Chinese Science Bulletin

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ARTICLES

Effects of negative emotion and its correlated neural activity on secretory immunoglobulin A

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In this study, we investigated how the negative emotional arousal induced by watching a number of unpleasant images altered the concentration of secretory immunoglobulin A (SIgA) in saliva. Although our results found discrepancies among participants' SIgA concentration (i.e. some participants' SIgA decreased after watching unpleasant pictures, whereas others increased), further analysis revealed correlations among participants' changes in SIgA concentration, their general coping styles and their actual strategies for emotion regulation when perceiving unpleasant pictures, and the event-related potentials (ERPs) associated with viewing unpleasant pictures. The participants whose SIgA increased after watching unpleasant pictures (the "Increasers") had higher positive coping scores in the Trait Coping Styles Questionnaire (TCSQ) than those whose SIgA decreased (the "Decreasers"). Also, relative to the "Decreasers", the "Increasers" tended to use more emotion regulation strategies, particularly with extremely negative pictures. The amplitude of their late positive potentials (LPPs) exhibited a reverse dissociation pattern for extremely negative pictures versus least negative ones and was related to the cognitive evaluation of the stimuli's meaning. In sum, this research revealed the psychological mechanisms by which negative emotional states influence the immune system and the related ERP changes.

secretory immunoglobulin A, negative emotion, emotion regulation, cognitive reappraisal, LPP

PSYCHOLOGY

The immune system plays a key function in individual health. One important method used in the psychological study of emotional effect on immune system function is to determine mucosa immune levels, done by measuring the concentration of secretory immunoglobulin A (SIgA) in saliva. SIgA, an antibody involved in local mucosal immunity, prevents pathogens from sticking to cell surfaces by combining with bacteria and viruses, therefore preventing local infection[1]. SIgA is the first barrier against bacterial and viral infections of the upper respiratory tract[2], and an index of proper mucosa immune system function[3].

Previous researches have found that negative emotion is correlated with a decrease in the function of individual immune systems[4] and an increase in the likelihood of infection[5,6], while positive emotion is correlated with an increase in the function of the immune system and a decrease in the likelihood of infection[7,8]. However, researches on the effects of negative emotion on individual SIgA levels have reported mixed findings. Evans et al.[9] found that participants' SIgA concentrations increased with increases in negative emotions. In contrast, Stone et al.[10] found that undesirable events that increased negative emotions were correlated with decreases in SIgA level. Labott et al.[11] found that participants'

Received January 28, 2008; accepted March 23, 2008
doi: 10.1007/s11434-008-0233-5
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Supported by the National Hi-Tech Research and Development Program of China (Grant No. 2006AA02Z431), National Natural Science Foundation of China (Grants No. 30770708) and Knowledge Innovation Program of the Chinese Academy of Sciences (Grant No. KSCX2-YW-R-28)

SIgA concentrations decreased as they released their negative emotions by crying after watching a sad video. However, Martin et al.[12] found no evidence for changes of SIgA if participants cried while watching a sad video. In a study by Yang et al.[13], the researchers found that negative emotion pictures could lead to an apparent negative emotional reaction among the participants without any accompanying SIgA changes. Researchers have attributed these conflicting results to different experimental designs, different times for collecting saliva samples, and different methods of detecting SIgA[9,14].

Emotion is a kind of subjective individual experience that arises from the cognition of objective events. Because the brain's cognitive processes directly affect emotion, it makes sense for researchers investigating the effects of emotion on immune system function to study changes in brain electroencephalogram (EEG) activity. Several studies have adopted just such an approach. For example, Rosenkranz et al.[15] found that individuals with greater relative left-prefrontal EEG activation had a greater antibody response to an influenza vaccination following negative affect induction. Many event-related potentials (ERPs) studies have looked at the neural mechanisms of emotion cognition and regulation and have found that emotional stimuli elicit larger late positive potentials (LPPs) than neutral stimuli. Moreover, pictures of higher emotional intensity/arousal elicit larger LPPs than those of lower intensity/arousal[16−19]. These studies have also found that among participants who applied the strategy of cognitive reappraisal when watching negative emotional pictures, LPP amplitude was smaller than their counterparts in passive viewing conditions[20,21]. However, it should be noted that these ERP experiments have not studied the relation between ERP and individual immune level.

