Research Article

TWO SUCCESSIVE PHASES IN THE THREAT-RELATED ATTENTIONAL RESPONSE OF ANXIOUS SUBJECTS: NEURAL CORRELATES

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Background: *It is well known that highly anxious individuals are characterized by the allocation of a greater amount of attentional resources to threatening stimulation. However, neural data in relation to the time course of attentional biases in anxiety are still surprisingly scarce and preliminary. The present research explored attentional biases in anxious subjects (grouped according to their scores in trait and state forms of the State–Trait Anxiety Inventory) over time by using event-related potentials (ERPs) and through the application of source localization methodologies.* **Methods:** *Participants (n = 27) were asked to perform an indirect visual matching task in a cue-target paradigm. The targets consisted of three types of emotional pictures: positive arousing, negative arousing, and neutral. ERPs in response to target stimulation were submitted to temporal and spatial principal component analyses.* **Results:** *Statistical analyses revealed that negative targets elicited higher amplitudes than positive pictures in P200. Subsequently, greater amplitudes in response to positive than to negative pictures were observed in P500. Source analyses (standardized low resolution brain electro-magnetic tomography: sLORETA) indicated an involvement of visual association cortical areas (i.e., precuneus and cuneus) both in P200 and P500.* **Conclusions:** *The results might be interpreted in line with the orienting-avoidance hypothesis toward threatening events in anxious subjects. This attentional pattern was only manifested by individuals with high levels of both trait and state anxiety. Further investigation should be done in order to better understand the brain mechanisms underlying the attentional biases in anxiety and to apply this knowledge to the development of cognitive therapies. Depression and Anxiety 26:1141–1150, 2009.* © 2009 Wiley-Liss, Inc.

Key words: *anxiety; attentional biases; emotion; threat; event-related potentials*

INTRODUCTION

Successful behavior requires an individual to select relevant information at every moment in order to cope with environmental demands in an adaptive way. Among other relevant sources of stimulation, the processing of threatening events is of particular relevance due to its survival implications.[1,2] However,

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it is thought that when this adaptive allocation of resources turns into a constant monitoring of threatening cues, it may constitute a dysfunctional strategy underlying the maintenance and even the origin of pathological manifestations of anxiety.[3,4] A biased tendency to preferentially deploy attentional resources toward threat-related signals or even to provide a more negative interpretation of emotionally ambiguous events have been extensively documented in individuals with high anxiety (HA) levels [for a review see[5,6]].

The time course of attentional biases has become a key point in approaching the study of attention to threat in anxious individuals. Nevertheless, there is no agreement about whether deployment of attentional resources toward threat-related information by anxious individuals follows a steady pattern at all the stages of processing.[7] Several cognitive theories[4,8] and behavioral data[9–11] have indicated that anxiety is characterized by a dysfunctional attentional pattern composed of two phases. In other words, anxious people direct their automatic attentional resources to threatening stimulation at an early stage of processing, but subsequently, during a more elaborated step of such processing, they allocate attention away from the threat. Because avoidance hinders both a detailed evaluation and habituation to feared stimuli, it is considered as a primary factor in the etiology and maintenance of anxiety conditions.[12] This effect known as *vigilance-avoidance* attentional pattern is particularly striking in people with specific phobias.[13,14] In contrast, another line of evidence has postulated that attentional avoidance does not occur after the initial orientation to threat; rather, anxious subjects would maintain cognitive resources on threatening stimulation due to their difficulty to disengage attention from such stimuli.[15,16]

From a psychobiological approach, different aspects related to the attentional biases in anxiety have already been explored using event-related potentials (ERPs) methodologies. For instance, some studies have investigated spatial orienting responses in people with high levels of anxiety. An attentional bias reflected by the highest amplitudes of short latency components (peaking between 100–250 ms) in response to potentially dangerous stimulation has been found.[17,18] Other authors have focalized on the neural correlates of the processing bias showed by high-anxious individuals toward stimuli with a more social meaning such as emotional faces.[19,20] Specifically, Bar-Haim et al.[19] have found a modulation of P200 when threat-related faces were presented. However, and as far as authors know, a deep study of the spatio-temporal characteristics related to the attentional biases in anxiety has not been yet undertaken.

Another open question refers to the different role that both state and trait anxiety would play in the manifestation of these attentional patterns to threatening stimulation. Although some influential cognitive theories[21] have highlighted the contribution of trait anxiety in the increased tendency to allocate attention toward threat, more recent behavioral perspectives have extended this prediction to the combined effect of state and trait anxiety as being important sources of variance when trying to explain findings related to the intensification of attentional biases.[22] Till date, several ERP studies support the idea that cortical activity directly linked with attentional responses might be modulated by the influence of dispositional variables such as trait anxiety;[17] however, both state anxiety and interactive effects between trait and state measures of anxiety have been also found.[23] Additionally, neuroimaging data have confirmed that both trait[24] and state anxiety[25] can modulate cortical and subcortical functions while subjects perform attentional tasks.

