Psychophysiology, 43 (2006), 486–497. Blackwell Publishing Inc. Printed in the USA.

DOI: 10.1111/j.1469-8986.2006.00412.x

A multi-process account of startle modulation during affective perception

MARGARET M. BRADLEY,[a] MAURIZIO CODISPOTI,[b] AND PETER J. LANG[a]

[a]Center for the Study of Emotion and Attention, University of Florida, Gainesville, Florida, USA
[b]Department of Psychology, University of Bologna, Bologna, Italy

Abstract

Modulation of the startle reflex by sensory, attentional, and emotional processes was explored by presenting acoustic startle probes at various delays following picture onset. Within 500 ms of onset, blinks were first greatly facilitated and then inhibited, indicating prepulse facilitation and prepulse inhibition that did not vary with affect. Attention allocation to the picture continued across the viewing interval and was most pronounced for emotional pictures, as determined by attenuation of the P3 component to the startle probe. Startle potentiation for unpleasant pictures occurred later in the viewing interval and was strongest for highly arousing pictures. Taken together, the startle reflex during picture viewing is modulated by sequential and sometimes concurrent processes of prepulse facilitation, prepulse inhibition, attentional inhibition, and affective modulation, with reflex magnitude reflecting the net effect of multiple processes.

Descriptors: Startle, Prepulse, Emotion, Attention, ERP, Reflex, Pictures

The reflexive blink response to a sudden, startling probe is modulated by a number of different factors that are held to reflect specific sensory, attentional, and emotional processes. In particular, prepulse and affective modulation of the startle reflex constitute well-replicated phenomena that have received considerable experimental scrutiny. In the "prepulse" paradigm (e.g., Graham, 1975) a nonstartling stimulus is presented at different temporal delays before a startle probe. An acoustic prepulse stimulus inhibits blink magnitude for up to 500 ms, peaking around 150 ms after prepulse onset (see Anthony, 1985). This prepulse inhibition effect is usually interpreted as reflecting the operation of a low-level sensory gating process, as it occurs on the first trial in which a prepulse is presented, is resistant to habituation, and is not greatly affected by close temporal repetition of the prepulse stimulus (for a review, see Hackley & Boelhouwer, 1997). On the other hand, when attention is directed to a prepulse stimulus via instruction or task relevance, blink inhibition increases, indicating that attention can also modulate the degree of prepulse inhibition (e.g., DelPezzo & Hoffman, 1980; Filion, Dawson, & Schell, 1993; Hackley & Graham, 1987; Ison & Ashkenazi, 1980).

The startle reflex is also reliably modulated by the affective component of a stimulus, with larger blinks elicited when processing aversive events. In animals, for example, the startle reflex is reliably potentiated when presented in the context of a stimulus previously associated with shock (e.g., Davis, 1997). In humans, the startle reflex is also reliably potentiated by aversive stimulation, with larger reflexes not only elicited in contexts associated with shock (e.g., Greenwald, Bradley, Cuthbert, & Lang, 1998) but also when people simply view unpleasant pictures (Vrana, Spence, & Lang, 1988). We have hypothesized that affective modulation reflects priming of defensive reflexes such as startle when the motivational system mediating defense behavior is active (Lang, Bradley, & Cuthbert, 1997). Conversely, when appetitive motivation is dominant, as when people view pleasant, arousing pictures, the defensive startle reflex is inhibited (for an overview, see Bradley, Cuthbert, & Lang, 1999).

In a previous study (Bradley, Cuthbert, & Lang, 1993), we found evidence of both prepulse inhibition and affective modulation of the startle reflex when viewing pictures. Using an acoustic startle stimulus, probes were delivered at one of four delays following onset of an affective (pleasant or unpleasant) or neutral picture. Significant inhibition of the blink reflex was found for probes presented soon after picture onset (i.e., 300 ms) compared to probes presented later in the interval. In addition, inhibition was somewhat more pronounced for emotional (pleasant or unpleasant), compared to neutral pictures, suggesting that affectively engaging stimuli may draw more attentional resources than neutral stimuli, producing inhibition early in the viewing interval.

This prepulse effect was subsequently replicated in some studies (e.g., Dichter, Tomarken, Shelton, & Sutton, 2004; Levenston, Patrick, Bradley, & Lang, 2000), but not in others (Stanley & Knight, 2004; Vanman, Boehmelt, Dawson, & Schell, 1996). One hypothesis is that reflex modulation in the picture

This research was supported in part by grants from the National Institute of Mental Health (P50 MH 72850) and the National Institute of Dental Research (DE 13956).

Address reprint requests to: Margaret M. Bradley, Center for the Study of Emotion and Attention, Box 100165, Health Sciences Center, University of Florida, Gainesville, FL 32610, USA.

viewing paradigm reflects the net effects of multiple modulatory processes on the startle response, including those mediated by sensory, attentional, and affective factors. In the current experiments, we measured the reflexive blink response as well as the event-related potential to startle probes that more completely spanned the viewing interval in order to determine more precisely the nature of reflex modulation during picture perception.

Affective pictures are viewed here as cues that activate one of two motivational systems—either defensive or appetitive—that have evolved to mediate transactions in the environment that either threaten or promote physical survival (Lang, 1995; Lang et al., 1997). At extremely high levels of defensive or appetitive activation (e.g., as when an actual predator or food source is present), appropriate action (e.g., fleeing, consuming) ensues. At lower levels of defensive or appetitive activation, however, the primary adjustment is an increase in attentional resource allocation and sensory intake, which presumably increases the probability that an appropriate, life-saving (or sustaining) action will be selected. In the picture viewing context, in which the affective cue is merely symbolic and motivational activation is rarely intense enough to prompt actual action, a number of physiological and behavioral measures are consistent in suggesting increased attention allocation when viewing motivationally relevant pictures (for an overview, see Bradley & Lang, 2000). As startle inhibition is often held to reflect variations in attention allocation (e.g., Filion et al., 1993), it is likely that reflex inhibition may play a role in the affective modulation of the startle reflex during picture viewing, particularly early in the viewing interval.

In Experiment 1, 12 new probe delays were added to those reported by Bradley et al. (1993), including a set of probes presented immediately after picture onset to gauge very early sensory and attentional effects. Effects of the picture prepulse were assessed by comparing blink magnitude following picture onset to reflexes elicited in the absence of picture (i.e., no-picture probes). We hypothesized that prepulse effects due to sheer sensory stimulation (e.g., sensory gating) would be accompanied by magnitude inhibition but would not be affected by the hedonic content of the picture, whereas effects reflecting differences in attentional resource allocation would not only be accompanied by magnitude inhibition but would be heightened for emotional, compared to neutral, pictures. Onset latency of the blink reflex was also assessed to assist in interpreting the modulatory processes ongoing during prepulse processing.

Recent data suggest that the magnitude of reflex potentiation for unpleasant (compared to neutral) pictures increases with longer stimulation of the defensive motivational system. For example, Smith, Bradley, and Lang (2005) reported increased reflex potentiation following sustained exposure to a series of unpleasant pictures, compared to reflexes elicited early in exposure, and Sutton, Davidson, Donzella, Irwin, and Dottl (1997) found greater potentiation for probes presented later in a 12-s viewing of unpleasant pictures, compared to those presented earlier. Based on these findings, we expected defensive potentiation to be strongest later in the viewing interval. Relatedly, although Smith et al. (2005) found some support for an increase in blink inhibition as sustained exposure to pleasant pictures increased, the evidence was weaker, and do not prompt a strong prediction that inhibition should increase with longer duration of appetitive picture viewing.

The importance of emotional intensity in the affective modulation of the startle reflex is now clear, with a number of studies reporting that the most arousing picture contents (e.g., erotica, threat) prompt the greatest modulation of the blink reflex (e.g., Bradley, Codispoti, Cuthbert, & Lang, 2001; Cuthbert, Bradley, & Lang, 1996). We therefore compared early and late reflex modulation for pictures rated high or low in emotional arousal. We expected to find stronger effects of affective modulation late in the picture interval (e.g., potentiation for unpleasant pictures and inhibition for pleasant pictures) for pictures rated high in emotional arousal, whereas low arousal pictures were not expected to be strongly modulated by affect. To the extent that even low levels of motivational activation prompt increased attention (compared to neutral stimuli), we hypothesized that effects of early inhibition due to attentional allocation in this picture viewing context may be more similar for low and high arousal pictures. Finally, we expected that emotional arousal would not affect reflex modulation processes engaged solely by perceptual processing (e.g., sensory inhibition).

EXPERIMENT 1

Method

Participants

The subjects were 113 members (58 female) of University of Florida introductory psychology courses who participated for course credit. Probe delays were varied across three groups of subjects with $n = 40$ (18 female), $n = 38$ (22 female), and $n = 35$ (18 female) in each group.

Materials and Design

Fifty-four pictures—18 pleasant, 18 neutral, and 18 unpleasant—were selected from the International Affective Picture System on the basis of their affective valence ratings[1] (IAPS; Lang, Bradley, & Cuthbert, 2005). Eleven additional pictures that also varied in valence were presented on the trials that were not probed with a startle stimulus. The acoustic startle probe consisted of a 103-dB, 50-ms burst of white noise with instantaneous rise time and was presented binaurally over Telephonic headphones.