Although cognitive reappraisal is a strategy for emotion regulation, it has a close relation to individual's explanatory style. A study by Seligman et al.[22] suggested that individuals with optimistic explanatory style perceive negative events as temporary and confined to a certain condition, while individuals with pessimistic explanatory style may perceive such events as permanent and generalized to all aspects of life. It is known that education plays a greater role in shaping individuals' explanatory styles than genetic factors[23]. If we are to succeed in training individuals to form an optimistic explanatory style, then we must study the relationships between negative emotion, changes of EEG activity, and changes of SIgA concentrations. We must also account for the cognitive and psychological mechanisms that mediate these relationships.

Prior studies of emotion and SIgA concentrations have resulted in conflicting findings, and do not elucidate the cognitive or psychological mechanisms that explain how emotion affects immune level. However, these studies do suggest the importance of using ERP technology to study how emotion affects immune level. Therefore, our study combined EEG recordings with the collection of immune indexes, and also used the technologies of experimental interview and psychological measurement. The current experiment presented participants with unpleasant pictures as emotion-arousing stimuli. EEG data was recorded as the participants viewed the pictures, while changes in SIgA were determined by measuring levels before and after viewing of the pictures. Then on the basis of ERP data analysis, we determined the participants' psychological activities by revisiting them and investigated them with the Trait Coping Styles Questionnaire (TCSQ). Our study aimed to elucidate the relationship between changes in EEG activity and cognitive and psychological mechanisms to the immune changes induced by negative emotion. We were especially interested in explaining the conflicting findings reported by previous researchers, some of whom found that immune level increased when individuals faced a provocation event or stimulus, while others found precisely the opposite.

1 Methods

1.1 Participants

Twenty right-handed healthy female undergraduates with normal or corrected-to-normal vision in Beijing (mean age 21.85±1.31 years) participated in this study. Because several studies have suggested sex differences in degree of emotional arousal and memory for negative visual materials, we used only female participants to avoid this source of variability.

1.2 Stimuli

One hundred and fifty negative non-face pictures with clear interpretation were chosen from the Chinese Affective Picture System (CAPS) (valence means: 2.55; arousal means: 5.63). Since the afferent accent of the stimulus was not always located at the center of the picture, each stimulus was presented a second time in its mirror orientation, resulting in a total of 300 pictures (150 primary pictures plus 150 mirror-image pictures). The pictures were divided into 3 groups, each group consisting of 100 images (i.e. 50 primary images and the accompanying 50 mirror images). The first group, labeled EN (extremely negative), consisted of those images with the lowest values on the valence scale. The second group consisted of those images with intermediate values on the valence scale. Finally, the third group, labeled LN (least negative), consisted of those images with the highest values on the valence scale. A comparison of the EN group and the LN group found that there were significant differences in valence and arousal between these two groups (valence means: EN = 2.13, LN = 2.96, $t(98) = -24.27$, $P < 0.001$; arousal means: EN = 5.82, LN = 5.43, $t(98) = 2.46$, $P < 0.05$).

1.3 Methods of saliva collection and SIgA measurement

SIgA concentration typically decreases significantly during the first 4 h after awakening, before stabilizing and remaining constant in the following 6 h[27]. In the present study, saliva was collected from participants between 2:00pm and 4:00pm. The collection process involved participants first gargling, before having an aseptic cotton swab placed underneath their tongues. They were then instructed to avoid swallowing while chewing with mouth closed for 10 minutes to promote saliva secretion. After that, the saliva was extracted from the swab and placed in a centrifuge tube, which was sealed and frozen at −20℃ for later analysis.

Jemmott et al.[28] thought SIgA level was the appropriate index for measuring mucosal immune level. Meanwhile, other reported studies have shown that SIgA concentration and the SIgA secretion rate had the same changing tendency. That is, SIgA secreting rate (μg/min) = SIgA concentration (μg/mL) × volume (mL/min)[27,29,30]. Therefore, our study used SIgA concentration, as measured by enzyme-linked immunosorbent assay (ELISA).