To summarize, our main interest is to cast light not only on the temporal characteristics linked to attentional biases in nonclinical anxious subjects (with different levels of anxiety), but also to know which cortical areas are involved in the different steps of each stage of processing. Due to cognitive processes such as attention that occur in very short period of time, we need a temporally agile signal that allows us to record them. ERPs methodology constitutes a very useful tool for studying dynamics in the brain. Moreover, in order to determine the neural origin of ERP components (i.e., processing phases) a source-localization algorithm was applied. Neural correlates underlying this attentional bias to threat-related information were analyzed while subjects carried out an indirect visual matching task. Different attentional paradigms (e.g., emotional Stroop dot-probe or visual search) have been used to study attentional biases in anxiety. Recently, emotional adaptations of the classical cue-target paradigm[26] have been applied to investigate selective attention in anxious individuals.[17,27] In our investigation, we used an emotional and nonspatial cue-target task. This variant has been documented as a helpful task to investigate neural correlates of interactive processes between attention and emotion, such as some aspects related to vigilance or expectancy processes toward the following events.[28] Furthermore, the case of target stimulation consists of a visual image, and if its visual exploration is required to a correct performance in the task (to detect cue-target correspondences, as is the case in this study), ERP elicited by this stimulus has also shown to be able to reflect input processing-related attention to visual stimuli.[29]

Finally, and based on the revised literature on the potential influence of specific attentional patterns in the maintenance of anxiety, we hypothesize that high-anxious individuals will show a biased attentional response toward threatening stimulation reflected by "early" and "late" attention-related ERP components that would involve different components of attention.

MATERIALS AND METHODS
PARTICIPANTS

Thirty-two right-handed students took part in the experiment, but only the data from 27 of them were analyzed, as explained later. The participants were aged between 19 and 28 years old (mean: 21.3;

SD: 2.33). They were selected from a pool of 200 students from the Universidad Autónoma de Madrid on the basis of their scores on the trait form of the State–Trait Anxiety Inventory (STAI),[30] and grouped into a high-trait anxiety group (13 participants—11 females and 2 males—with trait centile over 50) and a low-trait anxiety group (14 participants—13 females and 1 male—with trait centile under 50). All of them completed this form of the STAI between 4 and 12 weeks before the recording session. Once in the laboratory, and just before the beginning of the electrophysiological recording, the participants completed the state form of the STAI (participants scored over centile 50—8 females and 1 male—and 18 under centile 50—16 females and 2 males). The mean trait anxiety for the HA individuals was 60.5 (*SD* = 5.3); for the low anxiety (LA) individuals, it was 37.8 (*SD* = 6.8). The mean state anxiety for the HA individuals was 58.9 (*SD* = 6.2) and for the LA individuals it was 35 (*SD* = 7.3). Both groups showed significantly different anxiety scores to trait [$t(22) = -10.98$, $P < .001$] and state [$t(20) = -5.55$, $P < .001$] variables. All the participants gave written informed consent for their involvement in the experiment, which was totally voluntary. All had normal or corrected-to-normal eyesight.

STIMULI AND PROCEDURE

The experimental paradigm (emotional cue-target) comprised the sequential presentation of two stimuli in each trial, the S1 or *cue* stimulus and the S2 or *target* stimulus. Through this procedure we studied the deployment of attention toward three categories of target stimulation that differed in the main dimensions (valence and arousal) explaining the principal variance of emotional information. The target stimuli were affective scenes: arousing-positive (A+), arousing-negative (A−), and neutral (N). In contrast, cue stimuli were devoid of emotional meaning, and informed only in an implicit way about the emotional content of target stimulation. Cue stimuli were simple white-on-black schematic line drawings or symbols. Each cue consisted of a pair (one above the other) of these schematic drawings. Each pair was made up of stimuli belonging to the same category. Thus, these cue stimuli would be less prone to having deep intrinsic emotional significance at least compared to photographs or realistic drawings. In order to give them a meaning, before the recording session, subjects had the opportunity to see each symbol to identify and associate it with a particular object (e.g., insects, cake, etc.) guided by the experimenter. Two drawings or symbols were designed for each category representing the emotional content of the forth-coming target stimuli: A+ drawings showed a "naked person" and a "cake," those corresponding to the A− category showed an "insect" and a "wolf jaw," and finally, those belonging to the N category showed a "building" and a "glass." Each cue presentation, with a size of $21.9°$ (high) × $15.1°$ (wide), lasted 250 ms and was centered on the screen.

