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\rrh*Cardiovascular responses to affective pictures*

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\a\Cardiovascular patterns associated with appetitive and defensive activation during affective picture viewing

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\e\Abstract

In this study we assessed blood pressure (BP), heart rate (HR), stroke volume (SV), cardiac output (CO), and total peripheral resistance (TPR) in response to 13 picture series in 37 participants in order to investigate their hemodynamic response associated with activation of the appetitive and defensive motivational systems underlying emotional experience. BP and SV, but not TPR, increased with increasing self-rated arousal, whereas HR decelerated more in response to negative than positive and neutral pictures. These findings suggest that modulation of the cardiovascular response to pictures is primarily myocardial. The observed response pattern is consistent with a configuration of cardiac sympathetic–parasympathetic coactivation. The relationships between self-rated arousal, BP, and SV were mainly exhibited by men, suggesting that increases in the sympathetic inotropic effect to the heart with self-rated arousal may be larger in men than in women.

Descriptors: Affective pictures; Arousal; Blood pressure; Cardiovascular reactivity; Emotion; Heart rate; Sex differences; Stroke volume

Several lines of research point to a hierarchical structure of emotion with the affective dimensions of valence (degree of pleasantness) and arousal (degree of activation) as strategic determinants of emotion (Russell & Barrett, 1999). In this context, valence defines a general disposition to approach positive stimuli (appetitive motivation) or avoid negative stimuli (defensive motivation), whereas arousal describes the intensity of the behavioral tendency (Bradley & Lang, 2007; Lang, Bradley, & Cuthbert, 1997).

Over the past few decades, empirical work has provided evidence for correlation between several neurophysiological measures (e.g., skin conductance, heart rate, facial muscle activity, cortical activity, startle reflex eyeblink magnitude, respiration) and changes in either reports of valence and arousal (e.g., Bradley & Lang, 2007; Gomez, Shafy, & Danuser, 2008). Significant relationships have been found for a wide range of emotional stimuli (e.g., music, sounds, imagery), but most of this research has been done using static affective pictures. Pictures are able to elicit a broad range of emotional reactions, varying in pleasantness and arousal, without engaging subjects in physically active tasks. According to Lang and co-workers (1997), the physiological responses that are observed in the context of affective picture viewing are primarily those supporting perception, and those that index the motivational strategy dictated by the pictures. Reactions of most individuals to images appear to activate phylogenetically primitive appetitive and defensive systems, reflecting the cognitive processes of orienting, affective mobilization, and the early stages of approach and defense. The present study used affective pictures as emotion induction procedure to investigate the cardiovascular patterns associated with activation of the appetitive and defensive systems.

Although heart rate (HR) is a standard cardiovascular measure in studies with affective pictures, it has been rarely measured in combination with other cardiovascular measures such as blood pressure (BP). In fact, we could locate only four investigations that assessed BP

reactivity to affective pictures (Globisch, Hamm, Esteves, & Öhman, 1999; Hempel, Tulen, van Beveren, Mulder, & Hengeveld, 2007; Hempel et al., 2005; Sarlo, Palomba, Buodo, Minghetti, & Stegagno, 2005). Findings from these four studies are not totally consistent. Sarlo et al. (2005) found that BP increased specifically in response to erotic pictures but not to negative, highly arousing pictures, pointing to an asymmetry between appetitive and defensive activation and, thus, a valence modulation of the BP response. However, Globisch et al. (1999) found that images of snakes and spiders induced large increases in mean arterial pressure (MAP) in fearful individuals, suggesting that also activation of the defensive system is associated with BP increases. Finally, Hempel et al. (2007) reported that systolic BP (SBP) increased when both pleasantness and arousal increased and contended that an increase in BP may also be induced by pleasant, arousing pictures without erotic content. Although very informative, results from these studies do not allow firm conclusions about the relationship between BP and valence and arousal, mainly because of the limited number of different semantic contents and the lack of some combinations of valence and arousal in their sets of stimuli.

Importantly, none of these studies have measured cardiovascular parameters other than HR and BP. A change in BP can be brought about in different ways through various centers in the central and peripheral nervous systems and can be regulated by local mechanisms in the heart, blood vessels, and other organs (Berntson, Quigley, & Lozano, 2007). Differential stimulation of muscarinic, alpha- and beta-adrenergic receptors can produce BP responses of similar magnitude but accompanied by different patterns of change in HR, stroke volume (SV), and total peripheral resistance (TPR). Therefore, a detailed hemodynamic profile can provide additional and potentially useful information not afforded by measuring HR or BP alone.

The present study aimed to determine the cardiovascular response patterns associated with activation of the appetitive and defensive motivational systems underlying emotional experience in the context of affective picture viewing. In particular, we were interested in establishing how cardiovascular measures relate to self-reported valence and arousal. Besides HR, we assessed SBP, diastolic BP, SV, TPR, and cardiac output (CO) with the Finometer device (FMS Finapres Medical Systems, Amsterdam, The Netherlands), which is the successor of the Finapres device. The technique used in the Finometer relies on the volume-clamp method of Penaz (1973), and the main feature of this instrument is that it allows the continuous beat-by-beat concurrent assessment of several cardiovascular measures. To the best of our knowledge, it is the first time that the Finometer is used to investigate cardiovascular responses associated with processing of affective pictures.

Besides cardiovascular measures, skin conductance level (SCL) was also measured. Because skin conductance is sympathetically regulated and has been consistently shown to increase with both appetitive and defensive activation and, thus, to correlate positively with self-reported arousal (cf., Bradley & Lang, 2007), its assessment will allow us to better interpret the hemodynamic responses to the pictures.

In accordance with the idea that both increasing appetitive and defensive activation are associated with increasing activation of the sympathetic nervous system in preparation for appetitive and defensive behavior (Bradley & Lang, 2007; Lang et al., 1997) and in line with previous findings (Globisch et al., 1999; Hempel et al., 2007), we hypothesized that BP would increase with increasing activation of both motivational systems and, thus, would covary with self-rated arousal. As to an influence of valence, we did not make any predictions given the current inconsistency of findings (e.g., Christie & Friedman, 2004; Globisch et al., 1999; Hempel et al., 2007; Neumann & Waldstein, 2001; Sarlo et al., 2005). Sympathetic activation may be expected to increase myocardial contractility and, thus, SV through its inotropic

control of the heart but also to prompt peripheral vasoconstriction mediated by alpha 1-adrenoreceptors. Thus, BP changes with increasing arousal may result from an increase in SV, TPR, or both. In animal studies, presentation of various "emotional" stimuli has not been consistently associated with either vasoconstriction or vasodilation, but the apparent inconsistencies may be due to lack of distinction between different forms of reaction (e.g., defense vs. vigilance reaction, cf., Fisher, 1991). Cardiac deceleration characterizes the HR response in the context of picture processing and is generally greater when viewing unpleasant, compared to either pleasant or neutral pictures (cf., Bradley & Lang, 2007). Therefore, we expected HR to decelerate with increasing unpleasantness.

