An fMRI study of cognitive regulation of reward processing in generalized anxiety disorder (GAD)

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Abstract

Background: Cognitive regulation can affect the process of decision making. Generalized anxiety disorder (GAD) patients seem to have an impairment in cognitive regulation of reward processing concerning food stimuli. This study aims to explore the impact of GAD in cognitive regulation of food-related rewards.

Methods: GAD patients (n=11) and healthy controls (n=15) performed a cognitive regulation craving task with food images while undergoing a functional magnetic resonance imaging (fMRI) acquisition. Between-group differences in functional connectivity were measured using dorsolateral prefrontal cortex (dlPFC) and ventromedial prefrontal cortex (vmPFC) seeds during cognitive regulation.

Results: During cognitive regulation, there was a significant interaction for functional connectivity between the right dlPFC and bilateral vmPFC with the thalamus. GAD patients had lower functional connectivity for cognitive regulation conditions (distance and indulge) than for the non-regulated condition in these clusters, while control participants presented the opposite pattern. GAD group presented fixed food valuation scores after cognitive regulation.

Conclusions: GAD participants showed inflexibility while valuating food images, that could be produced by cognitive regulation deficits underpinned by functional connectivity alterations between prefrontal regions and the thalamus. These results show cognitive inflexibility and difficulty in the modulation of cognitive responses during decision making in GAD patients.

Key words:

Cognitive regulation, reward processing, generalized anxiety disorder, neuroimaging, fMRI, prefrontal cortex, thalamus.

1. Introduction

Cognitive reward control, defined as the cognitive control of craving for hedonic stimuli, and the cognitive regulation of emotional states are examples of model-based decision making. These strategies seem to share common neurobiological underpinnings, implying the activation of the dorsolateral prefrontal cortex (dlPFC) among other prefrontal regions (Brandl et al., 2019). Moreover, previous functional magnetic resonance imaging (fMRI) studies demonstrated that participants could modulate decision making in various scenarios, such as food selection and craving, through valuation regulation and behavioral control (Ferreira et al., 2019; Hutcherson et al., 2012). The results of these studies suggested that the main areas involved in cognitive regulation are the ventromedial prefrontal cortex (vmPFC) and the dlPFC. The vmPFC is critical for the representation of reward and value-based decision making (Hiser and Koenigs, 2017), while the dlPFC acts in the regulation of the vmPFC during cognitive regulation (Hare et al., 2009; Hutcherson et al., 2012; Kober et al., 2010). The dlPFC is responsible for preserving choice goals while the vmPFC represents the decisions' value relative to the goals (Ochsner et al., 2012). Furthermore, there is evidence that the vmPFC is involved in the generation of negative emotion, such as anxiety and fear (Hiser and Koenigs, 2017). Regarding threat processing, the vmPFC seems to play a very important role as an integrative center essential to behavioral adaptation in both positive and negative contexts. Therefore, the vmPFC might be acting as a connector between anxiety, reward processing and threat systems (Hu, 2018), and, indeed, some studies suggest that individuals with higher levels of trait anxiety or generalized anxiety disorder (GAD) seem to have alterations in this brain area (Hu, 2018; Greenberg et al., 2013).

On this matter, GAD is one of the most common psychiatric disorders, with up to 20% of adults affected each year. It is characterized by a persistent overexpressed worry about everyday life ordinary events, becoming a permanent state of worry (Munir and Takov, 2021). GAD patients tend to overestimate the advantages (Ladouceur et al., 1997) and underestimate the disadvantages of worrying (Brown et al., 1993). They use worry as an ineffective cognitive attempt to problem solving. Anxiety levels seem to interfere with the responses associated with food consumption (Santa Cecília Silva et al., 2017). Regarding the relationship between anxiety and food perception, studies have shown that anxiety can both increase (Hakkarainen et al., 2004; Suzuki et al., 2016; Yannakoulia et al., 2008) or decrease (Deboer and Smits, 2013; Herman et al., 1987) food intake, appetite, and the enjoyment of food. Therefore, it is coherent to examine decision making and cognitive reward regulation in populations with anxiety through a food valuation assessment, as done in previous work with an obsessive-compulsive disorder population (Ferreira et al., 2021). Furthermore, the study of the neural substrates of approach and avoidance processes is relevant for

understanding dysfunctions associated with anxiety disorders. Accordingly, value-based decision making tasks, like food valuation, have been previously used to investigate these substrates (Aupperle and Paulus, 2010).Besides the vmPFC, some other relevant brain structures for anxiety, threat and fear are the amygdala, other limbic areas, and the thalamus (Goossen et al., 2019). In particular, the thalamus has a role in behavioral control and emotional processing. Its role in anxiety-like behavior as a regulator structure seems to be highly notorious and it has been shown that activations in some thalamic regions generate anxiety and aversive states (Barson et al., 2020; Kirouac, 2015). Hence, both vmPFC and the thalamus seem to play a crucial role in the regulation of emotional processing in anxious individuals.