1.4 Procedures

Upon arrival at the laboratory, participants were instructed to gargle and then rest for 10 minutes in a soundproof room. A baseline saliva sample (T1) was then collected from participants at the rest state. Following collection of saliva, subjects were asked to evaluate their degrees of pleasantness on a nine-point scale, from 1 (unpleasant) to 9 (pleasant). The 300 pictures, each sized 10 cm × 7 cm with resolution 72 pixels/inch, were randomly presented in 6 counterbalanced blocks using Eprime software, with each block containing 50 images. The distance between the participant's eyes and the color computer monitor was approximately 70 cm. Figure 1 shows the time sequence of the emotion elicited session. Each trial was preceded by a fixation cross (+) that appeared in the center of a black screen for 500 ms, followed by presentation of the target picture for 6 s. Following presentation of the picture, a black screen appeared, lasting until the participant had made a response by pressing one of two keys on the keyboard, either key 1 ("I feel uncomfortable") or key 2 ("I feel very uncomfortable"). This was done to keep them focusing on the pictures. Another black screen then came on for 500 ms, marking the completion of one full trial. All six blocks of stimuli were presented in this fashion. At the end of the emotion elicited session, a saliva sample (T2) was collected again. During this sample collection, participants were instructed to chew on a cotton swab while viewing 10 pictures selected from the stimuli on the screen. These images were presented in a procedure similar to that used during the test trials, only each picture lasted for 1 minute. This was done to maintain participants' emotion level. After the saliva sample had been collected, participants were asked to complete the self-appraisal for their emotion again on the nine-point scale.

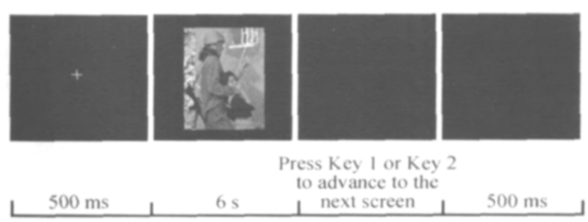

Figure 1 Schematic description of the experimental paradigm.

1.5 EEG recording and analysis

Electroencephalogram (EEG) recordings were taken from 64 scalp sites using Ag/AgCl electrodes mounted in an elastic cap (Neuroscan Inc.), with an on-line reference to the left mastoid, subsequently algebraically re-referenced to linked left and right mastoids. The vertical electrooculogram (EOG) was recorded supra- and infra-orbitally at the left eye. The horizontal EOG was recorded from the left versus right orbital rim. Electrode impedances were below 5 kΩ. A bandpass of 0.05—70 Hz was used for the recording amplifiers. EEG and EOG data were digitized continuously at a sampling rate of 500 Hz/channel.

ERP averages were computed off-line. Ocular artifacts were removed from the EEG signal using a regression procedure implemented in the Neuroscan software. Trials with various artifacts were rejected, with a criterion of ±100 μV. EEG data were epoched into periods of 1800 ms (including a 300 ms prestimulus baseline). EEG activity for all stimuli (300 pictures), for pictures in just the EN group (100 pictures) and for pictures in just the LN group (100 pictures) was overlapped and then averaged separately. ERP waveforms were time-locked to the onset of stimuli. The following 15 electrode sites were chosen for statistical analysis for LPP: Fz, FCz, Cz, CPz, Pz, F3, FC3, C3, CP3, P3, F4, FC4, C4, CP4, P4. The mean amplitude of LPP was measured in the 450—950 ms time window.

2 Results

2.1 Immune and self-appraisal

Means and standard deviations of SIgA concentrations during baseline and immediately following the emotion elicited session are shown in Table 1. The change of total SIgA among the entire sample of 20 females is not significant, with 8 individuals showing decreased SIgA concentration and 12 showing increased SIgA concentration. In order to study the difference in EEG activities between the individuals with decreased SIgA and those with increased SIgA, we rearranged the participants according to SIgA concentration (No. 1 was the one with most decreased SIgA, while No. 20 was the one with most increased SIgA). Participants were then divided into terciles, with the front 1/3 of participants (i.e. No. 1 through No. 7) defined as the "Decreasers" and the back 1/3 of participants (i.e. No. 14 through No. 20) defined as the "Increasers". Data for the middle tercile was discarded. The difference in baseline SIgA concentration between the two groups reached marginal significance ($t(12) = -2.04$, $P = 0.064$), with concentration ranging from 96.25 to 377.48 μg/mL. This range, however, falls within the normal range for healthy adults (79.26—679.50 μg/mL)[31].

As Figure 2 shows, there was a significant difference in scores of self-appraisal for emotion before and after presenting stimuli for all the participants (20 females), just the "Decreaser", and just the "Increaser" respectively ($t(19) = 6.84$, $P < 0.001$; $t(6) = 4.10$, $P < 0.01$; $t(6) = 4.58$, $P < 0.01$). That is, subjects in all 3 cohorts exhibited a significant decrease in the degree of pleasantness. Analysis of covariance indicated no significant difference in the self-appraisal score for emotion before and after stimuli between the "Decreasers" and the "Increasers" ($F(1,10) = 0.428$, $P > 0.05$).