Each picture was presented for a 6-s viewing interval, followed by a randomly determined 10–20 s intertrial interval. Startle probes were presented at one of six delays on each trial, distributed across groups such that probes were presented both early and late in the viewing period, as well as during the interpicture interval in each group. In Group 1, probes were delivered at 300, 800, 1300, or 3800 ms after picture onset or 300 or 3800 ms after picture offset. In Group 2, probes were delivered at 500, 1800, 2300, or 5300 ms after picture onset, 500 ms after picture offset, or 500 ms before picture onset. In Group 3, probes were delivered at 50, 150, 3000, or 4500 ms after picture onset, 3800 ms after picture offset, or 500 ms before picture onset. Thus, across the three groups of subjects, 16 different probe delays were used.

[1]The International Affective Picture System and technical manuals (Lang et al., 2005) are available on CD-ROM and can be obtained on request from the authors. IAPS numbers for unpleasant pictures used in Experiment 1 are: 1070, 1090, 1120, 1300, 2120, 3000, 3010, 3100, 3130, 3150, 3530, 6020, 6190, 6200, 6230, 6370, 9040, 9490; for neutral pictures: 2190, 2200, 5500, 7000, 7010, 7020, 7050, 7080, 7090, 7100, 7130, 7150, 7160, 7170, 7180, 7500, 7550, 7700; for pleasant pictures: 1600, 2080, 2250, 4650, 4660, 4680, 7200, 7330, 7350, 8030, 8080, 8200, 8510; 4180, 4210, 4250, 4290, and 4310 for males; 4470, 4490, 4500, 4520, and 4550 for females.

Pictures were arranged in nine blocks of six, such that (1) each of the six probe conditions occurred once in each block and (2) two pictures of each of the three types of valence occurred in each block. Two presentation orders varied the serial position of specific pictures across subjects, and six different timing orders completely counterbalanced probe delay for specific pictures and serial positions across subjects. Pictures were presented using a Kodak Ektagraphic III projector situated in a room adjacent to the experimental room, which rendered slide changes inaudible. Picture offset resulted in a blank (i.e., nonilluminated) screen.

Stimulus control and physiological data acquisition were accomplished using an IBM-compatible computer running VPM data acquisition and reduction software (Cook, 1997). The eyeblink component of the startle response was measured by recording EMG activity from the orbicularis oculi muscle beneath the eye.[2] The raw EMG signal at each site was amplified (30,000), and frequencies below 90 Hz and above 250 Hz were filtered, using a Coulbourn S75-01 bioamplifier. The raw signal was rectified and integrated using a Coulbourn S76-01 contour-following integrator, with a calibrated time constant of 123 ms. The blink response was sampled at 1000 Hz for 50 ms prior to the onset of the startle probe and for 250 ms after probe onset. The startle data were reduced off-line by a program that scored each trial for magnitude and onset latency, using an algorithm developed by Globisch et al. (1993). Trials with zero magnitude were omitted in analyses of onset latency.

Procedure

Following completion of the informed consent and several questionnaires, physiological sensors were attached while the subject reclined in a comfortable chair. The subject was instructed that a series of pictures would be presented, and that each picture should be viewed the entire time it was on the screen. Shortly after picture offset (i.e., 5 s), the subject rated the emotional reaction to the picture in terms of pleasure, arousal, and dominance, using a 20-point line rating displayed on a computer screen; these ratings are not reported here. Finally, the subject was told that occasional noises heard over the headphones could be ignored. After the picture series was finished, a number of additional questionnaires were completed and the subject was debriefed.

Data Analysis and Reduction

Blinks were standardized for each subject, using the mean and standard deviation of reflexes elicited on trials in which the startle probe was presented in the intertrial inteval, when no picture served as a foreground stimulus. For Groups 1 and 3, no-picture startle data were those elicited 3800 ms after picture offset; for Group 2, the no-picture startle data were elicited 500 ms before picture onset.[3] This *z*-standardization procedure removes effects due to normal intersubject variability in blink magnitude across subjects, and yet continues to provide an appropriate comparison condition within each subject (i.e., relative to blinks elicited in the no-picture intertrial interval) which is necessary in order to assess prepulse inhibition effects. These *z* scores were then expressed as *t* scores (i.e., (z * 10)+50) to provide unidirectional data. The raw magnitude data for Group 1 were previously published as a brief report (Bradley et al., 1993).

Because not all subjects contributed data at each probe delay, the main ANOVAs were conducted using picture (i.e., $n = 54$) as the random factor, rather than the subject. In these analyses, standardized *T* scores (as described above) were determined for each subject and for each trial and were averaged across subjects for each picture and probe delay condition. An ANOVA was conducted using picture content (pleasant, neutral, unpleasant) as a between-picture factor and probe delay (15 levels) as a within-picture factor.[4] There were at least 35 subjects contributing to each data point in these analyses. When specific comparisons included data from the same set of subjects (e.g., the simple main effect of picture content at each probe delay), two analyses were conducted and are reported, one using picture as the random factor (labeled F_{pic}), and the more typical analysis that used subject (labeled F_{sub}) as the random factor.

For analyses assessing differences in emotional arousal, the pictures in each of the pleasant and unpleasant picture categories were divided into two sets, based on a median split of their arousal ratings in the IAPS (Lang et al., 2005). For unpleasant pictures, the mean arousal ratings were 5.9 and 6.9 for pictures low and high in arousal, respectively, $F(1,16) = 21.2$, $p = .0003$; for pleasant pictures, the mean arousal ratings were 5.0 and 5.8 for pictures low and high in arousal, respectively, $F(1,23) = 4.42$, $p = .04$. For the 18 neutral pictures, the mean arousal was 2.7. Tests comparing blink magnitude at different probe delays for high or low arousal pictures were conducted with ANOVAS that used picture as the random factor.

Greenhouse–Geisser corrections were applied where relevant.

Results

Figure 1 illustrates blink magnitude for startle probes presented at each delay (from 50 ms after picture onset to 500 ms after picture offset) for pleasant, neutral, and unpleasant pictures. In the main analysis, there were significant effects of probe delay, $F_{pic}(14,714) = 21.99$, $p < .001$, picture content, $F_{pic}(2,51) = 7.80$, $p < .001$, and their interaction $F_{pic}(14,714) = 1.73$, $p < .05$. The interaction of probe delay and picture content was decomposed by assessing (1) simple main effects of probe delay for each picture content, in order to determine the nature of the temporal function for pleasant, neutral, and unpleasant pictures; and (2) simple main effects of picture content at each probe delay, in order to determine the nature of modulation at each time interval.

Temporal Modulation of Startle

There was a significant effect of probe delay for each picture content, $F_{pic}(14,238) = 12.89$, 6.79, and 6.40 for pleasant, neu-

[2]Blinks were measured from both the left and right eyes. ANOVAs conducted on raw blinks with site of measurement (left, right eye) as a factor resulted in no main effects or interactions involving measurement site, and we averaged over site in the main analyses primarily to make use of all the data. Magnitude was standardized for each eye based on the data from the intertrial interval for that eye. Analyses conducted using data from either one eye alone or averaged over eyes produced the same statistical outcome.

[3]The raw magnitude of blinks elicited during the no-picture probe trials were analyzed to determine that there were no effects due to picture content at these probe delays, which was the case. For Group 1, $F_{sub}(2,78) = 0.08$, $p = .92$; for Group 2, $F_{sub}(2,74) = 1.04$, $p = .36$; for Group 3, $F_{sub}(2,68) = 1.08$, $p = .35$. Thus, the mean and standard deviation of blink magnitude for blinks elicited on the no-picture probe trials was used to standardize the distribution of blink data for each subject.

[4]Analyses that could be conducted using raw blink magnitude (i.e., within-group) were similar in all major aspects to those reported here for standardized blink scores.