The target stimuli were displayed after a time interval of 1500 ms. This stimulation consisted of color pictures ($22.9° × 15.6°$). Photographs could be an "ice cream cake" or an "opposite-sex nude" in the A+ category, an "insect" or an "open mouth of a wolf in an aggressive expression" in the A− type and a "building" or a "glass" in the N category. Therefore, photographs always represented one of the motives cued by the two drawings of the cue presentation. The participants were told to identify verbally which of the two drawings of the cue corresponded to the target saying aloud "A" if the photograph corresponded to the top part of the cue presentation or "B" if it corresponded to the drawing located at the bottom. The target presentation also lasted 250 ms. Finally, an auditory stimulus (beep) was presented 2000 ms after the target offset, indicating to participants the moment from which they had to give a response about the match between cue and target stimuli. They were requested to avoid blinking as much as possible and to look continuously at a small cross situated in the center of the screen. Intertrial interval was 2500 ms. A total of 96 S1–S2 trials were presented (32 for each emotional category: A+, A−, and N). The subjective content of valence and arousal associated with the stimuli was assessed by each participant after the recording session by means of a bidimensional scaling test. Finally, the study of input processing-related attention required that only attended stimuli (cues and targets) were analyzed. To ensure this, as will be seen later, recordings obtained in trials in which subjects responded erroneously were eliminated.

ELECTROPHYSIOLOGICAL RECORDING

Brain electrical activity was recorded from 58 homogeneously distributed scalp electrodes. All these electrodes were referenced to linked mastoids. For the entire sample of cephalic electrodes, originally linked mastoids-referenced data were algebraically re-referenced offline using a global average reference.[31] Vertical and horizontal eye movements were controlled through an electrooculo-graphic recording. Electrodes were located infra- and supra-orbitally as well as at the left and right outer canthi. All electrode impedances were kept below 5 kΩ. A bandpass filter of 0.1–50 Hz was applied. Channels were continuously digitizing data at a sampling rate of 250 Hz throughout the entire recording session. The continuous recording was divided into 800 ms epochs for each trial, beginning 200 ms before S2 onset. Baseline correction and EEG visual inspection were also carried out eliminating epochs with ocular artifacts for further analysis. Trials where subjects answered incorrectly were rejected as well. Data from 5 out of the 32 participants were eliminated due to the high rate of deleted trials (over 20%). ERP averages were categorized according to the type of stimulus.

SOURCE ESTIMATION

In order to explore the cortical regions that could account for the experimental effects, standardized low-resolution brain electro-magnetic tomography (sLORETA) was applied to the components of the ERP in accordance with the analysis of variance (ANOVA) results, as will be explained later. sLORETA is a 3D, discrete linear solution for the EEG inverse problem.[32] Under ideal conditions, solutions provided by sLORETA, being based on distributed brain activity, have no localization bias. sLORETA refers to a three-shell spherical model registered with the MNI305 digitized structural human brain atlas template. These solutions are given, therefore, in three coordinates: "*x*" is the distance in millimeters to the right (+) or left (−) of midline, "*y*" is the distance anterior (+) or posterior (−) to the anterior commissure, and "*z*" is the distance above (+) or below (−) a horizontal plane through the anterior and posterior commissures.

RESULTS

Grand averages at four selected scalp sites for A+ and A− targets once the N category had been subtracted from each ERP are displayed in Figure 1. This subtraction operation is recommended, given the fact that variability between groups in ERP research is often high, and not linked exclusively to the dependent variable under study.[33] In our particular case, ERPs for neutral targets clearly showed higher amplitudes in the low-state and low-trait anxiety groups, as compared to the high-state and high-trait anxiety groups (see Fig. 2). As neutral events have not been previously reported as being associated with any attentional bias in
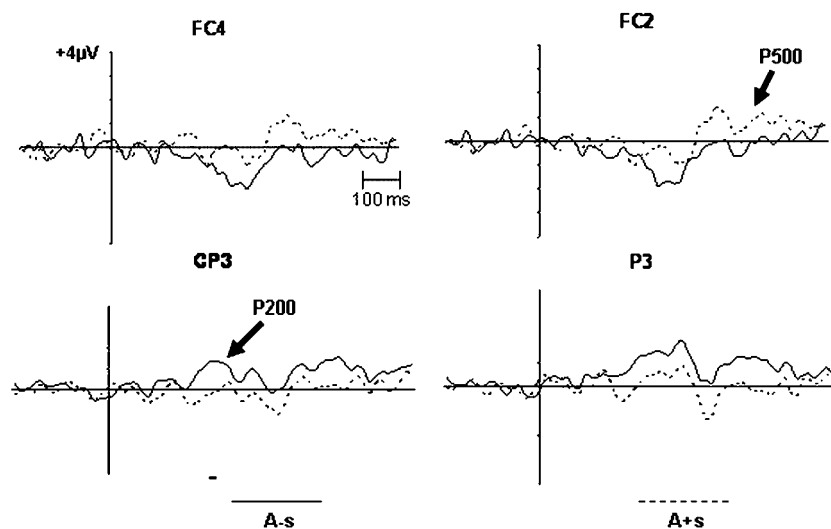
Figure 1. Grand averages corresponding to high- and low-trait anxious participants and high- and low-state anxious participants in response to A−s (threatening) and A+s (positive) stimuli. Scales and polarity are shown at FC4.
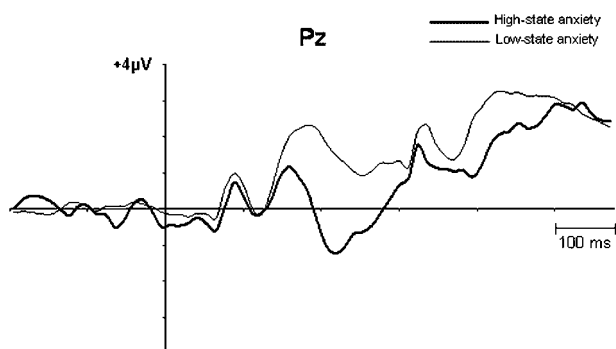