2.2 The result of ERP

(i) Differences between "Decreasers" and "Increasers". The differences of mean amplitude of LPP elicited by just the EN pictures, just the LN pictures, and all 300 pictures were analyzed using repeated-measures ANOVA between the "Decreasers" and "Increasers". The ANOVA factors included Group ("Decreasers", "Increasers") × Laterality (left, middle, right) × Anterior-Posterior (F, FC, C, CP, P). The Greenhouse-Geisser epsilon correction was applied to adjust the degrees of freedom of the F-ratios.

There was no main effect of mean amplitude of LPP elicited by the 300 pictures between the "Decreasers" and the "Increasers" ($F(1,12) = 0.214$, $P > 0.05$). There were no interaction effects either, with both Group × Laterality and Group×Anterior-Posterior failing to reach significance ($F(2,24) = 0.548$, $P > 0.05$; $F(4,48) = 0.314$, $P > 0.05$). The main effect of mean amplitude of LPP elicited by EN pictures did not reach significance ($F(1,12) = 2.59$, $P > 0.05$), and neither the Group× Laterality interaction nor the Group× Anterior-Posterior interaction reached significance ($F(2,24) = 0.515$, $P > 0.05$; $F(4,48) = 1.04$, $P > 0.05$). The main effect of mean amplitude of LPP elicited by LN pictures did not reach significance ($F(1,12) = 0.01$, $P > 0.05$). The Group× Laterality interaction and the Group× Anterior-Posterior interaction did not reach significance either ($F(2,24) = 0.79$, $P > 0.05$; $F(4,48) = 0.10$, $P > 0.05$).

Table 1 Means and standard deviations of SIgA concentration during baseline and immediately following the emotion elicited session (μg/mL)

	Baseline	After stimuli	$t^{a)}$
Total concentration of SIgA	237.54 (72.60)	261.34 (95.05)	–1.47
Concentration of SIgA in "Decreasers"	275.92 (53.49)	227.45 (72.23)	4.17*
Concentration of SIgA in "Increasers"	198.29 (85.56)	296.93 (131.70)	–5.12*

a) *, $P < 0.01$.

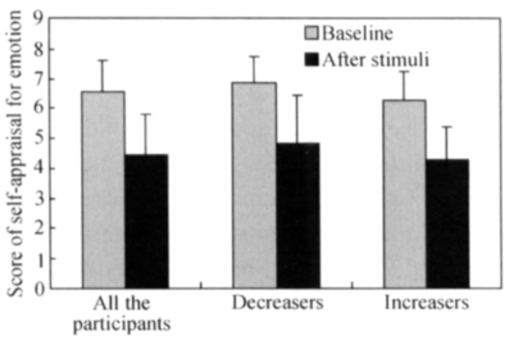

Figure 2 Scores of self-appraisal for emotion before and after presenting stimuli for all the participants (n = 20), the "Decreasers" (n = 7) and the "Increasers" (n = 7).

(ii) Difference within "Decreasers" and "Increasers". The differences in mean amplitudes of LPP elicited by EN pictures and LN pictures were also analyzed using repeated-measures ANOVA within the two groups separately. The same 15 electrodes were selected for statistical analysis. The ANOVA factors included: Pictures Group (EN, LN) × Laterality (left, middle, right) × Anterior-Posterior (F, FC, C, CP, P). The Greenhouse-Geisser epsilon correction was applied to adjust the degrees of freedom of the F-ratios.

As Figure 3 shows, the main effect of mean amplitude of LPP for Picture Group of "Decreasers" did not reach significance ($F(1,6) = 2.31$, $P > 0.05$), while the Picture Group × Anterior-Posterior interaction reached significance ($F(4,24) = 12.22$, $P < 0.01$). Simple effect analyses indicated that at the frontal and front-central locations, the mean amplitude of LPP elicited by EN pictures was larger than that elicited by LN pictures ($F(1,6) = 7.47$, $P< 0.05$; $F(1,6) = 11.98$, $P < 0.05$). The largest difference in LPP amplitude was recorded at front-central electrode sites (e.g. FC3, FCz, FC4).