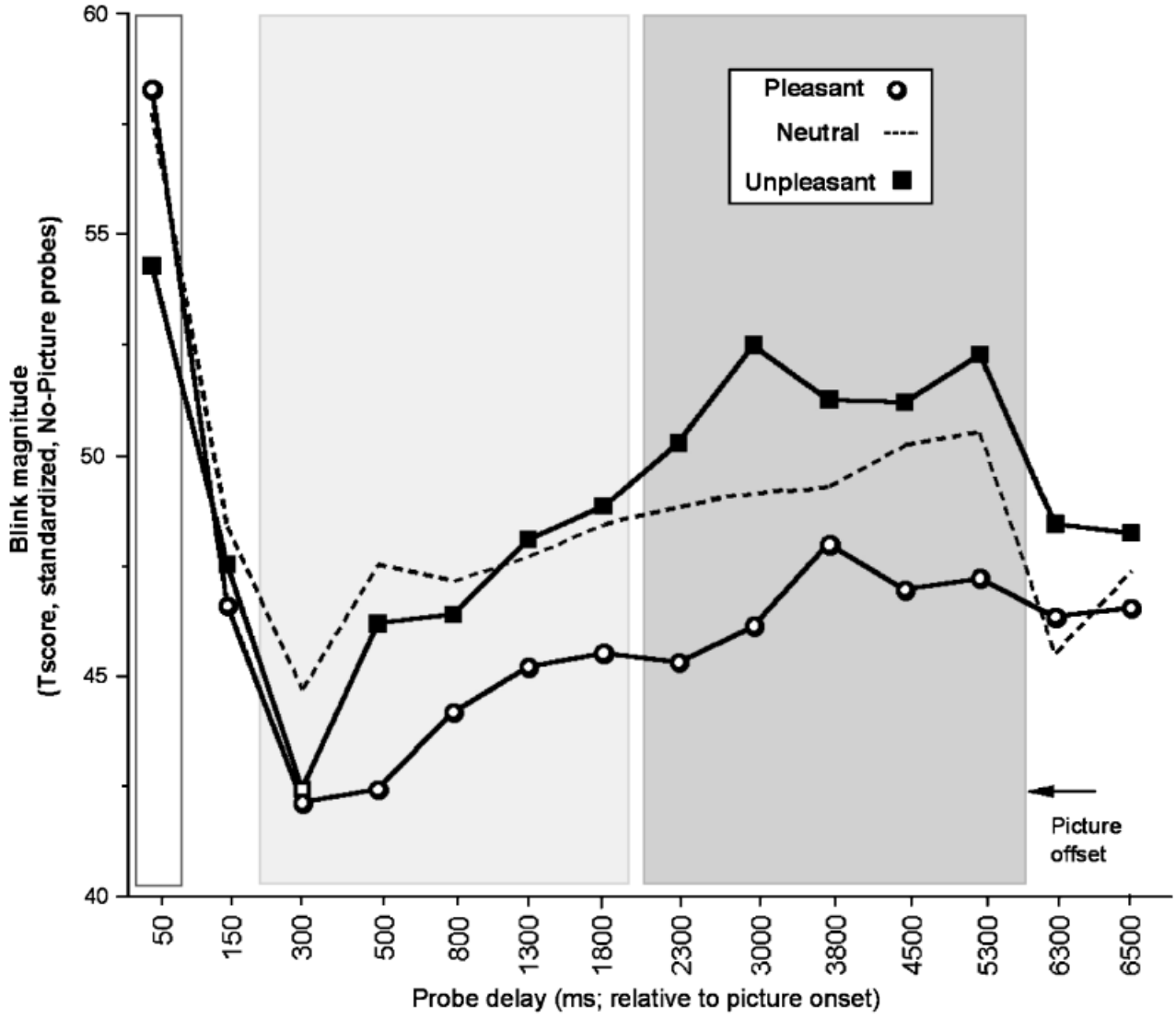

Figure 1. The magnitude of the blink reflex to an acoustic startle probe (standardized with respect to blinks elicited during the no-picture intertrial interval) presented during and after viewing of pleasant, neutral, or unpleasant pictures.

tral, and unpleasant pictures, respectively, all $ps < .001$. Two general effects of probe delay, significant in comparisons evaluating differences across time for each content, are apparent in Figure 1: For all three picture contents, probes presented at 50 ms after picture onset were significantly facilitated, compared to blinks elicited at every other delay, whereas blinks elicited at 300 ms after picture onset were significantly inhibited, compared to blinks elicited at every other delay. Thus, within 250 ms of picture onset, blinks went from maximum facilitation (at 50 ms) to maximum inhibition (at 300 ms) for all picture contents.

Thus, blinks elicited at 50 ms were significantly facilitated, compared to those elicited in the no-picture control distribution (i.e., a mean of 50; $t[53] = 7.4$, $p < .01$), whereas blinks elicited at 150 and 300 ms following picture onset were significantly inhibited (i.e., $t[53] = -2.4$ and -11.6, $ps < .01$). In fact, compared to the no-picture probes, blinks were inhibited by the picture prepulse until 3 s after picture onset ($t[53] = -7.5$, $-7.1, -5.4, -3.4$, and -2.1 for probes presented 500, 800, 1300, 1800, and 2300 after picture onset, respectively). Moreover, immediately following picture offset, blinks again showed inhibition, compared to the no-picture control conditions ($t[53] = -5.1$ and -3.7, $ps < .01$, for probes elicited 300 and 500 ms after picture offset, respectively).

In general, blinks increased from 300 ms to the end of the picture viewing interval, linear $F(1,51) = 73.5$, $p < .001$, and a significant linear trend over this interval was obtained for each picture content. On the other hand, the size of the linear increase varied with picture content, Content $\times$ Probe Delay, linear $F(2,51) = 3.24$, $p < .05$. Unpleasant pictures prompted a steeper linear across across time than did neutral pictures, $F(1,34) = 4.84$, $p < .05$, and tended to show a steeper increase than pleasant pictures, $F(1,34) = 3.26$, $p = .08$, whereas reflexes elicited when viewing neutral and pleasant pictures clearly did not differ in slope, $F < 1$.

Picture Content Effects

For probes presented at 50 or 150 ms after picture onset, there were no significant effects of picture content, indicating that initial reflex facilitation and inhibition are not modulated by the emotionality of the picture.

At 300 ms after picture onset, a quadratic trend suggested that reflexes were more inhibited for emotional (pleasant or unpleasant), compared to neutral, pictures, $F_{pic}(1,52) = 4.04$, $p < .05$. By 500 ms, however, blinks were significantly larger when viewing unpleasant, compared to pleasant, pictures, $F_{pic}(1,34) = 9.4$, $p < .005$, and blinks continued to be potentiated when viewing unpleasant, compared to pleasant, pictures throughout picture viewing ($F_{pic}[1,34] = 9.3$, 2.7, 4.6, 5.1, 8.4, 8.9, 3.6, 5.5, and 8.9 for probes presented at 500, 800, 1300, 1800, 2300, 3000, 3800, 4500, and 5300, respectively). For probes presented at 800 and 4500 ms, these effects were marginal when picture served as a random factor ($p = .11$, .07), but significant when subject served as the random factor ($ps < .05$). When compared to blinks elicited during neutral pictures, blinks were significantly inhibited for pleasant materials from 500 ms to picture offset (with a single exception at 3800 ms); blinks elicited when viewing unpleasant pictures were not significantly different from those elicited during neutral pictures at any delay in either set of analyses.

Figure 1 illustrates that emotional content of the picture began to affect reflex magnitude around 300 ms and continued throughout picture presentation. Based on these modulatory effects in Figure 1, reflexes were averaged into an initial viewing region (300 ms to 1 s) and a later viewing region (1 s to 6 s). Figure 2 illustrates these data. In the initial processing region, a significant effect of picture content, $F_{pic}(2,51) = 7.30$, $p < .005$, $F_{sub}(2,154) = 12.43$, $p < .001$, was accompanied by a significant quadratic trend, $F_{pic}(1,52) = 8.29$, $p < .005$, $F_{sub}(1,77) = 9.85$, $p < .001$. Blinks elicited during pleasant pictures were inhibited, compared to neutral pictures, $F_{pic}(1,34) = 18.42$, $p < .001$, $F_{sub}(1,77) = 19.24$, $p < .001$, as well as to unpleasant pictures, $F_{pic}(1,34) = 4.77$, $p = .036$, $F_{sub}(1,77) = 16.76$, $p < .001$. For unpleasant pictures, blinks were not different from those elicited during neutral picture processing, $F_{pic}(1,34) = 2.07$, $p = .16$, $F_{sub}(1,77) = 1.54$, $p = .22$.

Later in the viewing interval (>1 s), a significant effect of picture content, $F_{pic}(2,51) = 10.74$, $p < .001$, $F_{sub}(2,224) = 31.76$, $p < .001$, indicated that blinks elicited during unpleasant pictures were significantly larger than those elicited during pleasant pictures, $F_{pic}(1,34) = 17.41$, $p < .001$, $F_{sub}(1,112) = 60.01$, $p < .001$.

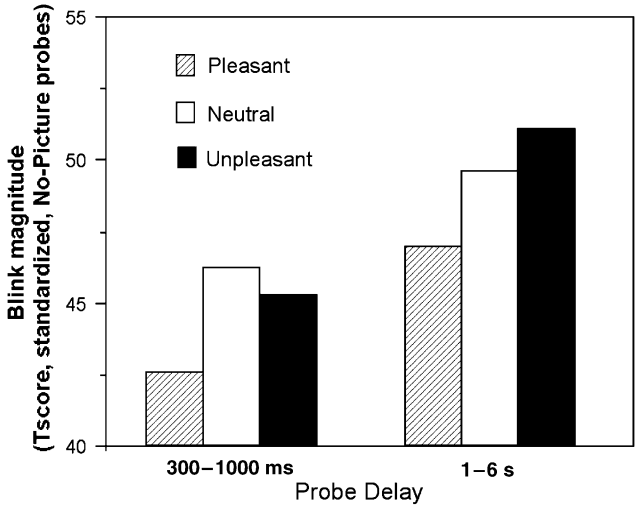

Figure 2. Startle blink magnitude averaged over an early region of picture viewing (300 ms to 1 s) and a later region (1–6 s) shows effects of early inhibition that is greater for emotional, compared to neutral, pictures, and affective modulation in the later region.

Compared to blinks elicited during neutral pictures, blinks were inhibited when viewing pleasant pictures, $F_{pic}(1,34) = 11.32$, $p < .005$, $F_{sub}(1,112) = 35.14$, $p < .001$, and facilitated when viewing unpleasant pictures in one analysis, $F_{sub}(1,110) = 6.66$, $p < .05$, and marginal in the other, $F_{pic}(1,34) = 2.31$, $p = .14$.

