THE EFFECTS OF LEFT VS. RIGHT EAR STIMULATION ON HUMAN STARTLE REFLEX MODULATION

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Abstract- It has been reported previously that modulatory effects of pleasant and unpleasant slides on the human acoustic reflex are observed only if startle probes are presented to the left ear or with binaural presentation. The present study examined the effects of the left and the right ear stimulation on modulated startle reflex (as indexed by eyeblink component, measured at the right eye) employing short (2-min) film-clips to elicit emotions. Sixteen right-handed subjects served as volunteers. The experiment included two consecutive sessions, on a single occasion. The acoustic stimuli were presented monaurally to one of the ears in each session, counterbalanced across order. The laterality phenomenon in startle modulation previously observed with affect-toned slides was not confirmed in this experiment. The probable discrepancy will be discussed methodologically.
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Key words: Lateral stimulation, laterality, startle response, emotional manipulation, film clips

INTRODUCTION

Traditionally, the emotional processing has been ascribed to the right hemisphere activity; this assumption has been proved by recent studies (1). From a theoretical viewpoint, examining the laterality effect on modulated startle reflex might contribute to research on hemispheric specialization during emotional states, hence contributing to the investigation of the neuroanatomy of acoustic startle pathway in man, as found in animals (2). From a methodological viewpoint, if there is any laterality effect, the use of binaural acoustic probes together with a monocular recording procedure might not reveal all the important information. The matter of brain lateralization in affective processing has already been of interest to researchers in many areas. Tucker and Williamson concluded that the right hemisphere has a general advantage in processing emotional (whether positive or negative) stimuli (3). According to some models, the right hemisphere is involved in negative emotions (4, 5) and the left hemisphere in positive emotions (6).

The modulated startle reflex, as an established tool for assessing emotional reactivity, offers an interesting paradigm in probing such lateralization effects (7, 8). Larger startle reflexes with shorter latency were elicited by binaural acoustic probes than by monaural probes, with no overall difference in reflex response for the left and right ears (9). Bradley *et al.* found that the reflexes elicited by the right ear stimulation did not vary with foreground valence (i.e. there was no effect of valence), whereas probe presentation to the left ear elicited blink magnitude that linearly increased from pleasant through neutral to unpleasant slide foregrounds (7). They hypothesized that a lateralized affect-startle system might exist in the central nervous system, suggesting that reflex magnitude and foreground valence depends on right hemisphere processing. Similar effects have recently been observed by Bradley *et al.*, confirming their previous findings with respect to

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lateralization of emotional processing (8).

The present study was designed to replicate lateral acoustic startle probes in the study of emotion using film-clips in place of slides (7, 8). It also aimed to examine the discrepancy between the results of the studies by Grillon and Davis (2) and Bradley *et al.* (7, 8), using an affect-eliciting method with a higher intensity level of emotions, including anxiety, than would be expected in response to static slides.

MATEIRALS AND METHODS

Subjects

Sixteen right-handed volunteers (8 men, mean age 29.25 yrs; 8 women, mean age 27.38 yrs) were recruited by advertisement and from an existing subject pool. They met all criteria for recruitment as described in Kaviani *et al.* (10).

Handedness was simply established on the basis of handwriting preferences. Although, a more comprehensive handedness questionnaire might have been used, this was deemed unnecessary because even among left-handers, the left hemisphere is usually dominant. Five subjects with missing data during one or more film-clips were eliminated from the analyses, leaving 6 men and 5 women; they were retained for the analyses of affective ratings.

Apparatus and materials

The film-set, the same as used in the previous studies in our lab (10-13), consisted of 9 clips separated by blank intervals (dark blue screen) 10-25 seconds long. The first three clips were used only to familiarize subjects with experimental procedure. The last six clips, used to induce emotions experimentally, were presented in two blocks and in the order neutral (N), pleasant (P), unpleasant (D), N, D, P. Each film-clip lasted about 2 mins. The set, shown on a Sharp video recorder (VC-A30HM) connected to a Sharp color TV monitor, 20 inch (DV-5101 A), was viewed from a distance of 2 m.