Figure 3 also shows that the main effect of mean amplitude of LPP for Picture Group of "Increasers" was significant ($F(1,6) = 46.63$, $P < 0.001$), and the Picture Group × Laterality interaction was of significance ($F(2,12) = 5.23$, $P < 0.05$). Simple effect analyses indicated that whether in the left side, or midline or right side of the brain, the mean amplitude of LPP elicited by EN pictures was smaller than that elicited by LN pictures ($F(1,6) = 14.64$, $P < 0.01$; $F(1,6) = 82.09$, $P < 0.001$; $F(1,6) = 42.00$, $P < 0.01$). The largest difference in LPP amplitude was recorded in the midline of the brain (e.g. Fz, FCz, Cz, CPz, Pz). The Picture Group× Anterior-Posterior interaction was of significance as well ($F(4,24) = 5.89$, $P < 0.05$). Simple effect analysis indicated that no matter at the frontal, or front-central, or central, or centro-parietal locations, the mean amplitude of LPP elicited by EN pictures at each of these sites was smaller than that elicited by LN pictures ($F(1,6) = 32.3$, $P < 0.01$; $F(1,6) = 40.64$, $P < 0.01$; $F(1,6) = 134.63$, $P < 0.001$; $F(1,6) = 19.29$, $P < 0.01$). The largest difference in LPP amplitude was recorded at central electrode sites (e.g. C3, Cz, C4).

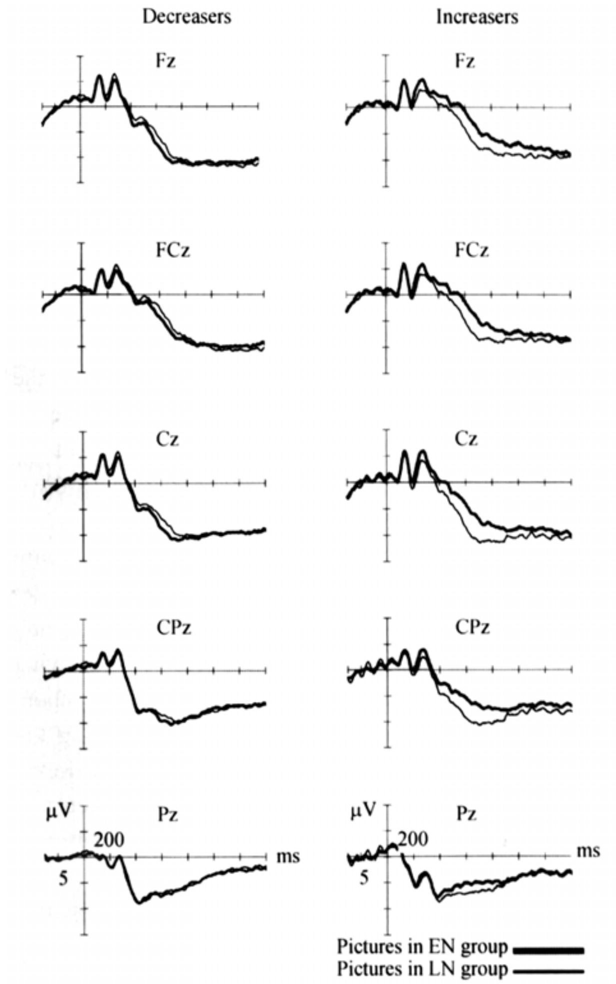

Figure 3 LPP induced by EN pictures and LN pictures in "Decreasers" and "Increasers".

2.3 Follow-Up Interview

An analysis of our data, particularly the within-group difference of LPP mean amplitude, suggested a similarity to the use of emotion regulation strategies uncovered in other studies[20,21]. In order to further investigate and understand the participants' true psychological activities as they were viewing the negative pictures, we revisited the participants involved in our study. We were particularly interested in learning whether and how the participants regulated their emotions. Interviews could not be completed for all of the participants, as a result of missing or incorrect contact information. Of the 12 participants in the increasing group, interviews were completed for 9 of them. Of the 8 participants in the decreasing group, interviews were completed for 7 of them.

To do this, a semi-structured interview, in which the interviewer used formalized questions but flexible set of answers, was employed. All participants involved in our study were called back for the interview the day following the completion of the ERP experiment.

The conversation combined open questions with closed questions. The first question was: "Please describe in detail your subjective feelings, including any thoughts or other mental activities, as you were viewing the pictures. It is very important for us to know about your psychological activities as you were viewing the pictures, so please do not exclude any details, however seemingly unimportant." If the participant revealed that she had used a certain strategy of emotion regulation, a follow-up question was asked: "During the experiment, did you use some methods to cope with your negative emotion? If you did, what kind of method did you use? Did you use the same method or a similar method for every picture?" The entire duration of the interview was recorded.