Figure 2. Grand averages obtained in response to neutral targets at Pz. Recordings distinguish between high- and low-state anxiety.

anxious populations, it could be concluded that this effect reflects the influence of interfering variables (i.e., variables unrelated to attention biases). The new subtracted categories will be labelled hereafter as A+s and A−s.

CONTROL ANALYSES

Assessments given by the participants on the valence and the arousal content of the stimuli used as target were analyzed. These analyses were carried out in order to confirm that the stimulus affective valence was that assumed a priori and that A+ and A− photographs had similar levels of arousal. One-way repeated-measures ANOVAs were computed for valence and arousal dimensions, using Stimuli (three levels: A+, A−, and N) as factor. Post hoc comparisons were made to determine the significance of pairwise contrasts, using the Bonferroni test ($\alpha = .05$). ANOVAs yielded significant differences in both valence [$F(2, 54) = 38.029$,

$P < .001$] and arousal [$F(2, 55) = 30.960$, $P < .001$]. Post hoc contrasts indicated that A+ and A− showed different valence but not different arousal. Furthermore, A+ and A− differed from N in both arousal and valence.

DETECTION AND QUANTIFICATION OF ERPs: TEMPORAL PRINCIPAL COMPONENTS ANALYSIS

ERP components were detected and quantified through a temporal Principal Components Analysis (tPCA) using a covariance matrix. This technique has been strongly recommended for these tasks as its application avoids the subjectivity of selecting time windows for components analyses based on a visual inspection of grand-averaged ERPs that can lead to several types of misinterpretation, especially when high-density montages are employed [see[34] for a more detailed description of tPCA procedure and advantages].

The main advantage of tPCA is that it represents each ERP component with its "clean" shape, extracting and quantifying it free of the influences of adjacent or subjacent components. This analysis can also facilitate efforts of source location. In brief, the tPCA computes the covariance between all ERP time points, which tends to be high between those time points involved in the same component, and low between those belonging to different components. The solution is therefore a set of different factors made up of highly covarying time points, which ideally correspond to ERP components. Temporal factor score, the tPCA-derived parameter in which extracted temporal factors may be quantified, is equivalent to amplitude. Through application of the scree test,[35] three post-target ERP components were extracted (see Fig. 3). These components were Promax
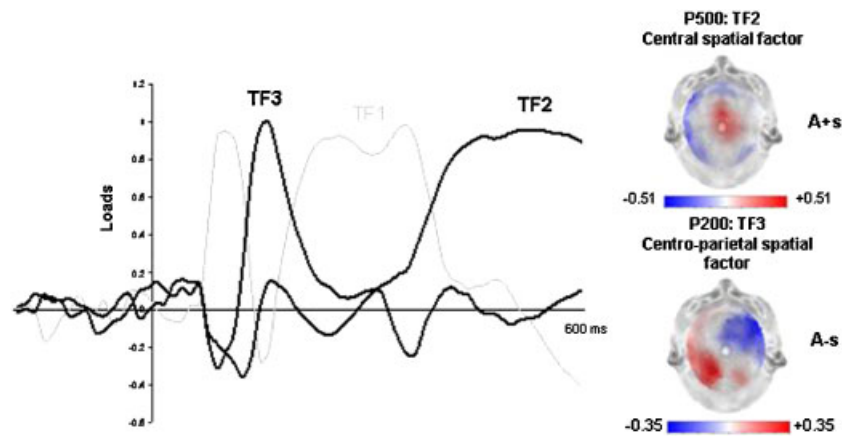
Figure 3. tPCA: Factor loadings after Promax rotation. Temporal factors 2 (P500) and 3 (P200) are highlighted in bold. 2D maps show topographical distribution of the spatial factor scores of temporal factors as a function of the particular experimental condition (P200: A−s; P500: A+s). Red areas reflect high activity.