Emotional Arousal

Consistent with the previous analyses, picture content did not significantly affect startle magnitude for probes presented 50 or 150 ms after picture onset in the analysis of either the high arousal or low arousal pictures. Figure 3 illustrates blink magnitude in the initial (300 ms to 1 s) and later (1 s to 6 s) viewing regions for pictures rated low and high in emotional arousal. In the early processing region, blink magnitude was modulated by picture content for both high and low arousal pictures, $F_{pic}(2,33) = 4.98$, $p < .05$ for highly arousing pictures and $F_{pic}(2,33) = 5.17$, $p < .05$ for low arousal pictures, with significant quadratic trends in each case, $F_{pic}(1,34) = 8.89$ and 5.61, $ps < .02$ for low and high arousal pictures, respectively. Separate comparisons indicated that, for both high and low arousal pictures, blinks elicited during pleasant pictures were significantly inhibited, compared to neutral stimuli, $F_{pic}(1,25) = 13.24$, $p < .005$ for high arousal, $F_{pic}(1,25) = 10.17$, $p < .005$ for low arousal. For unpleasant pictures, there was no significant difference from blinks elicited during neutral pictures.

Later in the viewing interval, blink modulation for pictures judged high and low in emotional arousal differed. For highly

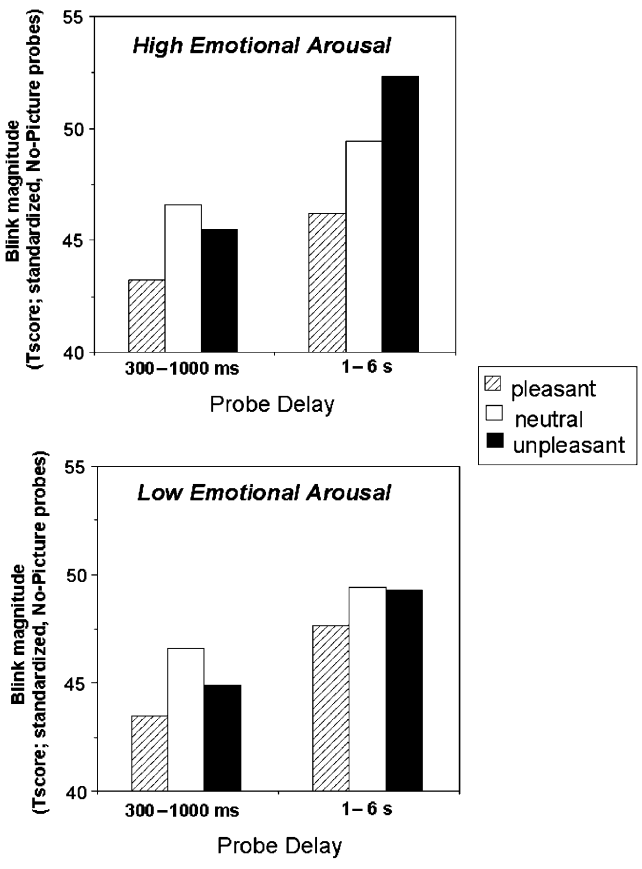

Figure 3. Experiment 1. Blink magnitude for high arousal pictures (top panel) and low arousal picture (bottom panel) for startle reflexes averaged over probes presented in an early region (300 ms–1 s) and a later region (1–6 s).

arousing pictures, a main effect of picture content, $F_{pic}(2,33) = 15.37$, $p < .001$, indicated that blinks were facilitated when viewing unpleasant, compared to neutral, pictures $F_{pic}(1,25) = 6.59$, $p < .05$, as well as inhibited when viewing pleasant, compared to neutral, pictures $F_{pic}(1,25) = 17.88$, $p < .001$. Clearly, blinks were potentiated when viewing unpleasant, compared to pleasant, pictures, $F_{pic}(1,16) = 24.37$, $p < .001$. For low arousal pictures, on the other hand, picture valence did not reach significance in this region, $F_{pic}(2,33) = 1.78$, $p = .18$.

Startle Onset Latency

Table 1 lists the mean onset latency for startle probes delivered at different delays before and after picture onset. In the overall analysis, there was a significant effect of probe delay, $F_{pic}(15,765) = 8.38$, $p < .001$. Blinks elicited at 50 ms and 150 ms after picture onset and those presented immediately following picture offset (300 ms) were significantly faster than probes presented at all other delays.

A marginally significant main effect of picture content, $F_{pic}(2,51) = 2.98$, $p = .06$, indicated that overall latencies were faster for probes presented during unpleasant pictures, compared to pleasant pictures, $F_{pic}(1,34) = 5.05$, $p < .05$ (and were marginally faster than when viewing neutral pictures, $F_{pic}[1,34] = 3.20$, $p = .08$). Separate tests at each probe delay indicated faster latency for unpleasant, compared to pleasant, pictures for all probe delays in a window from 2300 ms to 4500 ms after picture onset. Before and after this time window, onset latency did not differ as a function of picture content. In this time window, highly arousing unpleasant pictures prompted faster blinks (mean = 36.9 ms) than pleasant arousing materials (mean = 40.6; $F[1,16] = 9.07$, $p < .05$), but pleasant and unpleasant pictures low in arousal did not differ in onset latency (means = 39.5 and 38.2 ms, respectively).

EXPERIMENT 2

In Experiment 1, blink inhibition decreased across the viewing interval suggesting a general decrease in attentional resources. In Experiment 2, we more specifically assessed the allocation of attention over the picture interval using the P3 component of the event-related potential (ERP) to the startle probe as a measure of attention. Previously, we found that the P3 component to a startling acoustic probe is inhibited when pleasant or unpleasant pictures serve as the foreground stimulus, compared to neutral pictures (e.g., Schupp, Cuthbert, Bradley, Birbaumer, & Lang, 1997), and interpreted these findings as indicating that less attention is available for probe processing when emotional, compared to neutral, pictures were being viewed. In Experiment 2, we again varied the delay at which probes were delivered following picture onset to track attention allocation from early to late in the viewing interval and as it varies with picture emotionality.

As a further assessment of attentional differences prompted by stimulus processing, we included a set of visual stimuli that involved no semantic content, consisting of only an illuminated screen the same size as the pictures that was uniformly gray or colored. If blink magnitude reflects attentional processing prompted by the information content in the picture, we expected that these "blank" pictures would prompt less blink inhibition, particularly early in the viewing inteval, than pictures with semantic content (including neutral pictures). Based on the data from Experiment 1, all of the affective pictures presented in Experiment 2 were highly arousing, allowing the best opportunity to assess attentional differences during viewing of emotionally arousing, compared to neutral, materials.

Table 1. *Mean Onset Latency (in Milliseconds) at Each Probe Delay for Pleasant, Neutral, and Unpleasant Pictures*

	Picture content			
Probe delay (ms)	All pictures	Pleasant	Neutral	Unpleasant
50	34.9 (2.9)	34.5 (3.9)	35.1 (3.2)	35.1 (4.0)
150	36.2 (2.8)	36.4 (6.4)	37.1 (6.2)	35.0 (4.4)
300	39.9 (3.8)	40.7 (5.1)	38.8 (6.0)	40.2 (4.7)
500	41.7 (3.2)	41.3 (4.7)	41.8 (4.3)	41.9 (5.8)
800	39.3 (3.9)	40.7 (5.5)	38.3 (5.0)	38.9 (4.9)
1300	41.4 (2.9)	42.3 (3.9)	40.3 (2.4)	41.7 (6.1)
1800	38.2 (3.3)	38.2 (3.7)	38.3 (4.0)	38.0 (3.2)
2300	39.5 (2.4)	40.8 (4.0)	39.9 (4.0)	38.0 (2.3)
3000	39.8 (2.9)	40.7 (4.1)	39.7 (5.7)	39.0 (4.3)
3800	37.9 (3.2)	38.0 (3.5)	39.2 (5.0)	36.5 (3.4)
4500	39.2 (2.6)	40.6 (5.8)	40.2 (5.4)	36.8 (3.7)
5300	39.1 (2.9)	39.7 (2.9)	39.2 (2.1)	38.5 (4.1)
6300	37.2 (2.9)	35.6 (5.0)	38.3 (3.7)	37.5 (8.6)
6550	39.9 (3.3)	39.7 (4.3)	39.3 (3.6)	40.7 (4.9)
No picture	39.4 (1.9)	39.4 (2.8)	40.1 (2.4)	38.8 (2.6)

Notes: Standard deviations are in parentheses. Latencies underlined in the second column are significantly faster than those that are not underlined in that column.

Method

Participants

The participants were 40 (23 female) members of an Introductory Psychology course at the University of Florida. Because of equipment failures and/or excessive artifacts, data were missing, with the final *N*: ERPs: $n = 35$; startle reflexes, $n = 39$.

Materials and Design

Seventy-two pictures were selected from the International Affective Picture System (Lang et al., 2005) to comprise 24 pleasant, 24 neutral, and 24 unpleasant pictures.[5] Highly arousing picture contents were used that included erotic couples, opposite sex erotica, threatening humans, threatening animals, and mutilated bodies. Neutral contents included humans and objects. Twenty-four additional blank pictures consisted of either grayscale or color backgrounds, with no semantic content. Each picture was presented for 6 s, separated by a randomly determined 10–20-s intertrial interval.