Finally, even though the design of the study was not set to investigate sex differences, we also explored whether men and women differ in their cardiovascular responses. This was primarily motivated by Sarlo et al.'s (2005) observation that men responded with significantly greater BP changes to erotic stimuli compared to other contents, whereas women showed no differentiation among picture categories.

\2**Methods**

\3*Participants*

Participants were 18 men and 19 women, aged 18–34 years (mean age 25.9 years). Most participants were undergraduate students. None reported suffering from cardiovascular, respiratory, or psychiatric diseases. All were healthy on the day of testing and were not taking any drugs. Demographics and physiological baseline values for men and women are reported in Table 1\t1\. All participants appeared to be of normotensive BP status with the possible exception of one male participant whose mean SBP and DBP during baseline periods were 153 and 92 mmHg, respectively. All participants gave written informed consent and were paid for participation.

\3*Stimuli*

Stimuli were as described in Gomez et al. (2008). In short, 13 series of ten pictures, all selected from the International Affective Picture System (Lang, Bradley, & Cuthbert, 2005) were used. Each series had a different thematic content; these were: sport, nature, family, opposite-sex erotica, same-sex erotica, erotic couples (heterosexual), mutilation, accidents, loss, contamination, attacking humans, neutral faces, and household objects.

\3*Measurements*

Self-reports of valence and arousal were registered using the pencil-and-paper version of the 9-point Self-Assessment Manikin (Bradley & Lang, 1994). SBP, DBP, and MAP[1]\fn1\ **Q1** were recorded by finger-cuff photoplethysmography with the Finometer instrument. **Q2** Finometer measures finger arterial pressure on a non-invasive beat-to-beat basis, gives waveform measurements similar to intra-arterial recordings, and reconstructs brachial arterial pressure based on the volume-clamp method of Penaz (1973), the Physiocal criteria of **Q3** Wesseling (Wesseling, de Wit, van der Hoeven, van Goudoever, & Settels, 1995), and generalized waveform inverse modeling (Gizdulich, Prentza, & Wesseling, 1997). The finger cuff (small, medium, or large) was wrapped around the middle phalanx of the middle finger (index or ring finger for a few participants) of the left hand, which lay on the table during the entire experiment. The raw finger pressure waveform was sampled at 200 Hz. The hydrostatic height correction of the finger with respect to the heart level was active during the whole period of testing. Finger artery pressure wave was analyzed using Beatscope software that includes the Modelflow method (Jansen et al., 2001) generating the hemodynamic parameters SV, CO, and TPR. CO is the product of SV and HR expressed in lpm, and TPR is calculated as the ratio of MAP to CO expressed in mmHg * s/ml (medical unit, MU). Accuracy and precision of changes in both BP and hemodynamic parameters as measured with this technology have been shown reliable (e.g., Bogert & van Lieshout, 2005; Jansen et al., 2001).

HR[2]\fn2\ was extracted from the electrocardiogram (ECG), which was recorded with the Lifeshirt system (VivoMetrics Inc., Ventura, CA) using three electrodes placed directly onto the skin on the upper chest and on the lateral surface of the abdomen. It was sampled at 200 Hz.

SCL was recorded with Psylab (Contact Precision Instruments, London, UK). A Stand Alone Monitor was connected to a host computer via USB interface and a SC5 24 bit digital skin conductance amplifier. The SC5 amplifier measures, with an absolute accuracy within 0.1 µSiemens, a constant voltage electrode excitation (0.5 V) and a sampling rate of 40 Hz. Two pre-wired 8 mm diameter Ag/AgCl electrodes were placed adjacently on the hypothenar eminence of the left palmar surface. The electrodes were filled with TD-246 Skin Resistance–Skin Conductance Electrode Paste (Med Associates Inc., St. Albans, VT), formulated with 0.5% saline in a neutral base. Respiration and spontaneous eye-blinking were also recorded; the results for these measures are reported in Gomez et al. (2008).

Validated French versions of the Revised NEO Personality Inventory (NEO PI-R, Costa & McCrae, 1992) and the trait form of the State-Trait Anxiety Inventory (STAI Y-B, Spielberger, 1983) were also administered.

\3*Procedure*

The procedure has been described in Gomez et al. (2008). In short, after attachment of the sensors and a 15-min resting period, participants were presented with the 13-picture series, each lasting 1 min (6 s per picture), with 75-s pauses in between. At the end of each series, participants reported how they felt while watching the pictures by giving one valence and one arousal rating for each series. The order of the ten pictures in each series was randomly determined and maintained constant throughout the entire study. For each participant, a different presentation order of series was randomly constructed with the constraint that series of similar valence (positive, negative, neutral) had not to be presented consecutively. **Q4**

\3*Data Reduction and Response Definition*

The softwares VivoLogic (VivoMetrics Inc.), Beatscope 1.1a (FMS Finapres Medical Systems BV), and Psylab 8 (Contact Precision Instruments) were used to analyze the ECGs, the BP waves, and SCL, respectively. A few ECG artifacts of short duration were found **Q5** and replaced by linearly interpolated values in 11 subjects, and 26 BP artifacts (spiked or damped waveforms) lasting one to three beats were found in 13 subjects. These short artifacts were excluded from analyses. The Finometer data were not recorded for one subject due to technical failure. SCL was missing for two subjects due to a recording error. The final sample sizes were n = 37 for HR, valence ratings, arousal ratings, n = 36 for SBP, DBP, SV, CO, TPR, and n = 35 for SCL.

Median values of the physiological measures were calculated for each participant for the 30-s periods immediately before onset of each picture series (baseline) and for the entire 60-s series epoch. Medians instead of means were preferred because the median is a more robust estimate of the center of a data sample (Pagano & Gauvreau, 2000)[3]\fn3\. Change scores were computed by subtracting the baseline values from the corresponding series values and were used in the statistical analyses.