The acoustic startle stimuli (consisting of a 50-ms presentation of a 92.5-dB ‘A’ burst of white noise with quasi-instantaneous rise time) were superimposed on the sound tracks (ranging from 40 to 60 dB) of the film-clips, at moments of relatively low sound level, and presented mono aurally via head-phones (Telephonics TDH-39P). Three startle stimuli were presented during each clip (total = 27). To increase unpredictability, they were presented with varying inter-stimulus intervals of 20 to 90 secs after clip onset. The responses to the last 18 acoustic startle stimuli (during the last six clips) were included in the analyses, excluding the responses to the first 9 acoustic stimuli (during the first three clips which were only for habituation).

To record electromyographic (EMG) activity of the orbicular oculi muscle, two 6 mm-disc electrodes (Ag/AgCl) filled with electrolyte paste (SLE, Croydon, UK) were placed approximately 1 cm below the middle of the lower eyelid and 1 cm below the outer comer of the right eye, so that the second electrode was about 1 cm lateral and slightly higher than the first but both were parallel to the lower rim of the eyelid. An additional, ground electrode was placed behind the right ear over the mastoid. Raw EMG signals were recorded, amplified, filtered, stored and analyzed by a computerized startle response monitoring system (SR Instruments, San Diego, California).

The analytic program treats the first 20 ms after presentation of each startle stimulus as a baseline for that trial. It then calculates, relative to this baseline, latency (msec) to startle onset and peak EMG amplitude (in arbitrary analogue-to-digital units; 1 unit equals 1.2 microvolts, SR-Lab Program) over the 95 msec following startle onset. Trials with an unstable baseline (shift > 20 units) were eliminated. Samples are taken at 1 millisecond (1 KHz sampling rate). The lower band pass alternative provided by the apparatus (0-500 Hz) was used throughout (there is also a built-in standard 50-cycle filter). With respect to the 1.2 mV per AID unit, SD Instruments describe the logic as follows: The AID board is set for voltages from 0-5 volts. For maximal resolution a board with 12 bit resolution is used, which divides the 5 volts into 4095 AID units. Therefore a 1.2 millivolt input to the card is reported as 1 AID unit (5 divided by 4095).

To be consistent with previous studies (10-14), the scoring criteria were identical. Trials were rejected if there was evidence of excessive activity (including evidence of a premature eyeblink) during the baseline period. They were also rejected if there was no evidence of an eyeblink having been evoked by the startle probe. Altogether, 16.35% of trials were excluded on one or other of these criteria. Subjects were excluded from analysis if they showed missing data on all three startle probes within any of film clips.

The affective content of each clip was rated as each clip ended (during the blank interval) on a single II-point (-5 to +5) scale, from extremely unpleasant (*e.g.* depressed, disgusted, angry, anxious; scored as -5), through neutral (scored as -5) to extremely pleasant (e.g. happy, relaxed; scored as +5).

Experimental design and procedure

The study consisted of two consecutive sessions, on a single occasion. The acoustic stimuli were presented monaurally to one of the ears in each session.

Subjects (counterbalanced for sex) were randomly assigned in equal numbers to one of the two ear orders [left ear (session 1) - right ear (session 2); right ear (session 1) - left ear (session 2)], so that eight subjects (four men and four women) received acoustic probes as well as the sound track of the film-clips, first to the right and then to the left ear; the remaining eight subjects (four men and four women) received acoustic probes first to the left and then to the right ear.

Subjects were told in advance that they would be tested twice, once with left and once with right ear stimulation, while viewing a series of film-clips, with either pleasant, unpleasant or neutral content; that each sequence should be watched as long as it was on screen; and that throughout the experiment they would hear occasional bursts of noise through the headphones, which would be neither painful nor harmful and should be ignored.

The electrodes and headphones were then attached and subjects were asked to keep a comfortable position in the chair while watching the video, avoiding gross body movements, and to relax, concentrate and not to attempt to control their emotions, whether positive or negative. An experimenter was present throughout the session. The experimental procedures were approved by the ethical committee at the Institute of Psychiatry, London. In each session, the affective content of each clip was rated as each clip ended (during the blank interval) on the rating scale.