Interview recordings were transcribed, and participants were evaluated to judge whether they had used cognitive reappraisal strategies according to the following 3 criteria: (i) The participant can focus on the positive aspect of the pictures, or imagine the positive outcomes of the situation, or regard the content of the picture as false, allowing her to explain apparently negative pictures in a less negative way[20,32,33]. (ii) No single type of reinterpretation is universally applicable to all pictures, which is expected to close to the natural state that individuals using strategy in realistic life. The participants can choose automatically an effective reinterpretation method for relieving negative emotion depends on the specific emotion-arousing content[33]. (iii) The participant does not generate thoughts and images that are completely unrelated to the presented stimulus in order to produce a positive emotion to replace her initial emotional response to the picture[20]. All recordings were coded by two researchers independently and any disagreements were resolved by discussion. Inter-rater reliability was 93.8%.

The analysis of the transcriptions found that of the 9 individuals in the increasing group for whom interviews were completed, 6 participants mentioned regulating strategies similar to cognitive reappraisal strategy as they viewed the pictures. For example, upon viewing a picture of an injured person who needed surgery, some participants would think that while the person in the picture was in a serious condition, he would get better after surgery. Upon viewing a picture of war refugees, some participants would think that the war would come to an end and life for the refugees would be improved. Upon seeing a picture of mutated animals, some participants would think that such an incident seldom exists in the real world. Only 1 individual out of the 9 females interviewed in the increasing group reported using a regulating strategy for every picture, while the other 8 said that they used such a strategy for only the most emotionally arousing pictures. According to the self-descriptions of the individuals, 66.7% of the pictures to which regulating strategy was applied belonged to the EN group.

In contrast, of the 7 individuals who were interviewed in the decreasing group only 1 mentioned using a psychological regulating method. However, she was not able to recount specific details. Moreover, the immune data showed that her SIgA concentration decreased less than others in the decreasing group. This suggesting that her regulation of negative emotions had a somewhat weak influence.

2.4 Questionnaire

The Trait Coping Styles Questionnaire (TCSQ) was applied to all 16 individuals who came back for the follow-up interview (i.e. the 9 in the increasing group and the 7 in the decreasing group)[34]. The questionnaire included 2 sub-scales, negative coping and positive coping, with 20 items in total, and each item scored from 1 (absolutely no) to 5 (absolutely yes). The Cronbach coefficients of the negative coping and positive coping subscales are 0.69 and 0.70 respectively, indicating good internal concordance and content validity. The score on the positive coping scale for members of the "Increasers"(35.22 ± 3.93)was found to be significantly higher than for those of the "Decreasers"(29.00 ± 5.45), ($t(14) = -2.66$, $P < 0.05$). However, there was no significant difference of score on the negative coping scale between the two groups.

3 Discussion

Our study showed that viewing negative pictures induced negative emotions, therefore leading to a significant decrease in degree of pleasantness among the participants. Our finding of no significant change in total concentration of SIgA before and after viewing of the negative pictures was concordant with the finding reported by Yang[13]. Instead, we found that different individuals exhibited different changing tendencies of SIgA. Specifically, we found that for individuals whose SIgA levels decreased, the changes were consistent with those of individuals watching a sad video in the study by Labott et al.[11]. As for the individuals whose SIgA levels increased in the present study, the changing tendency of SIgA patterned liked those who accomplished the active coping task in other studies[30,35,36]. Although the difference in baseline SIgA concentration between the two groups reached marginal significance, we compared the two groups on their relative change in SIgA concentration levels before and after presentation of the stimuli. We also compared the different direction of change (increasing versus decreasing) in SIgA concentrations. So the baseline differences in different individuals would not affect the conclusions in this investigation.

Among the “Increasers” and the “Decreasers”, changes in SIgA concentrations were accompanied by changes in ERPs, suggesting that brain EEG activities are correlated with individuals’ reaction to negative emotion-arousing stimuli. The amplitude of LPP was directly related to the individual cognitive evaluation[17,37]. Emotional pictures elicited larger LPPs than neutral stimuli[20,21]. Moreover, pleasant and unpleasant pictures of high emotional intensity and arousal elicited larger LPPs than those of lower emotional intensity[16,17], which indicated that the individuals did more sufficient cognitive process to the emotional stimuli[20], and for stimuli with greater emotional intensity and arousal, individuals spent more psychological resources during cognitive processing. This suggests that LPPs reflects the evaluation deviation of participants’ emotion cognition in the course of their information processing. Our study found an opposite tendency for evaluation deviation of emotion cognition to pictures with different levels of emotion intensity of the participants between “Increasers” and “Decreasers”.