\3*Statistical Analyses*

All analyses were performed using Systat Version 10.2 for Windows (SYSTAT Software Inc., Chicago, IL). We performed two sets of analysis that are complementary to each other. **Q6** Analysis 1 assessed psychophysiological reactions as they vary when looking at pleasant, neutral, and unpleasant series, as defined by *a priori* groupings on the valence dimension. Moreover, appetitive and defensive activations were analyzed by comparing reactivity to the two neutral series with reactivity to the five pleasant and the five unpleasant series, respectively. In Analysis 2 we used an analytic strategy that correlates directly physiological responses with self-rated valence and arousal. The rationale for these analyses is given in Gomez et al. (2008).

\5\Analysis 1: Responses to a priori valence categories and appetitive and defensive activation. For each dependent measure, the same set of three multivariate repeated-measures analyses of variance (MANOVAs) were performed. In the first MANOVA, effects of emotional content were tested for pleasant, neutral, and unpleasant picture series by averaging responses over the different series in each valence category (pleasant: Family, Nature, Sport, Opposite-sex erotica, and Erotic couples; neutral: Household objects and Neutral faces; unpleasant: Loss, Contamination, Accidents, Attacking humans, and Mutilation). In this MANOVA, Valence Category (3 levels) was the within-subject factor and Sex was included as the between-subject factor. Linear and quadratic contrasts were tested for each measure. Where appropriate, paired *t*-tests were additionally conducted to compare the three valence categories to each other. Bonferroni-adjusted *p* values are reported (i.e., the *p* value of the simple *t*-test × 3 in accordance with the number of comparisons involved).

The second and third MANOVAs evaluated activation within the appetitive and defensive systems, respectively, from a common affectively neutral, low-arousal state. The MANOVA evaluating appetitive activation was performed over the neutral series (Household objects and Neutral faces averaged; in the following labeled "Neutral series") and the five pleasant series, and the MANOVA evaluating defensive activation was performed over the Neutral series and the five unpleasant series. The main interest of these MANOVAs was to test linear changes with increasing appetitive and defensive activation, respectively. Thus, for each MANOVA, series were ordered by increasing mean arousal of our participants. For appetitive activation the order was Neutral series, Family, Nature, Sport, Opposite-sex erotica, and Erotic couples, and for defensive activation the order was Neutral series, Loss, Contamination, Accidents, Attacking humans, and Mutilation. In each MANOVA, Series (6 levels) was the within-subject factor and Sex the between-subject factor. Because the series were not evenly spaced along the arousal dimension, the metric was adapted to the actual spacing using the mean arousal of the corresponding series; this was for appetitive activation

"3.04, 4.27, 4.46, 5.70, 6.60, 7.08" and for defensive activation "3.04, 4.51, 5.43, 5.62, 6.51, 7.65." Where appropriate, post hoc analyses were additionally conducted using paired *t*-tests that contrasted the Neutral series against each of the five pleasant and five unpleasant series. Bonferroni-adjusted *p* values are reported (i.e., the *p* value of the simple *t*-test × 5 in **Q7** accordance with the number of comparisons involved). All MANOVAs were also computed for men and women separately. For these analyses, the metric used for appetitive activation was "2.78, 4.00, 4.06, 5.56, 6.89, 7.11" for men and "3.29, 4.53, 4.84, 5.84, 6.32, 7.05" for women, whereas the metric for defensive activation was "2.78, 4.50, 5.56, 5.61, 6.39, 7.22" for men and "3.29, 4.53, 5.32, 5.63, 6.63, 8.05" for women.

For all MANOVAs, the significance level was set at 5%. The Same-sex erotica series was excluded from these analyses because pictures of attractive individuals of the same sex represent a content category that cannot be considered either typically pleasant, unpleasant, or neutral. Baseline means of all physiological measures were also analyzed in an ANOVA with Series as within-subject factor and Sex as between-subject factor. As measures of effect size, we report partial eta squared (η_p^2) and Cohen's *d*.

\5*Analysis 2: Relationships Between Affective Ratings and Physiological Responses.* Mixed effects regression models with marginal maximum likelihood estimation were calculated. The procedure involves a combination of the EM algorithm and Fisher **Q8** scoring (Hedeker & Gibbons, 1996). This statistical approach was used to take into account the multilevel structure of the data (13 observations nested within each participant). The general model can be described as $Y = \alpha + \beta_V V + \beta_A A + \beta_S S + \beta_{VA} (V \times A) + \beta_{VS} (V \times S) + \beta_{AS} (A \times S) + \beta_{VAS} (V \times A \times S) + E$. It includes a random intercept for each participant (α) and fixed effects for valence rating (V), arousal rating (A), sex (S), the interaction terms (V × A, V × S, A × S, V × A × S), and the random error (E). Y is the response score of the physiological measures as defined above. (V) and (A) are the subjective valence and arousal

judgments given by the participants. (S) is defined as categorical variable with the values +1 for female participants and –1 for male participants. Significant effects for (S) would indicate significant differences between men and women in the average level of the physiological responses, whereas significant effects for (V × S), (A × S), and (V × A × S) would indicate sex differences in the relationship between the physiological responses and the affective ratings. Models with and without the interaction terms (V × A) and (V × A × S) were tested. These were not significant for any variables so that only models without them are reported. The significance level was set at 5%. When interaction terms including (S) were significant, the model was tested for men and women separately.

Residual analysis indicated a good conformity to assumptions of normality and linearity. Under normal regression assumptions, studentized residuals have a *t* distribution. Systat looks at the *t* distribution and prints a warning when the *t* value exceeds the expected 99% level, which corresponds to a value of about ± 3. Few outlying observations were removed as according to Systat's warning (see Results section).

\2**Results**

\3*Baseline Analysis*

There were no significant baseline differences among the 13 picture series for any parameter (*F*s(12,408) = 1.65, 1.08, 1.43, 1.78, 0.66 for SBP, DBP, SV, CO, and TPR, respectively; *F*(12,420) = 1.07 for HR; *F*(12,396) = 0.80 for SCL). Sex main effects of baseline levels were found for SV, CO, and TPR (see Table 1). No Series × Sex interactions were significant (*p*s > .33).