The startle stimulus and measurement of the blink response were as described in Experiment 1. Probes were presented at 300, 1500, 3500, or 4500 ms after picture onset, or in the no-picture intertrial interval (5 s after picture offset). The P3 response to the startle probe was measured using a SanDiego amplifier and LabView software on a Macintosh computer. The International 10–20 system was followed for 21 electrode sites: Fp1, Fp2, Af7, Af8, F7, F3, Fz, F4, F8, T7, C3, Cz, C4, T8, P7, P3, Pz, P4, P8, O1,O2; in addition, electrodes were placed on the mastoids (A1, A2). All channels were referenced to Cz and digitally

[5]The IAPS numbers for unpleasant pictures used in Experiment 2 are: Pleasant: 4001, 4002, 4003, 4005, 4210, 4232, 4235, 4240, 4250, 4290, 4300, 4310, 4611, 4650, 4651, 4652, 4658, 4659, 4664, 4669, 4672, 4680, 4800, 4810; Neutral: 2190, 2200, 2210, 2230, 2270, 2381, 2440, 2480, 2570, 2830, 2850, 7002, 7009, 7010, 7020, 7030, 7040, 7050, 7080, 7175, 7190, 7233, 7235, 9070; Unpleasant: 1050, 1052, 1113, 1120, 1201, 1300, 1930, 3000, 3010, 3053, 3060, 3071, 3080, 3102, 3110, 3130, 3150, 3400, 3530, 6260, 6350, 6510, 6540, 9405.

re-referenced off-line to linked mastoids. Vertical and horizontal eye movements were recorded using Sensormedics silver/silver chloride miniature electrodes to account for ocular artifacts. A 35-Hz high-frequency cutoff and a 3-s time constant (0.053 Hz low-frequency cutoff) were used to record all cortical and ocular channels. The data sampling rate was 125 Hz/channel. To correct for vertical and horizontal ocular artifacts, an eye movement artifact correction procedure (Gratton, Coles, & Donchin, 1983; Miller, Gratton, & Yee, 1988) was applied to the EEG recordings.

Procedure

The procedure was similar to that described in Experiment 1. The main difference was that evaluative ratings were not acquired after picture presentation (as the pictures in each valence category were from very few categories). The intertrial interval varied from 10 to 20 s across trials.

Data Analysis

The blink response was scored and analyzed as described in Experiment 1. For the probe P3 response, separate epochs of stimulus-synchronized EEG traces were extracted from the 10-s EEG recording, extending from 120 ms before until 1 s after probe onset. The P3 wave of the ERP was scored by determining the base-to-peak amplitudes on averaged waveforms for each subject, valence category, startle probe time, and electrode site. The P3 component was scored within a window beginning at N2 latency and continuing until 504 ms. When an unequivocal positive peak could not be detected, an inflection point was calculated such that the smallest vertical distance between adjacent points within the time window defined the location for the latency of the P3, with the corresponding amplitude as the peak score. P3 responses to startle probes presented at 300 ms after picture onset were difficult to score, as these ERPs occurred at the same time as the P3 response to the picture stimulus itself. Thus, these P3 responses were excluded from further analysis.

Initial analyses of the P3 data used a three-way ANOVA that included the factors affective valence (pleasant, neutral, and unpleasant) and topography (posterior–anterior and left–right dimensions). Replicating previous results (e.g., Schupp et al., 1997) the probe P3 component was significantly modulated as a function of affective valence. No main effect or interactions were found involving the laterality (left–right) factor. Also, replicating previous effects, affective modulation was greatest at parietal recording sites for the probe P3. Accordingly, for brevity, ERP analyses for specific picture contents included Pz only.

Results

Startle Blink

Figure 4 (top) illustrates blink magnitude for startle stimuli presented when viewing pleasant, neutral, unpleasant, or blank pictures at different probe delays. Probe delay affected startle reflex magnitude, $F(3,36) = 10.19$, $p < .001$, with reflexes increasing in size from the earlier to the later probe positions, linear trend $F(1,38) = 31.38$, $p < .001$. As in Experiment 1, effects of picture content on blink magnitude were dependent on probe delay, interaction $F(9,30) = 3.13$, $p < .01$, and this interaction was decomposed by assessing (1) simple main effects of probe delay for each picture content and (2) simple main effects of picture content at each probe delay.

A main effect of probe delay was found for pleasant, neutral, and unpleasant pictures, Fs(3,36) = 3.89, 4.46, and 18.56, respectively. Compared to no-picture probes (i.e., T score = 50), reflexes were inhibited for probes presented at 300 or 1500 ms after picture onset, $t(38) = -6.5$ and -3.2, respectively, ps $< .005$, but not for those presented at 3500 or 4500 ms. A significant linear trend across delay was obtained for pictures of unpleasant, neutral, or pleasant content, indicating an increase in blink magnitude from 300 to 4500 ms after picture onset. For blank pictures, on the other hand, the main effect of probe delay was not significant, $F(3,36) = 2.047$, $p = .12$, suggesting relatively little change in reflex magnitude across time for these content-free stimuli.

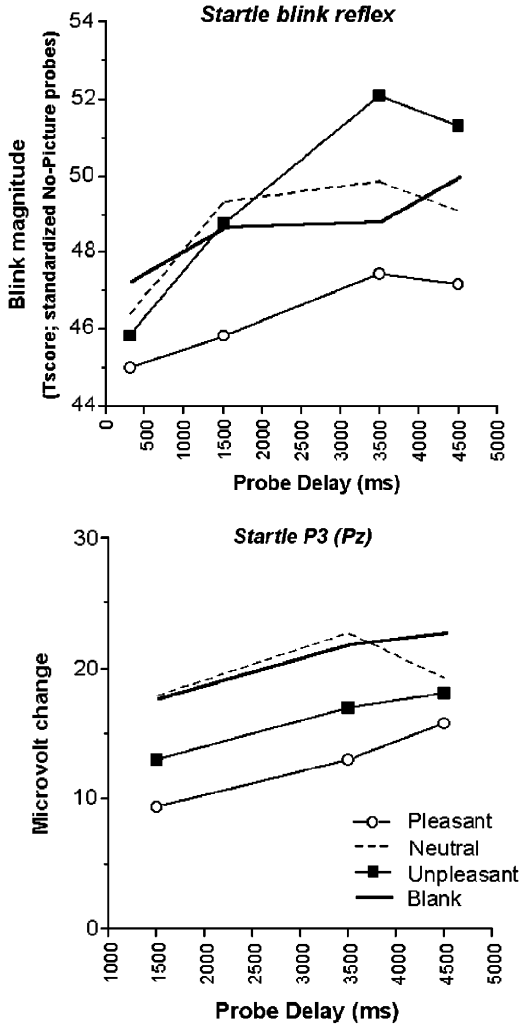

Figure 4. Experiment 2. Top: Blink magnitude when viewing emotional, neutral, and blank pictures at varying probe delays following picture onset. Bottom: Probe P3 magnitude when viewing emotional, neutral, or blank pictures at varying probe delays following picture onset.

Picture content affected blink magnitude for probes presented 300 ms after picture onset, with smaller blinks elicited when viewing unpleasant or pleasant pictures, compared to blank slides (quadratic $F[2,37] = 8.7$, $p = .001$; Fs[1,38] = 16.69 and 7.92 for pleasant and unpleasant pictures, respectively). Pleasant pictures also tended to prompt smaller reflexes than neutral pictures at this probe delay, $F(1,38) = 3.98$, $p = .05$, whereas blinks elicited when viewing neutral and blank pictures were not significantly different. At 1500 ms after picture onset, blinks were significantly inhibited when viewing pleasant pictures compared to each of the other picture contents, Fs(1,38) > 13, ps $< .001$. By

3500 ms, on the other hand, larger reflexes were elicited when viewing unpleasant, compared to neutral, $F(1,38) = 5.12$, $p < .05$, blank, $F(1,38) = 11.5$, $p < 0.01$, or pleasant pictures, $F(1,38) = 17.13$, $p < .001$, and this pattern continued for probes presented at 4500 ms (Fs[1,38] = 11.66 and 19.35 for unpleasant compared to neutral and pleasant pictures, respectively, $p < .05$). At the two later delays, pleasant pictures also prompted significantly smaller blink reflexes than neutral pictures, Fs(1, 38) = 4.10, 8.50, ps $< .05$.

Startle Probe ERPs

Figure 5 shows the general event-related potential (averaged over probe delays) elicited by the acoustic startle probe in different viewing conditions and illustrates that picture content affected ERP magnitude beginning around 250 ms after probe onset.

In the main analysis of P3 amplitude, there were main effects of probe delay, $F(2,33) = 16.71$, $p < .001$, and of picture content, $F(3,32) = 29.10$, $p < .001$, but the interaction of picture content and probe delay was not significant. As illustrated in Figure 4 (bottom), probe P3 amplitudes were smaller for probes presented at 1500 ms compared to probes presented later in the viewing interval (Fs[1,34] = 27.29 and 25.72 for 3500 ms and 4500 ms, respectively, ps $< .001$), suggesting greater attention allocation to picture foregrounds early in the viewing interval. The amplitude of the probe P3 did not significantly differ for probes presented at 3500 and 4500 ms postonset, but both were still significantly smaller than P3 amplitude for probes presented in the interpicture interval (mean = 22 mV; Fs(1,34) = 5.15, $p < .05$ for 3500 ms probes; 4.25, $p < .05$ for 4500 probes), suggesting sustained attention during picture viewing.

Probe P3 amplitudes when viewing pleasant and unpleasant pictures were smaller than when viewing neutral (F[1,35] = 56.61 and 12.16 for pleasant and unpleasant, respectively), or blank pictures (F[1,35] = 74.73 and 16.91 for pleasant and unpleasant, respectively) throughout the picture viewing interval. In addition, pleasant pictures prompted smaller probe P3 response than unpleasant stimuli, $F(1,35) = 12.57$, $p < .01$, whereas neutral and blank pictures never differed in probe P3 amplitude.