rotated. Another tPCA as well as the corresponding statistical analyses (ANOVAs) were also computed for the pretarget ERPs (cue-related activity).[1]

ANALYSIS OF THE EXPERIMENTAL EFFECTS

Analyses of experimental effects required the ERPs, recorded at 58 globally distributed scalp points, to be grouped into different scalp regions, as ERP components frequently behave differently in some scalp areas than in others (e.g., present opposite polarity). This regional grouping was determined through a covariance matrix-based spatial PCA (sPCA). This configuring and quantifying scalp regions system is preferable to an a priori subdivision into fixed scalp regions for all components, as sPCA demarcates scalp regions according to the real behavior of each scalp-point recording (basically, each region or spatial factor is formed with scalp points where recordings tend to covary). Consequently, the shape of the sPCA-configured regions is functionally based, and scarcely resembles the shape of the traditional, geometrically configured regions. The sPCAs were carried out, for each of the temporal factors, on their spatial factor

scores that, as earlier explained, represents a single parameter that reflects the amplitude of the whole spatial factor. Experimental effects were tested by computing repeated-measures ANOVAs on the spatial factor scores.

Three spatial factors (though with different shapes in each case) were also established through the scree test for each of the three components. As mentioned above we carried out statistical contrasts to arousing-negative vs. arousing-positive stimuli once brain activity in response to neutral stimulation was subtracted (i.e., A−s vs. A+s) and their possible interactions in function with the level of anxiety in order to test attention-related effects related to each ERP component. Therefore, ANOVAs on the spatial factor scores included two factors: Stimuli (within-subject factor with two levels: A+s and A−s) and Anxiety (between-subject factor with two levels too: high- and low-trait anxiety for one group of analysis, high- and low-state anxiety, for the second group, and a Combination of the two anxiety measures, people who scored high and low in both trait and state anxiety for the third group). As explained, HA groups were made up of participants that performed over centile 50 in the STAI, and LA groups by those who obtained a centile of 50 or under. Only temporal factors 2 and 3 were sensitive to experimental manipulations, according to the ANOVAs (see Table 1). Factor peak-latency (554 ms) and topography associate factor 2 with the wave labelled P500 in grand averages and factor 3 (168 ms) with P200 (see Fig. 3). Specifically, analyses on the centro-parietal spatial factor of P200 were significant for the interactions Stimuli by Trait Anxiety, Stimuli by State Anxiety, and Stimuli by Combination Trait-State Anxiety. Post hoc comparisons (Bonferroni; $\alpha = .05$) showed that A−s stimuli elicited higher amplitudes than A+s ones. However, this effect was significant only for high-state anxiety group and high trait–state combination group. In contrast, both low-trait and low-state anxious

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[1]The reviewers suggested the possibility that cue-related effects emerged as the content of these stimuli were related to the affective meaning of target stimulation (A+: positive-arousing; A−: negative-arousing). In order to explore this possibility we carried out a tPCA for pretarget ERPs. Following the same criterion used for the post-target ERPs selection three factors were extracted. These three factors explained 73.48% of total variance. Extracted components were submitted to promax rotation. We also carried out sPCAs for each of the temporal factors. Subsequently, we conducted repeated measures ANOVAs on the spatial factor scores of each spatial factor. These ANOVAs included two factors: Stimuli (A+s and A−s) and Anxiety (high-anxiety groups and low-anxiety groups). None of these analyses reached significance for the interaction between Stimuli and Anxiety ($F_{1,25} = 1.213$, $P = .235$).

TABLE 1. Statistical details of contrasts in which differences between A− and A+ were significant regarding the different groups of anxiety (Trait and State and their Combination, high and low in the three cases)

| Temporal factors	Spatial factors	Trait anxiety	State anxiety	Combination
TF1	SF1 (posterior)	No	No	No
	SF2 (frontal)	No	No	No
	SF3 (central)	No	No	No
TF2 (P500)	SF1 (posterior)	No	No	No
	SF2 (frontal)	$F(1, 25) = 5.014$, $P<.05$ $(P = .034)$	$F(1, 25) = 6.719$, $P<.05$ $(P = .016)$	$F(1, 25) = 8.951$, $P<.05$ $(P = .006)$
	SF3 (central)	No	No	No
TF3 (P200)	SF1 (frontal)	$F(1, 25) = 8.523$, $P<.01$ $(P = .008)$	$F(1, 25) = 15.756$, $P<.01$ $(P = .001)$	$F(1, 25) = 11.321$, $P<.01$ $(P = .002)$
	SF2 (central)	No	No	No
	SF3 (centro-parietal)	No	No	No

participants showed higher amplitudes to A+s than A−s stimuli. Significant differences were also found in the analyses on the central spatial factor of P500: interactions Stimuli by Trait Anxiety, Stimuli type by State Anxiety, and Stimuli by Combination Trait–State Anxiety. Post hoc tests indicated that the amplitude of this spatial factor of P500 was higher for A+s as compared to A−s stimuli for participants with high scores in both trait and state anxiety by itself as a combination of both of them.