\3*Analysis 1*

\5*Responses to a priori valence categories.* Table 2\t2\ presents mean changes for the dependent variables and the statistics for the first MANOVA. As expected, valence ratings decreased from pleasant to unpleasant series in a monotonic fashion ($F(1,35) = 372.72$, $p < .001$, $\eta_p^2 = .914$) and, on average, pleasant and unpleasant contents were rated as more

arousing than neutral contents ($F(1,35) = 136.98$, $p < .001$, $\eta_p^2 = .796$). There were no significant sex differences in the affective ratings.

For SBP there was a significant Valence Category × Sex interaction for the quadratic trend ($F(1,34) = 5.06$, $p < .05$, $\eta_p^2 = .130$). The latter was significant for men ($F(1,17) = 5.51$, $p < .05$, $\eta_p^2 = .245$) with higher SBP for both positive and negative series as compared to neutral ones, whereas it was not significant for women ($F(1,17) = 0.33$, $p = .57$, $\eta_p^2 = .019$). For DBP, both the linear and the quadratic contrasts were marginally significant ($F(1,34) = 3.70$, $p = .063$, $\eta_p^2 = .098$ and $F(1,34) = 3.22$, $p = .082$, $\eta_p^2 = .086$, respectively). Post hoc paired *t*-tests indicated that DBP for the positive series was marginally higher than for the neutral series ($p = .053$, $d = .57$)

HR decreased in a monotonic fashion from the positive to the negative series ($F(1,35) = 4.97$, $p < .05$, $\eta_p^2 = .124$). The Valence Category × Sex interaction for the linear trend was marginally significant ($F(1,35) = 3.19$, $p = .083$, $\eta_p^2 = .083$). Men had a significant linear trend ($F(1,17) = 9.57$, $p < .01$, $\eta_p^2 = .360$), whereas women did not ($F(1,18) = .09$, $p = .77$, $\eta_p^2 = .005$). The Valence Category × Sex interaction for the quadratic trend was significant ($F(1,35) = 4.53$, $p < .05$, $\eta_p^2 = .114$). Post hoc paired *t*-tests indicated that for men HR for the negative series was lower than HR for both the positive series ($p < .05$, $d = .66$) and the neutral series ($p < .05$, $d = .77$).

For SV both the Valence Category effect and the Valence Category × Sex interaction of the quadratic trend were marginally significant ($F(1,34) = 3.96$, $p = .055$, $\eta_p^2 = .104$ and $F(1,34) = 3.72$, $p = .062$, $\eta_p^2 = .099$, respectively). Men had a significant quadratic trend with higher values for positive and negative series as compared to neutral series ($F(1,17) = 5.43$, $p < .05$, $\eta_p^2 = .242$), whereas women did not ($F(1,17) = .00$, $p = .95$. $\eta_p^2 = .000$). There were no significant effects for CO.

For TPR the Valence Category effect of the linear trend was marginally significant ($p = .062$), and the Valence Category × Sex interaction for the quadratic trend was significant

($F(1,34) = 3.73$, $p = .062$, $\eta_p^2 = .099$ and $F(1,34) = 4.87$, $p < .05$, $\eta_p^2 = .125$, respectively). Men had a significant quadratic trend ($F(1,17) = 9.51$, $p < .01$) with higher TPR for both positive and negative series as compared to neutral series, whereas women did not ($F(1,17) = .54$, $p = .47$). For SCL, mean values for the positive and the negative series were higher than for the neutral ones (quadratic trend, $F(1,33) = 6.90$, $p < .05$, $\eta_p^2 = .173$), and there were no significant sex effects.

\5*Appetitive and defensive activation.* The mean valence rating of the Neutral series was significantly lower than the mean valence rating of all pleasant series and significantly higher than the mean valence rating of all unpleasant series (paired *t*-tests, all *p*s < .001). As series were ordered by increasing mean arousal rating, the Series main effects of the linear trends for arousal were highly significant ($F(1,35) = 176.42$, $p < .001$, $\eta_p^2 = .834$ for appetitive activation and $F(1,35) = 215.33$, $p < .001$, $\eta_p^2 = .860$ for defensive activation). There were no significant main effects of sex or Series × Sex interactions either for valence or arousal indicating that men's and women's ratings of the series were similar.

SBP linearly increased with both increasing appetitive and defensive activation ($F(1,34) = 14.72$, $p < .001$, $\eta_p^2 = .302$ and $F(1,34) = 17.67$, $p < .001$, $\eta_p^2 = .342$ respectively; see Figure 1\f1\). Moreover, the Series × Sex interactions for appetitive and defensive activation were significant ($F(1,34) = 4.39$, $p < .05$, $\eta_p^2 = .114$ and $F(1,34) = 11.59$, $p < .01$, $\eta_p^2 = .254$, respectively). Men had significant linear effects for appetitive activation ($F(1,17) = 10.96$, $p < .01$, $\eta_p^2 = .392$) and defensive activation ($F(1,17) = 14.04$, $p < .01$, $\eta_p^2 = .452$), whereas for women the linear trends for appetitive and defensive activation were not significant ($F(1,17) = 2.99$, $p = .10$, $\eta_p^2 = .149$ and $F(1,17) = 1.12$, $p = .31$, $\eta_p^2 = .062$, respectively). Post hoc paired *t*-tests indicated significant differences between the Neutral series and the Erotic couples series and the Mutilation series for the whole sample ($p < .01$, $d = .86$ and $p < .01$, $d = .76$, respectively). However, for men these differences were highly

significant ($p < .01$, $d = 1.55$ and $p < .001$, $d = 1.38$, respectively), whereas for women they were not ($p = 1.0$, $d = .16$ and $p = 1.0$, $d = .10$, respectively).

DBP increased with increasing appetitive activation ($F(1,34) = 19.98$, $p < .001$, $\eta_p^2 = .370$) with both men and women demonstrating a significant linear increase (men, $F(1,17) = 11.75$, $p < .01$, $\eta_p^2 = .409$; women, $F(1,17) = 7.51$; $p < .05$, $\eta_p^2 = .306$, see Figure 1). For defensive activation, the Series main effect was marginally significant ($F(1,34) = 3.02$, $p = .091$, $\eta_p^2 = .082$), and the Series × Sex interaction was significant ($F(1,34) = 6.15$, $p < .05$, $\eta_p^2 = .153$) reflecting a linear increase for men ($F(1,17) = 6.42$, $p < .05$, $\eta_p^2 = .274$) but not for women ($F(1,17) = 0.29$, $p = .60$, $\eta_p^2 = .017$). DBP was significantly higher for the Erotic couples series as compared to the Neutral series for the whole sample ($p < .001$, $d = 1.25$). For the negative contents, men showed significantly higher DBP for the Mutilation series in comparison to the Neutral series ($p < .05$, $d = 1.08$), whereas for women this difference was not significant ($p = 1.0$, $d = -0.25$).