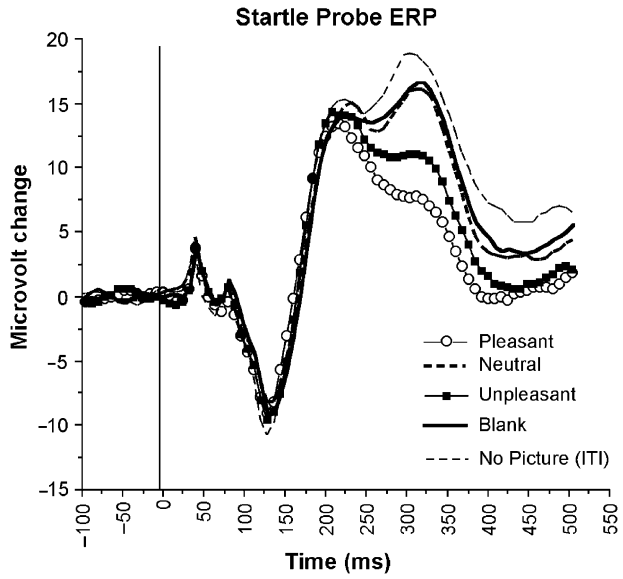

Figure 5. Experiment 2. Event-related potential (Pz) to an acoustic startle probe presented when viewing pleasant, neutral, unpleasant or blank pictures or during the interpicture interval.

General Discussion

A number of different modulatory blink effects occurred during a 6-s picture viewing interval, including (1) A very early facilitatory ($\leq$ 50 postonset) effect for both blink magnitude and onset latency that was not affected by picture content, (2) an early inhibitory effect (100–250 ms) on blink magnitude and facilitatory effect on latency that was not affected by picture content, (3) an inhibitory effect on blink magnitude (without accompanying latency changes) that peaked around 300 ms and was variably affected by picture content, and (4) affective modulation, in which unpleasant pictures prompted larger reflexes than pleasant pictures, beginning within a half second after picture onset and continuing throughout the viewing interval. At certain time periods, more than one of these processes appeared to be coactive, with net reflex magnitude presumably reflecting contributions from more than one factor. Below, we discuss these different modulatory effects in terms of hypothetical processes that reflect (1) intersensory (i.e., picture onset–probe onset) integration, (2) sensory prepulse inhibition, (3) attentional inhibition, and (4) affective modulation.

Picture-Probe Integration

Probes presented almost concurrent with picture onset (i.e., 50 ms postonset) elicited large, fast blink reflexes that constituted the largest modulatory effect in the study. Although blink inhibition has been the hallmark of prepulse studies, rapid *facilitation* of blink magnitude for probes presented shortly after stimulus onset was reported in 1933 by Hilgard, who found reflex potentiation when a visual prepulse was presented 25–50 ms before an acoustic startle probe. In fact, most reports of early magnitude facilitation in animals and humans involve cross-modal stimulation (Blumenthal & Gescheider, 1987; Campeau & Davis, 1995; Graham, 1980; Sanes, 1984; Sarno, Blumenthal, & Boelhouwer, 1997).

In their interstimulus integration account of prepulse facilitation, Boelhouwer and associates (1991) hypothesized that reflex facilitation reflects summation that occurs when activation from different sensory sources reaches a common neural location. For a visual prepulse and an acoustic startle probe, magnitude facilitation occurred between 10 and 50 ms after onset (Sarno et al., 1997), which is quite consistent with the temporal window in which reflexes were facilitated for picture-probe stimuli in the current study. On the other hand, a reflex priming account of prepulse facilitation (e.g., Sarno et al., 1997; Schicatano, Peshor, Gopalaswamy, Sahay, & Evinger, 2000) posits that blinks are facilitated when a prepulse engages the reflex circuit, priming the blink response. Gating picture onset in the current paradigm would provide a test between reflex priming and intersensory integration accounts of early reflex facilitation. Regardless of which mechanism is supported, the data indicate that this early facilitation is quite large, very brief, and unaffected by stimulus content.

Sensory Prepulse Inhibition

By about 150 ms after picture onset, blink magnitude was significantly inhibited, whereas blink onset latency continued to be facilitated. Picture content still did not modulate these effects. In general, onset latency is facilitated in prepulse paradigms (Lipp, Blumenthal, & Adam, 2001) and the discordant effects of a prepulse on startle measures—magnitude inhibition and latency facilitation—have been interpreted as reflecting the operation of two neural systems, with magnitude reflecting a system sensitive to stimulus onset (a fast rise-time system) and latency reflecting a

system sensitive to stimulus duration (a slow time-constant system; Graham & Murray, 1977).

We interpret these reflex effects, which were not modulated by picture content, as reflecting a form of *sensory prepulse inhibition*—that is, an obligatory inhibition of reflex magnitude that occurs whenever another stimulus precedes the startle probe by a brief delay and which is often interpreted as due to sensory gating. Sensory prepulse inhibition is held to occur on the first trial a stimulus is presented (i.e., is not learned), is resistant to habituation, and is probably the predominant mechanism underlying blink inhibition when simple noninformative stimuli (e.g., tones or lights) serve as prepulse stimuli. The current data are consistent with a sensory inhibition (gating) hypothesis, as there was no evidence of modulation in this time window by picture content. Although not previously linked with latency facilitation, magnitude inhibition was also clearly accompanied by latency facilitation for probes presented shortly after picture *offset* in the current study, suggesting that a change in the sensory array is a primary variable underlying these startle effects.

Attentional Prepulse Inhibition

For all pictures in Experiment 1, startle probes presented between 300 ms and 1000 ms after onset elicited inhibited blinks, compared to those elicited in the no-picture intertrial interval, and significant inhibition of the startle reflex continued up to 3000 ms after picture onset, suggesting a relatively long inhibitory interval for these complex picture prepulses, compared to simpler tone stimuli. On the other hand, reflex latency was not facilitated in this time window, and picture content now produced significant effects on reflex magnitude. These general inhibitory effects—long-lasting and affected by stimulus content—are hypothesized here to reflect reflex inhibition by attention, in which resource allocation to affectively engaging foregrounds decreases the number of resources available for processing the acoustic startle probe.

Attention allocation across the viewing interval was measured in Experiment 2, using the amplitude of the P3 component of the ERP to the startle probe. Consistent with the hypothesis that attentional resource allocation is greatest during initial processing, probe P3 was smallest early in the viewing interval for all visual stimuli. Moreover, emotional pictures prompted greater attention allocation throughout the viewing interval than either neutral or blank pictures, as evidenced by significant P3 differences at every probe delay. It is of interest that pictures depicting affectively neutral content were no different from blank slides in either blink magnitude or P3 amplitude, suggesting little modulation by either attention or affect for pictures of low motivational relevance. On the other hand, the pronounced attenuation of the P3 response for affectively engaging pictures, compared to neutral or blank pictures throughout the viewing interval, suggests a sustained difference in attention allocation that presumably contributes an inhibitory component to the startle blink response at all probe delays.

The hypothesis that attention inhibits the startle reflex late in the picture viewing interval is different from a very common finding of late startle facilitation (compared to the intertrial interval) when simple prepulse stimuli (e.g., tones, letters, lights) precede the startle probe. Late prepulse facilitation is found in both task and no-task contexts, although it can be larger when attention is directed toward the prepulse stimulus (e.g., Lipp et al., 2001). Although it initially appeared that stimulus modality is important in modulating this effect, more recent data suggest modality is not central (e.g., Lipp, Siddle, & Dall, 2000). It is possible that, for simple prepulse stimuli such as tones or letters, prepulse facilitation might reflect postencoding processes such as perceptual memory, motor readiness, or response engagement rather than differences in resource allocation that only become apparent using more complex prepulse stimuli, such as affective pictures.

For pleasant pictures, blink inhibition was apparent early in the viewing interval (300 ms), and the amplitude of the probe P3 was smallest, replicating the strong P3 attenuation the startle probe found previously when viewing erotic stimuli (Schupp et al., 2004) and consistent with heightened attention. We previously reported that startle reflexes were significantly inhibited when viewing unpleasant, compared to neutral, pictures early in the viewing interval (Bradley et al., 1993). When blink magnitude during picture viewing was explicitly compared to blinks elicited by no-picture probes (via standardization) in the current analysis, this difference was less apparent, although, in Experiment 2, blinks continued to be significantly inhibited early in the viewing interval when viewing unpleasant, compared to blank, pictures, supporting the idea that reflex inhibition prompted by increased attention can be a component of early blink modulation for aversive contents. As noted below, however, concurrent potentiation due to reflex priming can attenuate these inhibitory effects. Taken together, it appears that attentional processing has sustained inhibitory effects on the startle blink reflex that are maximal early in the viewing period and for affectively engaging pictures.