SOURCE ESTIMATION

Figure 4 shows the solutions linked to the cortical activity of participants with high scores in both state anxiety (for P200) and the combination of trait and state anxiety (for P500) where it was the maximum. This maximum cortical activity represents the neural source for both P200 and P500 components in response to A−s and A+s, respectively. As it can be seen, the foci are the precuneus (Brodmann area 7: $x = 10$, $y = 80$, $z = 50$) for P200 and the cuneus (Brodmann area 19: $x = 10$, $y = 85$, $z = 40$) for P500.

DISCUSSION

ERP activity associated with attentional processing of emotional information has shown differences that are modulated by the level of anxiety. We have described a temporal pattern with two successive phases initially characterized by an "early" allocation of attentional resources at about 180 ms (P200), which is more prominent toward the threatening stimulation. This is followed by a "late" response at 550 ms (P500) aimed in greater extent to the processing of positive events. Source estimation algorithms suggest that areas located in the posterior cingulated cortex, such as the precuneus and visual primary cortices regions (cuneus) as responsible for the brain responses obtained in both the first and second phases, respectively. This attentional modulation revealed by P200 and P500 appears
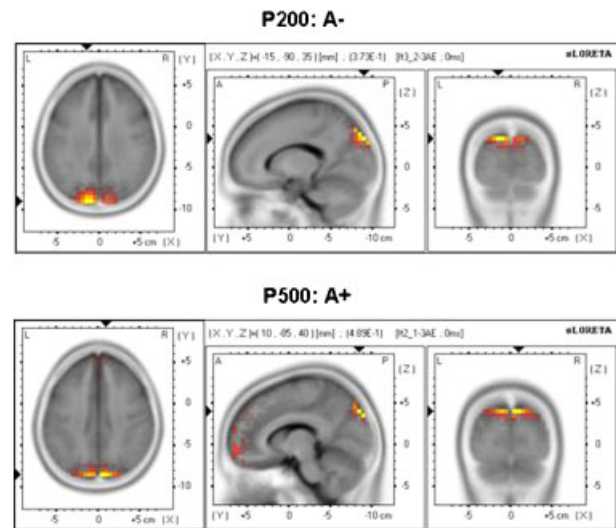
Figure 4. Images of neural activity computed with sLORETA on P200 and P500 factors scores. Three orthogonal brain views in MNI305 template, sliced through the region of maximum activity, are illustrated. In the image above is represented the neural source for P200 in response to A−s (high-state anxiety group). The image below represents the neural focus for P500 in response to A+s (high combination of high-trait and -state anxiety group).

to be a reflection of the brain mechanisms related to the time course of attentional biases in anxiety.

What can this *initial orienting* phase be reflecting? First attentional phase or *initial orienting* is defined by the highest amplitude of P200 to threat-related stimuli in people with high level of anxiety. This early attentional response reflected by P200 is in accordance with evolutionary perspectives, as there is no doubt that the setting in motion a rapid reaction in response to threat can have a considerable value for survival in order to face a potential danger.[1,36] As mentioned before, accumulated evidence about the time course of attentional biases in anxiety (mainly provided from behavioral experiments) has shown the existence of two

phases or components in the attentional response to affective stimulation.[9,10] All the available data point out to the initial phase, which is characterized by an automatic orientation to threatening events that would permit early detection of potentially dangerous stimulation in anxious individuals.

Spatial cueing paradigms developed by Posner et al. have been used to study attentional biases in anxiety.[26] As far as the authors know, there is no behavioral data related to attentional biases in anxiety derived from the use of our nonspatial variant of the classical cue-target paradigm. It could make difficult the straightforward comparison between the present data with those obtained from previous ERP studies, and the identification of typical components (P200 or P3b/slow waves) with the ones obtained here. In order to further the interpretation of these ERP components, it is important to note that task requirements (i.e., visual exploration of an emotional image) lead us to the consideration that the ERP components here analyzed reflect mainly stimulus processing-related attention. However, the experimental tasks usually employed in the study of visual attention to obtaining components with similar latency to P200 or P500, differ greatly from the one used here. Therefore, current components could be defined as components related to visual attention to previously cued emotional stimuli.