For HR (see Figure 2\f2\) there were no significant linear effects ($F(1,35) = 0.67$ and 0.00 for appetitive and defensive activation, respectively). SV linearly increased with both increasing appetitive activation ($F(1,34) = 8.80$, $p < .01$, $\eta_p^2 = .206$) and defensive activation ($F(1,34) = 19.26$, $p < .001$, $\eta_p^2 = .566$; see Figure 2). In addition, the Series × Sex interaction for appetitive activation ($F(1,34) = 3.27$, $p = .079$, $\eta_p^2 = .096$) and defensive activation ($F(1,34) = 8.84$, $p < .01$, $\eta_p^2 = .206$) indicated that men and women differed in their SV responses. Men had a significant linear trend for appetitive and defensive activation ($F(1,17) = 6.59$, $p < .05$, $\eta_p^2 = .279$; $F(1,17) = 18.09$, $p < .001$, $\eta_p^2 = .516$, respectively), whereas women did not ($F(1,17) = 1.34$, $p = .26$, $\eta_p^2 = .073$; $F(1,17) = 1.81$, $p = .20$, $\eta_p^2 = .106$, respectively). For the whole sample, post hoc paired *t*-tests indicated significant differences between the Neutral series and the Erotic couples series ($p < .05$, $d = .68$), the Mutilation series ($p < .05$, $d = .80$), and the Attacking humans series ($p < .05$, $d = .70$). When analyzed separately, men demonstrated significantly higher SV changes to the Erotic couples series and

to the Mutilation series in comparison to the Neutral series ($p < .05$, $d = 1.11$ and $p < .01$, $d = 1.33$, respectively), whereas women did not ($p = 1.0$, $d = .21$ and $p = 1.0$, $d = .22$, respectively).

CO significantly increased with increasing defensive activation ($F(1,34) = 14.58$, $p < .001$, $\eta_p^2 = .429$) but not with increasing appetitive activation ($F(1,34) = 2.36$). Both men and women had a significant increase with increasing defensive activation (men, $F(1,17) = 8.74$, $p < .01$, $\eta_p^2 = .340$; women, $F(1,17) = 4.75$, $p < .05$, $\eta_p^2 = .218$); whereas neither men nor women demonstrated a significant increase with increasing appetitive activation (men, $F(1,17) = 1.99$, $p = .18$, $\eta_p^2 = .105$; women, $F(1,17) = 0.26$, $p = .61$, $\eta_p^2 = .015$). Post hoc paired *t*-tests indicated a significant difference between the Neutral series and the Mutilation series for the whole sample ($p < .05$, $d = .65$). For TPR no linear trends were significant ($F(1,34) = 2.73$ and 2.07 for appetitive and defensive activation, respectively).

SCL linearly increased with increasing appetitive activation ($F(1,33) = 8.34$, $p < .01$, $\eta_p^2 = .202$) and defensive activation ($F(1,33) = 12.34$, $p < .001$, $\eta_p^2 = .374$) both for men and women (no significant Series × Sex interactions). For the whole sample, there were significant differences between the Neutral series and the Erotic couples series ($p < .05$, $d = .63$), the Opposite-sex erotica series ($p < .05$, $d = .64$), and the Mutilation series ($p < .05$, $d = .64$).

\3*Analysis 2*

\5*Models with data of all picture series.* The estimated models for the physiological measures are presented in Table 3\t3\. SBP and DBP increased with increasing self-reported arousal. However, this relationship was essentially due to the male participants (significant Arousal × Sex interaction). Systat sets the coefficient for the categorical variable (S) as follows: men = -1; women = +1. For men the overall coefficients for the arousal effect were 0.53 (0.24 + 0.29) for SBP and 0.31 (0.14 + 0.17) for DBP. For women, the overall coefficients were –0.05 (0.24 – 0.29) for SBP, and -0.03 (0.14 – 0.17) for DBP. Thus, men

demonstrated a significant positive correlation between arousal and the two BP measures, whereas women did not. These results were confirmed by estimating the models for men and women separately. The arousal coefficients were (standardized errors in parentheses): for men, SBP, 0.53 (0.17), $p < .01$; DBP, 0.31 (0.09), $p < .001$; for women, SBP, –0.04 (0.11), $p = .69$; DBP, -0.03 (0.08), $p = .71$. These coefficients can readily be interpreted in terms of BP changes in mmHg per unit of self-reported arousal on the 9-point Self-Assessment Manikin. Thus, men exhibited, on average, an increase in SBP of 0.53 mmHg in SBP and 0.31 mmHg in DBP per unit of self-reported arousal.

HR increased with increasing pleasantness as indicated by the significant Valence main effect. Similar to the BP measures, SV increased with increasing arousal ratings, and this relationship was essentially due to the male participants, as confirmed by separate model estimations. The correlation between SV and arousal ratings was highly significant for men (arousal coefficient, 0.53 (0.14), $p < .001$), and nonsignificant for women (arousal coefficient, 0.05 (0.09), $p = 0.58$).

CO increased significantly with increasing arousal, whereas TPR had no significant relationships to either valence ratings, arousal ratings, or sex (all *p*s > .28). Finally, SCL was positively correlated with self-reported arousal both for men and women (no significant Arousal × Sex interaction).

\5*Models without data of the Same-sex erotica series.* The estimated models were essentially the same as with data of all picture series. As shown in Table 3, there were slight changes in the significance level of two effects for SBP and DBP.

\2**Discussion*

The present study investigated the modulation of the cardiovascular response to affective pictures in relation to activation of the appetitive and defensive motivational systems in men and women. BP measures varied primarily with the intensity of the behavioral tendency (arousal) rather than with the motivational direction (positive or negative valence), in line

with our hypotheses based on previous findings (Globisch et al., 1999; Hempel et al., 2007). Results for SV largely paralleled those for BP; it increased with both appetitive and defensive activation and covaried with self-rated arousal. Importantly, the analyses revealed considerable sex differences for BP and SV, indicating that the above-mentioned effects were mainly exhibited by the male participants. As predicted, HR was mainly modulated by the valence tone of the pictures showing larger HR deceleration in response to negative contents as compared to neutral and pleasant ones and covarying significantly with self-rated valence. CO increased linearly with increasing defensive activation and correlated with self-rated arousal, whereas TPR showed neither linear changes with appetitive and defensive activation nor significant relationships with self-ratings of valence and arousal. Finally, SCL was larger for high-arousal series than low-arousal ones and covaried with self-rated arousal as anticipated.