Among participants in the “Decreasers”, EN pictures (defined as the 100 most negative pictures) induced larger LPPs than LN pictures. This result was consistent with the study reporting “pictures with more emotional intensity and arousal degree led to larger amplitude of LPPs”[16,17]. Past studies have found that the amplitude of LPP elicited by negative stimuli was higher than that elicited by neutral stimuli[38,39], which indicated that during the stage of individuals’ cognition and evaluation of environmental stimuli, negative stimuli use more psychological resources and get more cognitive processing so that individuals can cope with the challenges of surviving and developing. Here, the extremely negative stimuli presented the most severe threat to the individual’s survival [40]. Obeying the cognitive rule of emotion, it was of evolutionary significance for individuals to sufficiently process the most negative pictures, as shown by the EEG data. It is well known that the cognitive evaluation of environmental stimuli plays an important role in generating and regulating emotions. The sufficient appraisal and processing of the most negative pictures among participants in the “Decreasers” might lead to their increasing negative emotional reaction and decreasing SIgA.

As far as the participants in the “Increasers” are concerned, their changing tendency was the opposite of that found in the “Decreasers”. Among these individuals, EN pictures induced smaller LPPs than LN pictures. Although such a result does not follow the ‘negativity bias’ in the evaluation stage of emotion processing, it is consistent with the conclusion of the studies that the LPP of participants who actively used the emotion regulating strategy (cognitive reappraisal) was smaller than that of those individuals who viewed stimuli passively[20,21]. Gross introduced the idea of the strategy of cognitive reappraisal and pointed out two regulating methods at different stages of emotional expression. The first method is called antecedent-focused emotion regulation, which regulates emotion before the emotion is triggered. The second is called response-focused emotion regulation, which involves the inhibition of emotional response tendencies after the emotion has already been generated. Cognitive reappraisal is an example of the former[41], as it tries to understand the events leading to negative emotions such as frustration, anger, and antipathy in a positive way, or rationalize the events[42]. Gross proved that the application of reappraisal strategy could decrease the negative emotional experience of the participants with no hint of elevations in physiological responding[41].

Coinciding with the above results, 66.7% (6 of 9) of the participants in the "Increasers" said that they actively regulated their emotion when watching some pictures. Moreover, interviews suggested that the regulating methods they used were similar to Gross' strategy of cognitive reappraisal[20,32,33]. More statistical data showed that 66.7% of the pictures to which regulating strategies were applied belonged to the EN group. This suggests that the smaller amplitude of LPP induced by EN pictures may be attributed to the cognitive reappraisal strategy used by individuals in the "Increasers".

Past studies have also shown that SIgA increases when people take part in active coping tasks that require certain active cognitive effort such as performance of mental arithmetic, speech, and computer games, etc., while SIgA decreases during passive coping tasks such as the cold pressor test, watching the surgical videos, etc.[30,35,36]. This further suggests that unlike the participants in the "Increasers" who actively regulated their emotion while viewing pictures, individuals in the "Decreasers" just watched passively. Such a difference in active or passive processing would lead to the different changing tendency of SIgA of different groups.

In order to test if the changing tendency of SIgA was caused by using the strategy of cognitive reappraisal, our study included an additional intervention experiment looking at emotion regulating strategies. The 6 females with the lowest scores in the active coping subscale of the Trait Coping Styles Questionnaire (TCSQ) and watching the pictures without any regulating strategies were chosen to partake in this experiment.

The participants were asked to complete a task of passively viewing 100 negative pictures. They were just required to focus on the pictures and allow themselves to experience/feel any emotional response that might be elicited. Saliva samples were taken before and after viewing the pictures. A week later, the participants were retested, but they were asked to complete the task while regulating their emotions this time. Participants were first instructed how to use the strategy of cognitive reappraisal. After the individuals had mastered the strategy, they were asked to watch 100 negative pictures (different from the pictures seen in the first task of passive viewing, but matched for valence degree and arousal degree) while employing the regulating strategy for every picture. Again, saliva samples were taken before and after watching the pictures.