That these effects may reflect differential attention allocation to emotional pictures is supported by ERPs measured during picture viewing itself, which consistently find a larger late positive potential when viewing emotional (pleasant or unpleasant), compared to neutral pictures, which begins around 300 ms after picture onset and develops into a positive slow wave over centroparietal sensors (e.g., Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000; Palomba et al., 1997). Two additional relationships between the late positive potential during picture viewing and the probe P3 responses measured here are consistent with an attention allocation account: First, the late positive potential measured during picture viewing is larger for erotic content than for pictures depicting mutilation or threat (Schupp et al., 2004), which meshes well with the finding in Experiment 2 that the smallest probe P3 amplitudes were obtained when viewing erotica. Second, the positive slow wave that develops across picture viewing generally decreases in amplitude across time (Cuthbert et al., 2000) consistent with the finding in Experiment 2 that attention to the picture decreases across time. Taken together, the amplitude of the late positive potential measured during picture viewing and the amplitude of the P3 component to a secondary startle probe show a reciprocal relationship consistent with an interpretation of heightened attention/perceptual processing of affectively engaging pictures.

Coactivation of Sensory and Attentional Inhibition

If sensory inhibition and attentional inhibition reflect different inhibitory processes, reflex modulation early in the picture viewing should reflect their net effect (perhaps additive). In Experiment 1, maximum inhibition occurred between 150 and 500 ms, suggesting a relatively narrow window in which these processess might be coactive. On the other hand, attentional inhibition may be mediated by the same neurocircuitry as that implicated in studies of sensory gating. Thus far, in fact, the common assumption is that prepulse inhibition and attentional inhibition

reflect the same gating process (e.g., Filion et al., 1993). The routine use of simple prepulse stimuli (e.g., brief tones), may have made it difficult to discern independent effects, as simple prepulses are data limited (Norman & Bobrow, 1975) and may not show the benefits of additional resource allocation. The use of a relatively more complex picture as a prepulse stimulus, on the other hand, apparently results in a more extended processing period, allowing further tests of how a sensory prepulse and/or attention modulate reflex reactions. We hypothesize that sensory prepulse inhibition is brief, obligatory, and does not habituate, whereas attentional reflex inhibition, because it reflects resource requirements, is longer lasting, more susceptible to motivational control, and can habituate. It would not be surprising to find that these processes are subserved by different neural circuits. These hypotheses can clearly be tested in future psychophysiological and neuroimaging studies.

Aversive Potentiation: The Defense Cascade

Reflex potentiation when viewing unpleasant, compared to pleasant, pictures began around 500 ms after picture onset in Experiment 1 and continued throughout the picture viewing interval. On the other hand, compared to neutral pictures, reflexes elicited when viewing unpleasant pictures were not potentiated during the initial second of picture viewing for aversive contents that were either high or low in emotional arousal. Rather, blinks showed significant potentiation when viewing unpleasant, compared to neutral, pictures only in the later stages of picture viewing and only for highly arousing aversive materials. These data are consistent with previous studies finding greatest startle potentiation for highly arousing unpleasant pictures (e.g., Bradley et al., 2001).

That strength of defensive activation is important for hedonic modulation of the startle reflex is consistent with the defense cascade model (Lang et al., 1997). The defense cascade model also notes that at lower levels of defensive activation, reflexes associated with orienting, attention, and information intake dominate in aversive responding. That is, at this early stage of defense (i.e., when a threat is at distance), attention (with freezing), rather than action, is the primary process initiated by the brain's motivational system. Consistent with this view, unpleasant pictures rated low in arousal prompt startle reflex inhibition rather than the potentiation found for more arousing unpleasant stimuli (Bradley et al., 2001; Cuthbert et al., 1996). In the current study, a similar relationship appears to play out in time: Early in picture processing, defensive activation is at an early stage in which information intake and sensory procesing dominate, attenuating the reflexive startle response. This was obtained for unpleasant pictures rated low or high in arousal. With increased exposure, unpleasant pictures rated as highly arousing begin to prompt increasingly more defensive activation, resulting in reflex priming that leads to significant startle potentiation (over neutral pictures) later in the viewing interval. This later aversive potentiation is apparently due to the priming of defensive actions (rather than decreasing attentional allocation), as shown by the sustained difference in probe P3 amplitude between unpleasant and neutral pictures throughout picture viewing, which suggests a constant (inhibitory) attentional modulation for aversive cues.

When both of these modulatory processes are active (assuming their independence), net reflex magnitude for unpleasant pictures will reflect both differential attention allocation and defensive priming. Thus, inhibitory effects due to increased attention will subtract from defensive potentiation, rendering blink magnitude when viewing unpleasant pictures not significantly different from that elicited when viewing neutral pictures. This is the pattern generally obtained early in the picture viewing interval. Moreover, when pictures are presented briefly (e.g., for 500 ms), blink reflexes are potentiated when viewing unpleasant, compared to pleasant stimuli, but not compared to neutral, which is consistent with the hypothesis that brief presentation results in relatively weak defensive activation (Codispoti, Bradley, & Lang, 2001). Waters, Lipp, and Spence (2005) recently interpreted a similar lack of modulation for unpleasant, compared to neutral, pictures as resulting from the use of relatively low arousal pictures in their study, which included children as participants.

Taken together, the data suggest that reflex potentiation for unpleasant, compared to neutral, pictures early in the picture viewing interval occurs primarily when defensive activation is intense. Consistent with this, Globisch, Hamm, Stevens, and Öhman (1999) found significant startle potentiation at 250 ms after picture onset when snake phobics viewed snake pictures, which are presumably rapidly resolved and defensively very activating for these frightened folks. More recently, Stanley and Knight (2004) reported early potentiation for specifically fearful, compared to neutral, pictures (although the statistical test actually encompassed both early and late probe times). On the other hand, Levenston et al. (2000) and Dichter et al. (2005) both observed early inhibition for unpleasant, compared to neutral, pictures in a passive viewing context. Whether attentional inhibition or defensive priming will dominate in modulating the startle reflex during the initial stages of unpleasant picture viewing will depend upon the threat intensity of the aversive material.

Appetitive Inhibition

Reflexes were inhibited when viewing pleasant pictures, compared to neutral stimuli, for probes presented from 300 ms on, and these inhibitory effects were strong and of long duration in both Experiments 1 and 2. We have hypothesized that inhibition of the startle reflex during pleasant picture viewing may reflect attenuation of defensive reflexes when appetitive motivation is high (Lang, Bradley, & Cuthbert, 1990). Finding a similar pattern of modulation with a different defensive reflex (particularly one that is also not modulated by attention) would assist in addressing this question, as would finding potentiation of an appetitive reflex (e.g., salivation) in the context of pleasant stimuli (White, 1978).

On the other hand, it is possible that startle inhibition during pleasant picture viewing is primarily modulated by attentional processes. That is, we presume that, as for the defense system, appetitive activation initially prompts greater resource allocation, orienting, and information intake, in the service of selecting an appropriate consummatory response. Moreover, for symbolic picture cues, appropriate appetitive actions can never be instrumental (e.g., ingestion, copulation, etc.), suggesting that increased attention may be the primary mode of appetitive approach in this affective context. The finding of greater attenuation of the probe P3 response throughout the viewing interval for pleasant, compared to unpleasant, pictures in Experiment 2 is consistent with this interpretation, as is the finding that, unlike aversive potentiation, inhibition of the blink reflex during pleasant picture viewing did not increase across the viewing interval in either experiment. Whether reflex inhibition for pleasant pictures represents the summation of two processes—increased attention allocaton and inhibition of defensive reflexes due to appetitive activation—or is due to a single attentional process that dom-

inates during appetitive picture perception awaits further empirical test.

Startle: Blink and P3 Measures

These data highlight the utility of measuring two responses to the same startling probe—the reflexive eyeblink and the P3 component of the event-related potential—in understanding emotion and attention during picture viewing (see also Cuthbert et al., 1996; Schupp et al., 1997). The probe P3 measure is taken here as a relatively pure index of the amount of resources allocated during picture processing, with smaller P3 amplitude to the startle probe when greater attention is allocated to emotionally salient (pleasant and unpleasant) pictures. The reflexive eyeblink, on the other hand, appears to be modulated differently, with initial orienting and attention attenuating blink responses, and intense emotional engagement potentiating or inhibiting the blink response for unpleasant or pleasant pictures, respectively. Taken together, measuring the probe P3 allows one to estimate differences in picture viewing that are related to information intake, whereas the blink component further allows an estimate of affective engagement.

Summary

Using a complex picture as a prepulse stimulus, startle reflex magnitude was initially facilitated, then quickly inhibited, and later either facilitated or inhibited as a function of its affective content. We interpret these effects as reflecting a series of interacting sensory, attentional, and motivational processes engaged by the presentation of a picture stimulus, involving rapid interstimulus integration, sensory prepulse inhibition, attentional inhibition, and defensive or appetitive modulation. We propose that initial activation of either the defensive or appetitive motivational systems by an affective picture engages attentional orienting processes that prompt significant inhibition of the blink reflex, as well as a reduced P3 to the startle probe. With increasing defensive activation, however, defensive actions are primed, including the startle reflex, which potentiates the blink response. Taken together, the data suggest that the startle reflex is a complex index of defensive and appetitive motivation, reflecting initial sensory engagement, sustained attentional processing, and the organism's disposition to action when confronted by an emotionally arousing cue.

REFERENCES

Anthony, B. J. (1985). In the blink of an eye: Implications of reflex modification for information processing. *Advances in Psychophysiology*, *1*, 167–218.

Blumenthal, T. D., & Gescheider, G. A. (1987). Modification of the acoustic startle reflex by a tactile prepulse: The effects of stimulus onset asynchrony and prepulse intensity. *Psychophysiology*, *24*, 320–327.