The modulation of the cardiovascular response to affective pictures along the dimensions of valence and arousal appears to be primarily myocardial, and the pattern of cardiovascular response is consistent with a configuration of cardiac sympathetic-parasympathetic coactivation (i.e., concurrent increases in both vagal and sympathetic outflows; cf., Berntson, Cacioppo, & Quigley, 1991). The parasympathetic system dominates the control of cardiac chronotropy, whereas the sympathetic system has a more predominant effect on the inotropic state of the heart. Because of these asymmetries in autonomic influences on distinct functional dimensions of the heart, coactivation of both sympathetic and vagal controls can yield an increase in SV by increasing ventricular filling time in association with HR deceleration, a possibility that was confirmed in a study using direct electrical activation of vagal and sympathetic cardiac nerves (Koizumi, Terui, Kollai, & Brooks, 1982). Increased parasympathetic outflow mainly lowers HR and has only a limited secondary effect on BP via CO reduction (Berntson et al., 2007). HR deceleration in response to perceptual stimuli is largely mediated by increases in vagal activity (e.g., Quigley & Berntson, 1990;

Wilhelm, Kochar, Roth, & Gross, 2001). Increased sympathetic outflow to the heart increases SV and consequently BP (especially SBP). Our understanding of the findings is that increasing unpleasantness of the images was accompanied by increasing HR deceleration due to increasing parasympathetic activation to the heart. Pharmacological blockade studies of fear bradycardia in animals suggest that this deceleratory activity is mediated primarily by changes in parasympathetic activity (Campbell, Wood, & McBride, 1996). HR deceleration in the context of aversive picture processing is interpreted as indicating the heightened attention and sensory intake that occurs when the defensive system is alerted, but activation is relatively low (Bradley & Lang, 2007). At the same time, increasing arousal of the images was associated with increasing ventricular contractility due to increasing sympathetic inotropic outflow. The resulting increases in SV ultimately led to increases in BP with increasing self-rated arousal.

Thus, in the context of affective picture viewing there is a dissociation between HR and BP. Usually, BP changes follow HR changes in challenging or engaging tasks. Since BP is determined by HR, SV, and systemic vascular resistance, a decrease in HR often results in decreased BP. However, our data show that processing increasingly arousing pictures causes a significant increase in SV that overrides the BP drop that would be caused by HR deceleration. In contrast, the contribution of vascular changes as indexed by TPR to increases in BP seems less straightforward. TPR did not increase significantly with increasing appetitive and defensive activation and did not covary either with self-reported valence or arousal. Yet, TPR had a general tendency to increase from baseline, as indicated by a significant one-sample *t*-test of the average change from baseline against the null hypothesis of no change (mean change in MU = 0.009, $SEM = 0.003$, $p < 0.01$, $d = .71$, see also Table 2). Thus, on average, processing of the picture series was accompanied by a moderate peripheral vasoconstriction, which, however, was not significantly modulated either by the valence or arousal tone of the stimuli. Although we need to be cautious in comparing results from studies

using different emotion induction procedure, it is worth noting that Nyklicek, Thayer, & Van Doornen (1997) also found a general increase in TPR without any valence or arousal modulation in people listening to music excerpts, and Harrison and coauthors (2000) reported an increase in TPR from rest to film presentation. These observations are in line with an association between vasoconstriction and vigilant sensory intake (Williams, Bittker, Buchsbaum, & Wynne, 1975) and with findings in the animal literature suggesting that the vigilance reaction leads to decreased HR and increased TPR (Adams, Baccelli, Mancia, & Zanchetti, 1971; cf., Fisher, 1991).

The analyses also revealed sex differences for SBP, DBP, and SV. The sex effect for SV suggests that the increase in the sympathetic inotropic effect to the heart with increasing appetitive and defensive activation might be larger in men than in women, leading, ultimately, to larger BP increases in men. Biological differences in BP regulation between men and women may predispose men to show larger BP changes to high-arousal cues than women. Men do demonstrate, in general, larger BP changes than women in response to several behavioral challenges (e.g., Lawler, Wilcox, & Anderson, 1995). Sex differences in BP regulation seem evident, but their origin is not totally known. Studies have shown that women may have less sympathetic influence on BP (Barnett et al., 1999), and differences in the sensitivity and density of adrenoreceptors have been proposed (Freedman, Sabharwal, & Desai, 1987). Sex steroid hormones likely play a role, though still incompletely understood (Leinwand, 2003; Mendelsohn & Karas, 2005; Reckelhoff, 2001). However, men do not systematically show larger BP changes than women in emotional contexts (e.g., Fredrickson, Mancuso, Branigan, & Tugade, 2000; Neumann & Waldstein, 2001).

From a motivational perspective of emotion, cues that activate appetitive and defensive systems should be potent for both men and women, who share survival risks. Nonetheless, differential activation of these motivational systems could arise. Men and women seem to become physiologically aroused in different types of situations holding