As shown in Figure 4, after the completion of passive viewing, the SIgA concentrations of the participants showed a tendency to decrease, with a Paired-Samples T test showing marginal significance ($t(5) = 2.22$, $P = 0.077$). In contrast, after the completion of the emotion-regulating task (i.e. watching the negative pictures with the strategy of cognitive reappraisal), the concentrations of SIgA increased significantly ($t(5) = -6.10$, $P < 0.01$).

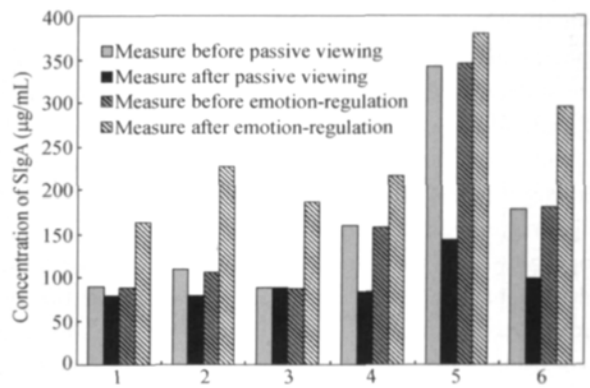

Figure 4 Concentration of SIgA before and after passive viewing task and emotion-regulating task.

Therefore, our study suggests that the participants in "Increasers" preferred using active emotion regulation to consume more psychological resources to the cognitive process during watching the extremely negative pictures, which soothed the emotional reaction to the extremely negative pictures and led to decrease of EEG activities and preferable immune changes (SIgA increased).

It should be pointed out that the above results were acquired on the basis of the participants in the "Increasers" reporting that the strategy of emotion regulation was only applied to those most intensely negative pictures, which indicated that although participants of "Increasers" applied the strategy just to parts of the pictures, it led a significantly different changing of SIgA from "Decreasers". The results of this study also show that the strategy of emotional regulation significantly affect individual immune system function. Our study suggests that individuals who use active emotion regulation in the face of negative emotion stimuli may experience significantly increases in immune system function, subsequently lowering the possibility of infection.

However, why is it that in the face of negative stimuli, only some individuals use such a strategy? The Trait Coping Styles Questionnaire (TCSQ) used in our study provided some clues, and suggested that the answer to this question lies in personality characteristics. The results of questionnaire indicated a higher score on the active coping subscale among individuals in the "Increasers" than in the "Decreasers". That is, individuals in the "Increasers" preferred to adopt a more active emotional coping style and belonged to the kind of personality termed "optimistic explanatory style" by Seligman[22]. Participants with this personality actively regulated their emotions with positive attitudes to relieve negative emotional experiences. In contrast, the score on the passive coping subscale did not differ significantly for participants in the two groups. This indicated that they were healthy psychologically, not belonging to the personality of pessimistic explanatory style[22], that is, they would not cope with environmental stimuli in their lives in a passive way. The results show that it is important to train individuals in the personality characteristics of the optimistic explanatory style so as to increase the level of individual immune function.

Our study proves the potential of combining ERPs, Trait Coping Styles Questionnaire (TCSQ), and revisiting participants to study the effect of psychological processes and EEG activities on the changes of SIgA led by negative emotions. Meanwhile, our study also provided an explanation for the different changing tendencies of SIgA induced by negative emotions, and it plays an important role in further studying the cognitive neural mechanisms of immune level in response to emotion. But the sample size of the current study was relatively small. Both more subjects and the investigation of a possible sex effect are needed in further research to make the results more representative. Also, it should be noted that the current research studied negative emotion without regard to the different latitudes of negative emotion such as sadness, anger, fear etc. In future studies, it may be interesting to focus on a specific latitude of negative emotion in the stimuli to study individuals' different cognitive responses and related EEG activities and immune level. Our study indicated that personality characteristics have a certain effect on the application of emotion regulation strategies. Therefore, a future study might want to select subjects with personality characteristics as an independent variable.

4 Conclusion

Our study investigated the change of SIgA levels and ERPs in a healthy population in response to negative emotion. We found:

(i) Individual negative emotion was induced successfully using negative pictures.

(ii) As far as immune changes are concerned, the preferable changing tendency of the participants in the "Increasers" (SIgA increased) may be related to the active emotion regulating strategy.

(iii) The more intensely negative the pictures shown to participants in the "Decreasers", the larger LPP amplitude. The opposite effect was obtained for those in the "Increasers".

(iv) Individuals of optimistic explanatory style prefer to use emotion-regulating strategies to cope with negative emotional stimuli.

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