm, future studies may investigate how neuroendocrine changes during affective states modulate cognitive processes.

REFERENCES


(Received August 2, 2002; Accepted April 22, 2003)