significant meanings for each of them (Brody, 1999). Some lines of research indicate that, in general, women show a stronger disposition to engage the defensive motivational system when exposed to aversive cues, whereas men display increased appetitive activation when viewing erotic stimuli (Baumeister, 2000; Bradley, Codispoti, Sabatinelli, & Lang, 2001; Hamann, Harman, Nolan, & Wallen, 2004; Rupp & Wallen, 2008; Sabatinelli, Flaisch, Bradley, Fitzsimmons, & Lang, 2004). Our findings for BP in response to pleasant arousing erotic pictures are in line with this view. They also accord with Sarlo et al.'s (2005) observation and with neurophysiological studies indicating that for men, more than women, visual sexual stimuli seem to preferentially recruit the amygdala, the hypothalamus (Hamann et al., 2004; Rupp & Wallen, 2008), and visual cortical regions (Sabatinelli et al., 2004). On the contrary, the results for defensive activation contrast with Sarlo et al.'s (2005) study, in which both sexes had no appreciable changes in response to either images of aimed weapons, injuries, and mutilations. Methodological differences between studies could explain these discrepancies (e.g., 60-s picture series in the present study vs. 6-s single pictures in Sarlo et al.'s study). However, it could also be hypothesized that the observed sex differences reflect different behavioral responses. Taylor et al. (2000) proposed that, in response to stress, men are more likely than women to show the "classic" fight-or-flight response and women more likely than men to show a "tend-and-befriend" response (i.e., nurturant and affiliative behaviors). Oxytocin appears to be at the core of this response, and studies point to a link between oxytocin and lower BP both in animals and humans (e.g., Light, Grewen, & Amico, 2005; Light et al., 2000; Petersson, Alster, Lundeberg, & Uvnäs-Moberg, 1996, Petersson, Lundeberg, & Uvnäs-Moberg, 1999; Uvnäs-Moberg, 1997). Exogenously administered oxytocin has been shown to attenuate neural responses to aversive pictures (Domes et al., 2007; Kirsch et al., 2005; Petrovic, Kalisch, Singer, & Dolan, 2008), but concomitant effects on cardiovascular measures have not yet been explored.

CO, which has to match changing metabolic demands of the body, did not appear to vary differently in men and women. In this study, minute ventilation, the respiratory indicator of metabolic activity, showed similar relationships with arousal as CO (Gomez et al., 2008). These findings suggest that processing of increasingly arousing picture series might be associated with largely comparable metabolic changes in both men and women, even if men and women may demonstrate differences in specific cardiovascular measures (BP and SV). SCL increased linearly with both increasing appetitive and defensive activation and covaried with self-rated arousal both in men and women, replicating many previous studies (cf., Bradley & Lang, 2007). Because of the heavy interconnections within the sympathetic chain ganglia, it was believed historically that the sympathetic system discharges as a whole, whereas the distinct parasympathetic ganglia allowed for a more organ-specific discharge. It is now clear that even the sympathetic system is capable of targeted actions, as microneurographic recordings in conscious subjects have demonstrated a striking specificity in the pattern of sympathetic discharge across organ systems (for review, see Vallbo, Hagbarth, & Wallin, 2004). Our findings for SCL suggest that sex differences in the affective modulation of the responses to pictures may be specific to some cardiovascular parameters and are in line with the contention that the sympathetic nervous system does not discharge as a whole.

Finally, sex differences in BP and SV were not paralleled by significant differences in ratings of valence and arousal. Previous studies have suggested that young men report more intense pleasure and arousal when viewing erotic stimuli, whereas young women report more intense displeasure when processing aversive cues (Bradley et al., 2001). Differences in the presentation mode, rating procedure, and sample size could explain these discrepancies.

In summary, the present study has shown how activation of the appetitive and defensive motivational systems in the context of affective picture viewing is associated with changes in BP, SV, HR, CO, and TPR. These parameters covary to a certain degree with the

dimensions of valence and arousal. More specifically, the modulation of the cardiovascular response to affective pictures appears to be primarily myocardial, and the observed response pattern is consistent with a configuration of cardiac sympathetic-parasympathetic coactivation. Further, the study suggests sex differences in the regulation of BP and SV responses: Men's sympathetic outflow to the heart seems to increase with increasing arousal more strongly than women's sympathetic outflow, leading to higher BP changes during processing of arousing contents in men as compared to women. However, although these sex effects appear to be relatively large as indicated by measures of effect size, we must be cautious in interpreting them in light of the small sample and of potential differences other than biological sex that were not assessed.

Footnotes

\fn1\[1]Results for MAP are not reported here because they were very similar to results for SBP and, thus, did not add any significant information.

\fn2\[2]We also analyzed heart period (HP) because HR and HP are not linearly related to each other and, thus, under certain circumstances, results may differ for the two cardiac parameters (cf., Berntson et al., 2007). Because the results for HR and HP were very similar, we only report the results for HR.

\fn3\[3]Similar response patterns were observed when mean values were calculated. There were slight changes of the significance level for three effects. In Analysis 1 the linear trend of defensive activation for CO was significant at the 1% level, and in Analysis 2 the arousal effect for SV was significant at the 1% level both with and without the Same-sex erotica series.

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Figure Captions

\f1**Figure 1.** Mean changes of systolic blood pressure (SBP) and diastolic blood pressure (DBP) for men (gray bars) and women (black bars) for the 13-picture series. Error bars refer to *SEM*s (n = 36).

\f2**Figure 2.** Mean changes of heart rate (HR) and stroke volume (SV) for men (gray bars) and women (black bars) for the 13-picture series. Error bars refer to *SEM*s (n = 37 for HR; n = 36 for SV).

Tables

\tc**Table 1.** *Demographics and Physiological Baseline Values for Men and Women (SDs in Parentheses)*

\tch\Measure	Men (n = 18)	Women (n = 19)	p value[c]
\tb\Age (years)	26.4 (3.6)	25.4 (4.3)	.42
Height (m)	1.80 (0.06)	1.67 (0.07)	< .001
Weight (kg)	71.9 (7.5)	57.6 (7.0)	< .001
BMI (kg/m^2)	22.2 (1.9)	20.7 (2.2)	< .05
Neuroticism (0-4)[a]	1.8 (0.5)	2.0 (0.5)	.16
Extraversion (0-4)[a]	2.3 (0.3)	2.4 (0.4)	.23
Openness to experience (0-4)[a]	2.7 (0.4)	2.6 (0.3)	.71
Agreeableness (0-4)[a]	2.6 (0.4)	2.5 (0.3)	.33
Conscientiousness (0-4)[a]	2.4 (0.4)	2.3 (0.5)	.61
Trait Anxiety (1-4)[b]	1.9 (0.4)	2.1 (0.4)	.26
SBP (mmHg)	118 (16)	112 (9)	.18
DBP (mmHg)	68 (9)	64 (5)	.20
HR (bpm)	66 (9)	72 (10)	.09
SV (ml)	97 (17)	75 (13)	< .001
CO (lpm)	6.3 (1.2)	5.3 (0.9)	< .01
TPR (MU)	0.85 (0.12)	0.98 (0.16)	< .01
SCL (µS)	4.2 (1.7)	3.7 (2.1)	.49

\tfn*Notes*: BMI, Body Mass Index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance; SCL, skin conductance level.
[a]NEO PI-R (Costa & McCrae Costa, 1992).
[b]STAI Y-B (Spielberger, 1983).
[c]Two-sample *t*-test.