Some investigations, however, have obtained a similar pattern of ERP components (P200 and P340) in healthy individuals when they performed during the same experimental task. In relation to P200 it has been suggested that its amplitude is modulated as a consequence of emotional valence of the stimulation (largest amplitudes to threatening would be reflecting early direction of attention) being associated with the so-called negativity bias.[28,37] Accordingly with previous data, several studies with nonanxious participants have shown a greater and more intense activation in visual cortices to negative events than other types of stimulations. This cortical response elicited by visual stimulation around 200 ms after the stimulus onset, has been quite often associated with a rapid capture of attention.[28,38,39] Although it is known that N1 and P1 components represent early indices of attentional processing related to automatic capture of attention, some authors have argued that this mechanism is reflected not only by N1 amplitude but also by P200.[40] This ERP wave has also been considered as the reflection of an automatic reaction triggered by threatening stimulation in high-anxious individuals.[41] In the light of the present results, we suggest that high levels of anxiety would facilitate sensory visual processing and in consequence it would enhance the amount of early attentional resources devoted to threat-related events. P200 could be considered, therefore, as an index of early attentional capture and processing resources allocation toward threatening stimuli that would be mobilized more intensely in anxious individuals.

As mentioned above, P500 followed the trend opposite to the one that has characterized P200, as it elicited the highest amplitudes (i.e., greatest processing resources) in response to pleasant stimuli. Selective attention studies have reported higher amplitudes in components peaking after 300 ms during the processing of appetitive stimuli comparing to threatening ones when similar experimental tasks to the one used here were employed.[28,42] Furthermore, late positive potentials (between 400 and 600 ms) measured while participants were presented to target emotional pictures have been repeatedly associated with an intense cortical activity over centro-parietal sites.[43] The occurrence of late ERP components (those are peaking around 300 after the stimulus onset) throughout different attention-related experimental paradigms have been considered as indices of evaluative and even response selection processes. Thus, this "late phase of attention" presumably would be oriented to a detailed processing that typically triggers approaching behavior.[44]

Taking anxiety into account, other studies have found that general emotional stimulation, such as both threatening and happy faces, are capable of producing an increase of attentional processing in high-anxious subjects[45] leading to hypothesize that even nonunpleasant stimuli can be perceived as a potential threat.[20] However, in our opinion, the fact that attentional processing is biased toward pleasant events in a late phase (around 500 ms after stimulus onset) in HA groups converges with data from recent behavioral and cognitive neuroscience studies where HA participants directed attention away from threat.[9,10] Although present data should be taken cautiously, it might be indicating that attentional biases during the processing of threatening stimuli undergo changes over time following the so-called orienting-avoidance attentional pattern. Thereby, present data could be interpreted from a different point of view in which high-anxious subjects would allocate attentional resources toward nonaversive information (avoiding threatening events) in a more strategic stage of processing with the aim of reducing affective distress. This factor is regarded as a major variable for the maintenance of anxiety conditions in the long term, as it would result in an inadequate processing preventing the habituation to the threat and its subjective re-assessment.[6,46]

Another relevant question for this study was to provide data about the role of the anxiety type in the processing of affective information. As indicated in some cognitive theories[47] our results show that not only trait anxiety, but also state anxiety and the combination of trait-state anxiety modulate attention to threat-related information reflected in both early and late ERP activity (P200 and P500). Although previous electrophysiological studies have found a trait anxiety-related attentional bias,[17,19] it is very common that anxious individuals show high-state anxiety levels too that may contribute to the attentional bias manifestation due to trait and state anxiety that are frequently highly correlated (state anxiety scores are at least partially explained by trait anxiety) being difficult

to separate their specific effects especially when clinical patients are tested. The employment of natural stressful situations or methods to experimentally induce high levels of state anxiety might help to circumvent this limitation. As a consequence of its limited temporal stability some studies assess state anxiety several times during the experimental session (although trait anxiety is considered a more stable measure, even to this variable more than one assessment should be taken along with the experimental study). This strategy should be considered in order to increase the reliability of anxiety measurement procedures.[7,22]

In agreement with many studies, the present results suggest that the best predictor in the manifestation of attentional biases (reflected by the amplitude of P200 and P500) is the trait–state combination (subjects who scoring high in both factors). It is likely that the combined trait-state measure is the most appropriate index of the subjects' level of anxiety and could be considered as a measure of general anxiety. These *general effects* of anxiety would be, therefore, responsible for the attentional biases in the present sample of participants. When examined, behavioral,[48] neuroimaging,[25] and also ERP[23] studies have demonstrated that high levels of state anxiety can be considered as well, along with trait anxiety, as responsible for the mediation of attentional biases to threatening stimulation. These data are in accordance with the interaction theory.[3] More refined investigation, however, is needed to confirm the role played by both trait and state anxiety.