On top of that, a recent publication has shown evidence regarding functional connectivity between cortical and subcortical regions in cognitive emotional regulation. The proposed cortical regions implicated are the dlPFC, vmPFC, ventrolateral prefrontal cortex (vlPFC) and the presupplementary motor area (preSMA). Their findings support the vmPFC as the primary conduit through which these regions directly modulate amygdala activity (Steward et al., 2021).

Besides theoretical knowledge, the neurobiological basis of GAD has not been substantially studied yet and there is still a need to go further to bridge the gap between fundamental research and clinical practice (Goossen et al., 2019; Mochcovitch et al., 2014). The aim of this study is to explore cognitive reward control differences between GAD patients and healthy controls (HC) in a food reward-processing task in terms of behavior and brain function and functional connectivity. We used an adaptation of Hutcherson's et al., 2012 task described in Ferreira's et al., 2019 study, which involves cognitive regulation of craving before valuating food pictures. We examined whole-brain activation as well as functional connectivity with the vmPFC and dlPFC, as both areas are relevant for cognitive reward control and model-based decision making processes. We expected to find differences in the behavior between groups, observing them both in self-reported measurements and task activations. In addition, in the GAD group we anticipated to notice less functional connectivity between cortical regions ─ related to reward processing, decision making and cognitive regulation (vmPFC and dlPFC) ─ and limbic regions, because of emotional arousal and cognitive regulation impairments related to this population of patients.

2. Methods

2.1 Participants

The study included Portuguese, Caucasian, mostly right-handed (1 left-handed GAD patient) participants. They were eligible if they were at least 18 years old and reported no history of traumatic brain lesion or substance abuse and MRI contraindications.

The GAD group included 11 participants (6 females) with median (interquartile range) age of 29.0 (17.0) years (21 to 44 years) and 12.0 (5.0) years of education (5 to 17 years). GAD participants were recruited at *Hospital de Braga* and diagnosed by experienced psychiatrists, using a semi-structured interview based on the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). This clinical assessment allowed to exclude the presence of other psychiatric diagnoses, particularly depression, eating disorders and other anxiety disorders. All patients were medicated with a selective serotonin reuptake inhibitor (SSRI) antidepressant and had no comorbidities. No other medications were allowed.

The healthy control (HC) group included 15 participants (8 females) with no history of psychiatric or neurological conditions, not taking any psychiatric medication, with age of 26.0 (21.5) years (21 to 58 years) and 17.0 (2.0) years of education (11 to 20 years).

The groups were matched for sex (chi-squared test $\chi^2(1) = 4.0\times10^{-3}$, $p = 0.951$) and age (Mann-Whitney test $U = 84.0$, $p = 0.958$), but not for education ($U = 129.0$, $p = 0.015$, rank-biserial correlation 0.6), with GAD patients having lower education than controls. Thus, education was used as a covariate in further statistical analyses comparing the groups.

2.2 Sociodemographic and psychological scales

Information on sex, age, educational level and handedness was collected. Weight and height were also measured to prevent the inclusion of participants with an out of normal range body mass index.

Participants also filled the 10 items Perceived Stress Scale (PSS) (Cohen et al., 1983; Morgado et al., 2013), the Beck Anxiety Inventory (BAI) (Beck et al., 1988), and the Beck Depression Inventory (BDI) (Beck et al., 1996). The PSS measures last month's perception of unpredictable, uncontrollable, and overloaded life. The higher the score, the greater the intensity of perceived stress. The BAI measures last week severity of an individual’s anxiety. Scores lower than 7 indicate minimal anxiety. Scores higher than 7, 15, and 25 indicate mild, moderate, and severe anxiety, respectively. The BDI measures the severity of depression and can be used as a screening tool. Scores lower than 14 indicate minimal depression. Higher scores indicate more severe depressive symptoms. The psychometric differences between-groups were analyzed with ANCOVA using education as a covariate.