Data reduction and analysis

In order to detect any interaction effect between block and valence found in Kaviani *et al.* (10), first the overall data was subjected to a two-way ANOVA, with valence and block as within-subject variable. Since an interaction effect again was found for the data on startle amplitude, the following analyses were performed.

The data on each of the dependent measures - affective ratings, response amplitude and latency to response onset of each block were separately analyzed by a three-way (valence [pleasant, neutral, and unpleasant] x ear side [left and right] x ear order [left-right and right-left]) ANOVA, with valence and ear side as within-subjects variables and ear order as a between-subjects factor. As there was no main or interaction effects of ear order, this variable was excluded from all further analyses and the data in each block were subjected separately to a three-way (sex [men and women] x ear side [left and right] x valence [pleasant, neutral, and unpleasant]) ANOVA with ear and valence as within-subjects variables and sex as a between-subjects factor.

Since no significant main or interaction effects were found for the measures of baseline EMG and latency to response onset, only the findings on affective ratings and startle amplitude are reported in the result section. Lang's group detected a linear trend of valence effect separately for each ear (7). Although no significant interaction effect appeared in the above analysis, only in order to compare the present data with that of Lang's group, the ear side variable was dropped from further analyses and the data of each block for each ear separately were subjected to a two-way (sex [men and women] x valence [pleasant, neutral, and unpleasant]) ANOVA, with valence as a within-subjects variable and sex as a between-subjects factor, followed by polynomial contrast tests (assessed by t) on valence effects.

Table 1. Affective ratings for men and women in right and left ear conditions*

		Film-clips	Men	Women
Right ear	Block 1	Mr Bean	1.63 (0.68)	2.87 (0.64)
		Housewares	0.00 (0.19)	-0.50 (0.46)
		Toe surgery	01.00 (0.53)	-3.50 (0.60)
	Block 2	Ice dancing	1.00 (0.53)	2.88 (0.58)
		Street scenes	-0.38 (0.28)	0.00 (0.33)
		Gangsters	-2.25 (0.37)	-3.63 (0.38)
Left ear	Block 1	Mr Bean	2.00 (0.46)	3.00 (0.93)
		Housewares	-0.13 (0.13)	-0.50 (0.46)
		Toe surgery	-1.25 (0.53)	-2.88 (1.04)
	Block 2	Ice dancing	1.00 (0.50)	2.75 (0.65)
		Street scenes	0.00 (0.00)	-0.13 (0.23)
		Gangsters	-2.00 (0.38)	-3.38 (0.50)

*Data are given as mean (standard error of mean).

RESULTS

Affective ratings

There appeared significant valence effect ($F[2, 30] = 48.12$, $P< 0.001$) and a block x valence effect on overall data. The next analyses yielded no significant effects except for the main effect of valence in both blocks (block 1: $F [2, 13] = 23.79$, $P < 0.001$; block 2: $F [2, 13] = 32.20$, $P < .001$).

In block 1, there were significant valence effects for each ear (right ear: $F [2,13] = 28.99$, $P < 0.001$; left ear: $F [2,13] = 32.26$, $P < 0.001$), with highly significant linear trends (right ear: $t = -7.64$, $P < 0.0001$; left ear: $t = -4.58$, $P < 0.001$); no significant sex x valence effect was found for the left ear ($P > 0.1$), but this was significant for the right ear ($F [2, 13] = 4.74$, $P < 0.05$). In block 2, there were significant valence effects for both ears (right ear: $F [2, 13] = 48.02$, $P < 0.001$; left ear: $F [2, 13] = 43.79$, $P < 0.001$), with highly significant linear trends (right ear: $t = -9.02$, $P < 0.0001$; left ear: $t = -8.23$, $P < 0.0001$); significant sex x valence effects were observed for both ears (right ear: $F [2, 13] = 4.99$, $P < 0.05$; left ear: $F [2,13] = 3.77$, $P = 0.05$). The results indicated that women found both pleasant film-clips more pleasant (right ear: $t [14] = 2.38$, $P < 0.05$; left ear: $t [14] = 2.14$, $P = 0.05$) and unpleasant film-clips more unpleasant than did men (right ear: $t [14] = 2.62$, $P < 0.05$; left ear: $t [14] = 2.20$, $P < 0.05$). Table 1 shows the mean affective ratings (error bars indicate ± 1 standard error of the mean) of film-clips classified by ear side and sex of subjects.