A final issue refers to the neural sources underlying the attentional processes reported. Spatial characteristics associated with the time course of attentional biases in anxiety have not yet been described. In this sense, present data offer a preliminary approach to this issue. Source estimation solutions suggest the involvement of precuneus and cuneus in the origin of the two threat-related attentional phases reflected by P200 and P500, respectively. Data provided by source estimation algorithms should always be interpreted cautiously despite the application of LORETA that have demonstrated high correlation indices with those provided by brain haemodynamic measures during performance in the same tasks.[49] These results fit well with Posner and Petersen"s (1990) model.[50] Their proposal postulates that visual primary cortices and visual associative cortices (VAC) participate in recognition and categorization tasks as the one used in this study. Further studies have confirmed the involvement of VAC in categorization processes.[51] More specifically, it has been found that the precuneus (apart from its other cognitive functions) is related to attentional orientation [for a review, see[52]]. Posterior parietal areas have been implicated in amygdalar-cortical networks for emotional attention in anxious individuals.[24] These cortical regions would receive projections from the amygdale, enhancing attention

for the processing of threatening events.[53] These brain structures play a crucial role in the neural underlying mechanisms of visual selective attention in anxiety.[54] In this line, visual primary areas such as the cuneus have also shown increased activation for affective stimuli in nonanxious people.[55] Although our data need to be complemented with evidence coming from neuroimaging studies, it leaves opened a research line in order to test the possible involvement of VAC and primary visual regions in the attentional bias in anxiety.

Some shortcomings and limitations of this study must also be considered. First, although all subjects were selected using the STAI (a valid and reliable test to measure anxiety in adults: feelings of apprehension, tension, nervousness, and worry), the participants were not screened for anxiety disorders. It can represent a problem for interpretation of the results obtained, especially when individuals who belong to the HA group reach quite high scores. It is therefore probable that at least some of the individuals included in that group of high level of anxiety had features as part of a clinical disorder that goes beyond high scores in trait/state anxiety. As a consequence of this circumstance, the pool of healthy participants grouped in function of trait anxiety could be different in one more factor apart from the one mentioned. Therefore, and taking into account this possibility, caution should be taken when interpreting the present findings, and replication of our results is necessary. Second, the paradigm used in the present investigation constitutes an emotional modification of the traditional cue-target paradigm. As already mentioned in the Introduction, experimental tasks that require recognition of already presented visual stimuli have been successfully used to study input processing-related attention.[29] Several investigations have indicated that the increase of activation in VAC regions appear to be a key point in the manifestation of this kind of attention.[50,56] According to these data, our results also suggest and support that the VAC might be associated with the input processing-related attention. However, the fact that the paradigm used here differs from those usually designed to investigate selective attention, it is a point to take into account in order to interpret the obtained results and the conclusions reached in this investigation must be established keeping in mind some constraint inherent to the methodology employed in this study. Thus, it should not be discarded that processes other than affective and attentional could contribute to configure the effects observed here. For example, long-term memory encoding processes, which have been studied following a similar paradigm.[57] Future research using more traditional paradigms should contrast these findings. Third, regarding a potential limitation of the investigation, the fact that both factor 1 of the ERP did not reach statistical significance to the conducted contrasts and that trait anxiety did not play a role in the early attentional orienting to threat (reflected by the

P200 component) could be explained, at least partially by the criterion used to split the sample in to HA and LA groups (i.e., percentile 50th). It is important to note the possibility that the modest difference between groups referred to anxiety scores may have played a role in the obtained results leading to a loss of power to detect anxiety-related differences. The selection of high- and low-anxious subjects using a more restrictive criterion could serve to reach a greater explicative power linked to the relationship between attentional biases and trait anxiety. Furthermore, large sample sizes would be required for raising the statistical power to make comparisons. Additional experiments need to be conducted keeping in mind these limitations in order to solve them.

CONCLUSION

In summary, the present investigation provides new evidence on the neural correlates related to the different processing stages in anxiety. Attentional bias in anxious subjects would start with a vigilant threat-related mode that would enable them to rapidly and automatically activate their defence mechanisms,[2] and subsequently, it would be followed by an attentional response more prominent toward positive events that could be acting as a mechanism to prevent further processing of threatening stimulation in an attempt to reduce the associated anxious mood states. This dual-attentional response, reflected in a strong involvement of visual association areas, is particularly striking in individuals with high levels of anxiety. Present data support the hypothesis that threat-related attentional bias involves different cognitive processes, or at least, different phases of the same process[5] going beyond clinical observations. Further research is needed to confirm these results and to fully understand biased attention processes in anxiety in order to refine cognitive therapies.

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