157 *2.3 fMRI task*

158 The task was adapted from Hutcherson et al., 2012 and a full description can be found in previous
159 work (Ferreira et al., 2019). It consisted in the valuation of 150 food pictures in two phases: pre-
160 scan valuation task and in-scan regulation task.

161 The pre-scan valuation task provided a measure of the subjective baseline for food value (from 1,
162 "Don't want it at all" to 4, "Want it a lot"). The in-scan regulation task measured food value (0, 1, 2
163 or 3€) after the cognitive regulation of craving (4s) for the same pictures randomly separated in
164 three blocks: indulge, distance and natural; where participants tried to increment their craving,
165 decrease it, or just allow spontaneous thoughts, respectively (**Figure 1**). To increase craving and
166 ensure truthful valuation during the task, participants were instructed to fast for at least 4h before
167 the experiment and were informed that they would be rewarded with the food they obtained using
168 an adapted version of Becker-DeGroot-Marschak auction (Becker et al., 1964; Plassmann et al.,
169 2007).

170 *2.4 Behavioral task fMRI data analysis*

171 Differences in the reaction time during the task while participants bid for food were studied. A
172 mixed-design ANCOVA was used with condition (distance, natural, and indulge) as within-subject
173 factor, group as between-subject factor, and education as covariate.

174 Additionally, the variation of the food valuation scores after cognitive regulation was analyzed. A
175 mixed-design ANCOVA was used with two within-subject factors (time [before and after cognitive
176 regulation] and condition [distance, natural, and indulge]), group as between-subject factor, and
177 education as covariate. The pre-regulation scores corresponded to the ratings of the food pictures
178 before entering the scanner. The post-regulation scores were the participants' bids after cognitive
179 regulation. Before the statistical analysis, the valuation scores were normalized by dividing them by
180 the maximum value (pre-regulation scores 4, and post-regulation scores 3€).

181 Post-hoc repeated measures ANOVAs and paired *t*-tests were performed to explore statistically
182 significant effects for interaction and within-subject effects, respectively, using Bonferroni
183 correction for multiple comparisons (p_{bonf}).

184 *2.5 MRI data acquisition*

185 Scans were acquired on a clinical approved 1.5 T Siemens Magnetom Avanto system (Siemens
186 Medical Solutions, Germany) using a 12-channel receive-only head array coil. For the functional

acquisition, we used a T2* weighted echo-planar imaging acquisition: 38 interleaved axial slices, repetition time 2750 ms, echo time 30 ms, field of view 224 mm × 224 mm, flip angle 90º, in-plane resolution 3.5 mm × 3.5 mm, slice thickness 3.5 mm, and between-slice gap 0.5 mm. To optimize the sensitivity in the orbitofrontal cortex, a tilted acquisition in an oblique orientation of 30° relative to the anterior-posterior commissure line was used. A total of 650 volumes were acquired during the task. The task stimulus was presented using the fully integrated fMRI system IFIS-SA (Invivo Corporation, United States) and the same system was used to record participants' key-press responses. One high-resolution T1-weighted Magnetization-Prepared Rapid Acquisition with Gradient Echo sequence, with 1 mm × 1 mm × 1 mm voxel size, repetition time 2.73 s, echo time 3.48 ms, flip angle 7º, field of view 234 mm × 234 mm, and 176 slices was acquired. This anatomical sequence was used to project the functional maps.

2.6 fMRI data analysis

The functional scans were preprocessed with the Statistical Parametric Mapping (SPM) version 12 (Wellcome Department of Imaging Neuroscience, Institute of Neurology, United Kingdom) using MATLAB version R2018a (The MathWorks Inc., United States). Preprocessing included: slice-timing correction using the first slice as reference; realignment to the mean volume of the acquisition; nonlinear spatial normalization to Montreal Neurological Institute (MNI) standard space and resampling to 2 mm × 2 mm × 2 mm voxel size; spatial smoothing with an 8 mm full-width at half-maximum Gaussian kernel; high pass temporal filtering at 128 s.

For the first-level analysis, one general linear model (GLM) was computed per participant. For this GLM, the regressors of interest included: the type of cognitive regulation trial (1 – distance, 2 – natural, and 3 – indulge) and the corresponding bid (4 – bids after distance trials, 5 – bids after natural trials, and 6 – bids after indulge trials). The bid regressors were parametrically modulated by the bid value (0, 1, 2, and 3€), the pre-rating score before the task (1 to 4), and the reaction time. Additional regressors included: 7 – the cue; 8 – the interstimulus interval; 9 – the omission bids; 10 – 16 the motion parameters estimated during the realignment step. The onset and duration of the regressors were defined according to the stimulus represented in **Figure 1** with a boxcar function and the regressors were convolved with the canonical hemodynamic response function.