Startle amplitude

The analyses showed significant effects for valence ($F [2, 20] = 3.85$, $P< 0.05$) and block x valence ($F [2, 20] = 5.99$, $P< 0.01$) on overall data. The next analyses yei1ded no significant interaction effect between ear order and the other factors in either block. No significant overall valence effect in block 1 was observed, but there was a significant overall valence effect in block 2 ($F [2, 8] = 6.15$, $P < 0.03$). Table 2 presents mean startle amplitude (error bars indicate ± 1 standard error of the mean) for the two ears in both blocks. In block 1, no significant main or interaction effects were observed, whereas in block 2, there was a significant valence effect ($F [2, 8] = 11.12$, $P < 0.01$). No other significant effects were found in block 2. No significant valence or sex effects were found in block 1 for either ear; there were significant valence effects in block 2 for both right ($F [2, 10] = 5.32$, $P < 0.03$) and left ($F [2, 10] = 5.66$, $P < 0.03$) ears, with a significant linear effect (right ear: $t = 3.02$, $P < 0.01$); left ear: ($t = 2.89$, $P < 0.02$). When analyzing the data for the left ear, three cases in block 1 and four cases in block 2 were excluded from analysis due to missing amplitude data; the missing data for the right ear were two and three in blocks 1 and 2, respectively.

DISCUSSION

Employing the emotion-eliciting film method, the present study extends the previous findings (7, 8) to probe the effect of ear laterality on the modulated startle reflex. The overall data (collapsed over left and right monaural probe presentation) replicated the main finding of Kaviani *et al.* (10-12): eye blink amplitude to acoustic startle probes varied linearly with the emotional valence of film-clips in block 2. This affect-startle effect is clearly robust.

The overall affective measures over pleasant, neutral, and unpleasant film-clips showed a similar linear variance, that is pleasant clips were rated more positive and unpleasant clips were more negative, relative to neutral clips. In addition, women rated the pleasant conditions as more pleasant and the unpleasant conditions as more unpleasant than men. There was a significant interaction between valence and block, again suggesting that the unpleasant film-clips (one with disgusting and the other with frightening contents) in the two blocks modulate startle amplitude differently. This issue has been discussed in Kaviani *et al.* (10).

Previous studies investigating the impact of monaural acoustic probes, administered to the left and right ears, reported significant affective modulation for probes presented to the left ear, but no significant effect for probes presented to the right ear (7, 8). The authors speculated that the data were consistent with the notion that right hemisphere activation is dominant for affective stimuli; when startle probes were presented to the left ear (processed by right-hemisphere neural structures), larger blink amplitude were observed in the context of foreground unpleasant stimuli in comparison with foreground pleasant stimuli.

Table 2. Startle amplitude in the two blocks of clips for right and left ear*

		Film- clips	Mean (SEM)
Right ear	Block 1	Mr. Bean	36.96 (11.29)
		Housewares	47.21 (14.49)
		Toe surgery	37.00 (8.42)
	Block 2	Ice Dancing	27.58 (3.64)
		Street scenes	51.56 (13.52)
		Gangsters	98.76 (20.01)
Left Ear	Block 1	Mr Ben	29.24 (4.68)
		Housewares	34.29 (8.10)
		Toe surgery	34.51 (5.79)
	Block 2	Ice Dancing	24.07 (4.62)
		Street scenes	38.52 (8.75)
		Gangsters	75.49 (19.32)

*Data are given as mean (±1 standard error of the mean).