\tc**Table 2.** *Mean Valence Ratings, Arousal Ratings, and Changes of the Physiological Measures (SEMs In Parentheses) When Viewing Pleasant, Neutral, and Unpleasant Picture Series and F-Values and Significance Levels of Univariate Linear and Quadratic Contrasts*

	\tch\Men			Women			Linear		Quadratic		
Dependent measure	Pleasant	Neutral	Unpleasant	Pleasant	Neutral	Unpleasant	Sex	V	V × Sex	V	V × Sex
\tb\Valence	6.9 (0.2)	4.7 (0.2)	2.8 (0.2)	7.3 (0.2)	4.6 (0.3)	2.5 (0.2)	0.06	372.72***	2.50	1.26	0.31
Arousal	5.5 (0.3)	2.8 (0.2)	5.9 (0.3)	5.7 (0.2)	3.3 (0.3)	6.0 (0.3)	0.89	3.00	0.00	136.98***	0.48
SBP (mmHg)	2.3 (0.7)	-0.3 (0.9)	1.6 (1.0)	0.0 (0.4)	0.1 (0.6)	-0.5 (0.6)	3.76	0.68	0.04	2.53	5.06*
DBP (mmHg)	0.5 (0.4)	-0.8 (0.4)	0.1 (0.4)	0.3 (0.3)	-0.2 (0.4)	-0.5 (0.5)	0.03	3.70	0.44	3.22	1.89
HR (bpm)	-0.7 (0.4)	-0.4 (0.5)	-1.9 (0.4)	-1.3 (0.3)	-1.9 (0.5)	-1.4 (0.3)	1.80	4.97*	3.19	0.22	4.53*
SV (ml)	2.2 (0.6)	0.3 (0.7)	2.5 (0.5)	0.5 (0.4)	0.5 (0.5)	0.5 (0.3)	7.69**	0.14	0.11	3.96	3.72
CO (lpm)	0.04 (0.06)	0.01 (0.05)	0.00 (0.07)	-0.07(0.03)	-0.12(0.05)	-0.05 (0.03)	3.21	0.09	0.48	1.07	0.50
TPR (MU)	0.009 (0.005)	-0.009 (0.004)	0.004 (0.007)	0.018 (0.006)	0.017(0.008)	0.004 (0.005)	4.24*	3.73	0.89	0.89	4.87*
SCL (µS)	-0.09 (0.03)	-0.14 (0.06)	-0.12 (0.08)	-0.19 (0.05)	-0.36 (0.07)	-0.21 (0.08)	3.68	0.24	0.03	6.90*	2.85

\tfn*Notes*: For valence and arousal ratings, higher values indicate more positive valence and higher arousal on a 1–9 scale. SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance; SCL, skin conductance level; Sex, Sex main effect; V, Valence Category main effect; V × Sex, Valence Category × Sex interaction.
Degrees of freedom were as follows: SBP, DBP, SV, CO, TPR: linear contrast, quadratic contrast, and Sex main effect (1,34); valence, arousal, HR: linear contrast, quadratic contrast, and Sex main effect (1,35); SCL: linear contrast, quadratic contrast, and Sex main effect (1,33).
*$p < .05$, **$p < .01$, ***$p < .001$.

\tc**Table 3.** *Estimated Models for the Physiological Measures*

Dependent variable	Predictor	\tch\With all picture series		Without Same-sex erotica series	
		Estimate	*SE*	Estimate	*SE*
\tb\SBP	V	0.04	0.09	0.05	0.09
	A	0.24*	0.10	0.31**	0.11
	S	1.08	0.80	1.41	0.81
	V × S	-0.08	0.09	-0.09	0.09
	A × S	-0.29**	0.10	-0.32**	0.11
DBP	V	0.09	0.05	0.10	0.05
	A	0.14*	0.06	0.19**	0.06
	S	0.58	0.46	0.72	0.47
	V × S	0.02	0.05	0.02	0.05
	A × S	-0.17**	0.06	-0.19**	0.06
HR	V	0.14*	0.06	0.14*	0.06
	A	-0.04	0.07	-0.04	0.07
	S	-0.02	0.49	-0.28	0.49
	V × S	-0.09	0.06	-0.06	0.06
	A × S	0.05	0.07	0.08	0.07
SV	V	-0.10	0.07	-0.10	0.07
	A	0.29***	0.08	0.30***	0.09
	S	0.34	0.64	0.68	0.66
	V × S	0.02	0.07	-0.01	0.07
	A × S	-0.23**	0.08	-0.27**	0.09
CO	V	0.03	0.07	0.03	0.07
	A	0.16*	0.08	0.16*	0.08
	S	0.19	0.60	0.15	0.06
	V × S	-0.04	0.07	-0.04	0.07
	A × S	-0.08	0.08	-0.07	0.08
TPR	V	0.01	0.01	0.01	0.01
	A	0.01	0.01	0.01	0.01
	S	0.06	0.08	0.08	0.08
	V × S	0.00	0.01	0.01	0.01
	A× S	-0.01	0.01	-0.01	0.01
SCL	V	-0.05	0.08	-0.04	0.08
	A	0.24**	0.09	0.25**	0.09
	S	-1.16	0.68	-0.94	0.69
	V × S	0.04	0.08	0.03	0.08
	A × S	0.05	0.09	0.04	0.09

tfn*Notes*: SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance; SCL, skin conductance

level; V, valence rating; A, arousal rating; S, sex; V × S, Valence Rating × Sex interaction; A × S, Arousal Rating × Sex interaction; *SE*, standardized error.
The number of outlying observations removed were SBP: 2, DBP: 1, HR: 2, SV: 0, CO: 1, TPR: 3, SCL: 4. For CO, TPR, and SCL, estimate and *SE* were multiplied by 10.
*$p < .05$, **$p < .01$, ***$p < .001$.

PSYP 0295

Q1 Acronyms have been used throughout when previously introduced.

Q2 Trademark symbol deleted per journal style. Manufacturer and location have been provided previously.

Q3 Please confirm spelling of Physiocal

Q4 Can either of these phrases be inserted in place of "had not to be presented" – "were not presented"– "or did not have to be presented"?

Q5 "Few" changed to "A few" – OK?

Q6 Please confirm introduction of location of SYSTAT.

Q7 "multiplied by 5" changed to "× 5" – OK?

Q8 Please spell out EM if applicable.