At the group level (second-level analysis), a random-effects analysis was performed using a mixed-design ANCOVA model for cognitive regulation during the task (enabled comparisons in average activation for each regulation trial between and within groups). The group (GAD vs. control) was introduced as the between-subject factor and each trial during cognitive regulation (distance vs. natural vs. indulge) as the within-subject factor. Education was used as a covariate. The model was

implemented with the GLMFlex toolbox which uses partitioned error terms for within-group and between-group comparisons, enabling the estimation of all the effects of interest with a single model. Results were considered statistically significant after correcting for multiple comparisons using cluster correction (minimum cluster size of 88 voxels). The minimum cluster size was determined with 3DClustSim (AFNI version 17.0.13; National Institute of Mental Health). This program determines a minimum cluster size with Monte Carlo Simulation to achieve a corrected significance of $p < 0.05$ with an initial voxel-wise threshold of $p < 0.001$. The Automated Anatomical Labeling plugin for SPM was used to classify the brain regions.

2.7 Functional connectivity (FC) analysis

The FC of the dlPFC and vmPFC during the task was also studied by performing generalized psychophysiological (gPPI) analyses (McLaren et al., 2012). Four seed regions with 10 mm radius based on the results from Hutcherson et al., 2012 were defined: right (MNI 6, 39, 0) and left (MNI -6, 39, 0) dlPFC and right (MNI 48, 36, 24) and left (MNI -48, 36, 24) vmPFC. The gPPI beta maps were estimated for the task conditions (distance, natural, and indulge) during cognitive regulation. The GLMFlex toolbox was used to calculate differences between groups in FC using the ANCOVA described above (minimum cluster size of 88 voxels to correct for multiple comparisons) (Do and Telzer, 2019; Humbert and McLaren, 2014; Olivé et al., 2015).

2.8 Statistical analysis

The statistical analysis of psychometric, demographic, and behavioral data was performed with JASP (version 0.11.1.0; JASP Team [2018], The Netherlands). Differences were considered statistically significant if $p < 0.05$.

3. Results

3.1 Psychological and behavioral analysis

The GAD group presented higher values in all psychometric scales, namely, PSS, BAI, and BDI (**Table 1**).

We did not find statistically significant differences between and within groups nor interaction effects for the reaction time during food valuation (**Table 2**).

Concerning the valuation score, we found a significant group × time × condition interaction ($F_{(1.3,}$

$_{30.0)}$ = 6.4, p = 0.011, η^2 = 1.1×10^{-2}, Greenhouse-Geisser correction for non-sphericity). Post-hoc repeated measures ANOVA (time and condition as within-subjects factors) demonstrated no statistically significant differences in the interaction time × condition for the GAD group ($F_{(2, 20)}$ = 1.5, p_{bonf} = 0.508). For the control group this interaction effect was statistically significant ($F_{(1.2, 16.5)}$ = 18.7, p_{bonf} = 5.932×10^{-4}, η^2 = 0.1; Greenhouse-Geisser correction for non-sphericity). Further post-hoc repeated measures ANOVA (condition as within-subject factor) yielded statistically significant results after cognitive regulation ($F_{(1.3, 18.1)}$ = 19.1, p_{bonf} = 3.104×10^{-4}, η^2 = 0.3; Greenhouse-Geisser correction for non-sphericity) but not before cognitive regulation ($F_{(2, 28)}$ = 1.8, p_{bonf} = 0.378) in the control group. Post-hoc paired *t*-test revealed differences among all conditions after cognitive regulation in the control group (distance vs. indulge t = -5.0, p_{bonf} = 5.895×10^{-4}, d = -1.3; distance vs. natural t = -3.2, p_{bonf} = 0.019, d = -0.8; indulge vs. natural t = 5.0, p_{bonf} = 6.000×10^{-4}, d = 1.3). Thus, the control participants modulated their bids for food according to the regulation condition while the GAD group presented fixed food valuation scores after cognitive regulation (**Table 2** and **Figure 2**).