Boelhouwer, A. J., Teurlings, R. J., & Brunia, C. H. (1991). The effect of an acoustic warning stimulus upon the electrically elicited blink reflex in humans. *Psychophysiology*, *28*, 133–139.

Bradley, M. M., Codispoti, M., Cuthbert, B. N., & Lang, P. J. (2001). Emotion and motivation I: Defensive and appetitive reactions in picture processing. *Emotion*, *1*, 276–298.

Bradley, M. M., Cuthbert, B. N., & Lang, P. J. (1993). Pictures as prepulse: Attention and emotion in startle modification. *Psychophysiology*, *30*, 541–545.

Bradley, M. M., Cuthbert, B. N., & Lang, P. J. (1999). Affect and the startle reflex. In M. E. Dawson, A. Schell, & A. Boehmelt (Eds.) *Startle modification: Implications for neuroscience, cognitive science and clinical science* (pp. 157–183). Stanford, CA: Cambridge.

Bradley, M. M., & Lang, P. J. (2000). Measuring emotion: Behavior, feeling and physiology. In R. Lane & L. Nadel (Eds.), *Cognitive neuroscience of emotion* (pp. 242–276). New York: Oxford University Press.

Campeau, S., & Davis, M. (1995). Prepulse inhibition of the acoustic startle reflex using visual and auditory prepulses: Disruption by apomorphine. *Psychopharmacology*, *117*, 267–274.

Cook, E. W., III (1997). *VPM reference manual*. Birmingham, Alabama: Author.

Codispoti, M., Bradley, M. M., & Lang, P. J. (2001). Affective reactions to briefly presented pictures. *Psychophysiology*, *38*, 474–478.

Cuthbert, B. N., Bradley, M. M., & Lang, P. J. (1996). Probing picture perception: Activation and emotion. *Psychophysiology*, *33*, 103–111.

Cuthbert, B. N., Schupp, H. T., Bradley, M. M., Birbaumer, N., & Lang, P. J. (2000). Brain potentials in affective picture processing: Covariation with autonomic arousal and affective report. *Biological Psychology*, *52*, 95–111.

Davis, M. (1997). The neurophysiological basis of acoustic startle modulation: Research on fear motivation and sensory gating. In P. J. Lang, R. F. Simons, & M. T. Balaban (Eds.), *Attention and orienting: Sensory and motivational processes* (pp. 69–96). Mahwah, NJ: Erlbaum.

DelPezzo, E. M., & Hoffman, H. S. (1980). Attention factors in the inhibition of a reflex by a visual stimulus. *Science*, *210*, 673–674.

Dichter, G. S., Tomarken, A. J., Shelton, R. C., & Sutton, S. K. (2004). Early- and late-onset startle modulation in unipolar depression. *Psychophysiology*, *41*, 433–440.

Filion, D. L., Dawson, M. E., & Schell, A. M. (1993). Modification of the acoustic startle-reflex eyeblink: A tool for investigating early and late attentional processes. *Biological Psychology*, *35*, 185–200.

Globisch, J., Hamm, A., Schneider, R., & Vaitl, D. (1993). A computer program for scoring reflex eyeblink and electrodermal responses written in Pascal. *Psychophysiology*, *39*, S30.

Globisch, J., Hamm, A. O., Stevens, F., & Öhman, A. (1999). Fear appears fast: Temporal course of startle reflex potentiation in animal fearful subjects. *Psychophysiology*, *36*, 66–75.

Graham, F. K. (1975). The more or less startling effects of weak prestimulation. *Psychophysiology*, *12*, 238–248.

Graham, F. K. (1980). Control of reflex blink excitability. In R. F. Thompson, L. H. Hicks, & V. B. Shvyrkov (Eds.), *Neural mechanisms of goal-directed behavior and learning* (pp. 511–519). New York: Academic Press.

Graham, F. K., & Murray, M. (1977). Discordant effects of weak prestimulation on magnitude and latency of the reflex blink. *Physiological Psychology*, *5*, 108–114.

Gratton, G., Coles, M. G. H., & Donchin, E. (1983). A new method for off-line removal of ocular artifact. *Electroencephalography & Clinical Neurophysiolology*, *55*, 468–484.

Greenwald, M. K., Bradley, M. M., Cuthbert, B. N., & Lang, P. J. (1998). Sensitization of the startle reflex in humans following aversive electric shock exposure. *Behavioral Neuroscience*, *112*, 1069–1079.

Hackley, S. A., & Boelhouwer, A. J. W. (1997). The more or less startling effects of weak prestimulation—revisited: Prepulse modulation of multicomponent blink reflexes. In P. J. Lang, R. F. Simons, & M. T. Balaban (Eds.), *Attention and orienting: Sensory and motivational processes* (pp. 205–227). Mahwah, NJ: Erlbaum.

Hackley, S. A., & Graham, F. K. (1987). Effects of attending selectively to the spatial position of reflex-eliciting and reflex-modulating stimuli. *Journal of Experimental Psychology: Human Perception & Performance*, *13*, 411–424.

Hilgard, E. R. (1933). Reinforcement and inhibition of eyelid reflexes. *Journal of General Psychology*, *8*, 85–113.

Ison, J. R., & Ashkenazi, B. (1980). Effects of a warning stimulus on reflex elicitation and reflex inhibition. *Psychophysiology*, *17*, 586–591.

Lang, P. J. (1995). The emotion probe: Studies of motivation and attention. *American Psychologist*, *50*, 371–385.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1990). Emotion, attention, and the startle reflex. *Psychological Review*, *97*, 377–395.

Lang, P. J., Bradley, M. M., & Cuthbert, M. M. (1997). Motivated attention: Affect, activation and action. In P. J. Lang, R. F. Simons, & M. T. Balaban (Eds.) *Attention and orienting: Sensory and motivational processes* (pp. 97–135). Hillsdale, NJ: Erlbaum.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (2005). International affective picture system (IAPS): Affective ratings of pictures and instruction manual. Technical Report A-6. Gainesville, FL: University of Florida.

Levenston, G. K., Patrick, C. J., Bradley, M. M., & Lang, P. J. (2000). The psychopath as observer: Emotion and attention in picture processing. *Journal of Abnormal Psychology*, *109*, 373–385.

Lipp, O. V., Blumenthal, T. D., & Adam, A. R. (2001). Attentional modulation of blink startle at long, short, and very short lead intervals. *Biological Psychology*, *58*, 89–103.

Lipp, O. V., Siddle, D. A., & Dall, P. J. (2000). The effects of change in lead stimulus modality on the modulation of acoustic blink startle. *Psychophysiology*, *37*, 715–732.

Miller, G. A., Gratton, G., & Yee, C. M. (1988). Generalized implementation of an eye movement correction procedure. *Psychophysiology*, *25*, 241–243.

Norman, D. A., & Bobrow, D. G. (1975). On data-limited and resource-limited processes. *Cognitive Psychology*, *7*, 44–64.

Palomba, D., Angrilli, A., & Mini, A. (1997). Visual evoked potentials, heart rate responses and memory to emotional pictorial stimuli. *International Journal of Psychophysiology*, *27*, 55–67.

Sanes, J. N. (1984). Voluntary movement and excitability of cutaneous eyeblink reflexes. *Psychophysiology*, *21*, 653–664.

Sarno, A. J., Blumenthal, T. D., & Boelhouwer, A. J. W. (1997). Modification of the electrically elicited eyeblink by acoustic, visual, and vibrotactile pulses. *Psychobiology*, *25*, 253–265.

Schicatano, E. J., Peshori, K. R., Gopalaswamy, R., Sahay, E., & Evinger, C. (2000). Reflex excitability regulate prepulse inhibition. *The Journal of Neuroscience*, *20*, 4240–4247.

Schupp, H. T., Cuthbert, B. N., Bradley, M. M., Birbaumer, N., & Lang, P. J. (1997). Probe P3 and blinks: Two measures of affective startle modulation. *Psychophysiology*, *34*, 1–6.

Schupp, H. T., Cuthbert, B. N., Bradley, M. M., Hillman, C. H., Hamm, A. O., & Lang, P. J. (2004). Brain processes in emotional perception: Motivated attention. *Cognition and Emotion*, *18*, 593–611.

Smith, J. C., Bradley, M. M., & Lang, P. J. (2005). State anxiety an affective physiology: Effects of sustained exposure to affective pictures. *Biological Psychology*, *69*, 247–260.

Stanley, J., & Knight, R. G. (2004). Emotional specificity of startle potentiation during the early stages of picture viewing. *Psychophysiology*, *41*, 935–940.

Sutton, S. K., Davidson, R. J., Donzella, B., Irwin, W., & Dottl, D. A. (1997). Manipulating affective state using extended picture presentations. *Psychophysiology*, *34*, 217–226.

Vanman, E. J., Boehmelt, A. H., Dawson, M. E., & Schell, A. M. (1996). The varying time courses of attentional and affective modulation of the startle blink reflex. *Psychophysiology*, *33*, 691–697.

Vrana, S. R., Spence, E. L., & Lang, P. J. (1988). The startle probe response: A new measure of emotion? *Journal of Abnormal Psychology*, *97*, 487–491.

Waters, A. M., Lipp, O. V., & Spence, S. (2005). The effects of affective picture stimuli on blink modulation in adults and children. *Biological Psychology*, *68*, 257–281.

White, K. D. (1978). Salivation: The significance of imagery in its voluntary control. *Psychophysiology*, *15*, 196–203.

(Received January 19, 2006; Accepted May 9, 2006)