The startle amplitude findings obtained in the present study did not show a statistically significant interaction between monaural probe side and foreground valence, suggesting no ear laterality effect in the affective modulation of the startle reflex. Therefore, the phenomenon of lateralized startle reflex modulation put forward by Lang's group (7) is not supported here. One reason for this discrepancy may be the greater modulation of the startle responses by the film contents, which seem to be more effective in mood induction. This claim will appear more logical if one takes into account some anatomical data. As pointed out by Bradley *et al.* (7), roughly two-thirds of the transmitting fibers from each ear cross the brain, and one-third proceed ipsilaterally. This means that the ipsilateral hemisphere is not completely silent while the stimulus has already activated the contralateral hemisphere. Bradley and colleagues raised this question: does right ear input fail to produce the effect because the one-third of fibers directed to the ipsilateral hemisphere carry insufficient information? It is possible that the robustness of film material compensates for the insufficient information. Bradley *et al.* (8), in a metanalytic review, showed the effect sizes for the differences in reflex magnitude between pleasant and unpleasant picture categories in their previous study (7). Overall effect sizes for the left and right ears were 0.52 and 0.17 (n.s.) respectively. However, the same analysis in the present study reveals appreciably larger effect sizes for both ears (left: 0.78 and right: 0.87).

A lack of a valence x ear effect, as observed here, has also been reported by Hawk and Cook (15). In a study of the laterality of emotion, they applied tactile probes (an airpuff to the side of the face) in place of acoustic probes, using the slide paradigm. Although the modulatory effect of valence was significant for tactile probes presented on the left side and not significant for probes presented to the right side, no interaction was found between valence and probe side. The latter result is in agreement with the lack of a valence x ear side effect observed in the present study.

Grillon and Davis used threat of shock as an aversive context to modulate startle response (2). Although startle potentiation was obtained for reflexes elicited under shock threat (compared with a no-threat condition), they reported greater potentiation when startle stimuli were delivered to the right than to the left ear, implying that the left hemisphere is probably involved in aversive emotional processing. Similarly, in the present study, the startle amplitudes when acoustic probes were presented to the right ear in all three valences were somewhat higher than when presented to the left ear. This finding, can be regarded as consistent with Grillon and Davis' findings (2). The present study also found that the basal tension of the orbicular oculi was higher during viewing of unpleasant film-clips compared to neutral film-clips when the acoustic probes were delivered to the left ear, suggesting greater activation of the right hemisphere in negative emotions. These findings provide support for theories claiming greater right hemisphere involvement in negative emotions (4) and left hemisphere specialization in positive emotions (6, 16). Moreover, Kinsboume (17) and Davidson (18) emphasized the role of the left hemisphere in pleasant emotions, approach, and positively valent action, and the role of the right hemisphere in unpleasant emotions, withdrawal, and negatively valent actions.

If we attribute the affect-startle effect to a central valence matching process (19), the valence match would happen within 75 ms between the probe stimulus onset and response peak. This startle timing program methodologically imposes a critical time constraint on information processing. Lang and associates speculated that a startle stimulus, initiated at the right ear, fails to influence the startle modulation circuit (see Davis and File, 1984) because information reaches the right hemisphere only after the motor program for the obligatory blink has been already determined (19). Since, in the present experiment, a 95-ms interval (during which response peak would happen) was programmed to occur after probe stimulus onset, this might allow the information to reach the right hemisphere and be processed before the activation of the obligatory blink. This feature of the experimental design may provide a neurological basis for the discrepancy between the results obtained here and those observed by others (*e.g.* 7, 8 and 2) in showing lateralization of the effects of startle probes. In order to examine this possibility, in additional analyses every single startle response was manually checked and all responses measured after 75 ms (i.e., when a peak occurred 75 ms after probe onset) were excluded from the data. Again no ear x valence effect was found, confirming the main results reported in the present paper.

In the present study, the EMG activity of orbicular is occult was measured only from the right eye, which precludes the possibility of investigating ipsilateral or bilateral effects of eye and ear. Thus, to fully test theories concerned with brain laterality effects on emotional responses, a further experiment is required, examining ear and eye laterality simultaneously.

To summarize, the present experiment showed once more the film-clips' effectiveness in mood induction and consequently startle response modulation. This finding replicated the main result of an earlier study (10) in which the affect-startle effect proved to be robust in comparison with the slide paradigm.

The laterality phenomenon for probe input side (leading to different magnitudes during left and right ear presentation), which has been documented using slides (*e.g.* 7 and 8), was not confirmed in the present data using film-clips.

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