3.2 Neuroimaging results

We did not find statistically significant differences in activation within and between subjects in whole-brain responses for cognitive regulation during the task. However, we observed statistically significant differences in functional connectivity with the seed regions. There was a statistically significant group × condition interaction for functional connectivity between the right dlPFC with the left thalamus, and between the left and the right vmPFC with the right thalamus (**Table 3** and **Figure 3**). Post-hoc tests with repeated measure ANOVA (condition as within-subject factor) and paired *t*-tests between conditions for each group demonstrated that GAD patients had lower functional connectivity for regulated conditions (distance and indulge) than for the natural condition in these clusters, while the control participants presented the opposite pattern (**Table 4**).

4. Discussion

To explore the neurobiological correlates of cognitive reward control of model-based decision making between GAD patients and healthy controls, we performed a food valuation task focused on cognitive regulation.

As expected according to GAD symptomatic manifestations, we found that the GAD group presented more anxiety, stress, and depressive symptoms than the HC group. Patients with GAD

usually have comorbidities with other mental disorders, and its connectedness with depression seems to be notable (Price et al., 2019). Nonetheless, our study sample did not have comorbidities and these symptoms might be explained by the GAD diagnosis itself.

The results obtained during the food valuation task showed a tendency that the GAD group might have not performed an effective cognitive regulation, based on the lack of differences in valuation scores before and after applying the regulation. This may suggest inflexibility and that these individuals cannot recruit the brain areas required for the process of regulation, which is in line with previous fMRI studies (Görgen et al., 2014). Furthermore, some systematic reviews have suggested that emotional dysregulation as a cognitive dysfunction in GAD patients is related to prefrontal and anterior cingulate cortices hypofunction, as well as deficient cortex-amygdala functional connectivity (Goossen et al., 2019; Mochcovitch et al., 2014). Therefore, it would be interesting to further explore if cognitive reward control and emotional regulation are indeed interconnected processes. In this case, the cortex-amygdala-thalamus system might be impaired in GAD patients, who are also characterized by autonomic dysregulation as a main clinical manifestation (Makovac et al., 2016).

The subjective value given to food would be modified by personal perception or emotional value given to it. Coupled with the fact that anxiety levels seem to interfere with food associated responses (Santa Cecília Silva et al., 2017), GAD patients are characterized by having emotional dysregulations which make harder for them to solve some problematic situations (Behar et al., 2009). The above may be affecting the valuation part of the decision making process, making them unable to achieve differences even when they try to regulate their cognition. Consequently, value-based decision making tasks have been used to investigate the neural dysfunctions found in people with anxiety, as in the present study. In particular, prefrontal cortex dysfunction has been repeatedly implicated across anxiety disorders (Aupperle and Paulus, 2010).

The flexibility of dlPFC attribute representations may be especially important for compensating when regulation of the vmPFC fails, a finding also observed in other studies of cognitive regulation. Previous exploratory connectivity results suggested that this may derive, at least in part, from functional interactions with the vmPFC area that represented all choice-relevant attributes, with the strength of connectivity between dlPFC and vmPFC correlating with regulatory success (Tusche and Hutcherson, 2018). Moreover, it has been described that people with anxiety and related disorders show cognitive inflexibility and related impairments, which is directly related with the prefrontal cortex (Park and Moghaddam, 2017).

We sought for differences in FC using the dlPFC as seed. The dlPFC is key in cognitive regulation,

modulating vmPFC activity which is important for reward valuation, linked to emotional value encoding and valuation process (Hare et al., 2009; Hutcherson et al., 2012; Kober et al., 2010). Our results demonstrated a contrasting pattern of FC between groups during regulation and no-regulation conditions. While GAD patients had more FC between the dlPFC/vmPFC and thalamus during the natural condition, the HC group had lower FC, and, when the regulation (up- or down-regulation) started, the FC increased in the HC group and decreased in GAD group. The fact that GAD patients had stronger dlPFC/vmPFC-thalamic connections during the natural condition may be linked to the concept of GAD patients maintaining a permanent state of worry as a strategy for constant emotional regulation (Saviola et al., 2020). It is worth mentioning that another explanation for these changes in FC might involve the thalamus directly. Studies have shown that hyperactivation of the thalamus might be associated with an increase in emotional distress and dysregulation (Geng et al., 2018; Mizuno-Matsumoto et al., 2013). Our results suggest that thalamus is recruiting dlPFC and vmPFC for regulation, as it is known to happen in other studies (Hutcherson et al., 2012). As recently described, it seems that a network involving different prefrontal regions, including the dlPFC and the vmPFC, is modulating subcortical structures, as the amygdala (Steward et al., 2021). Hence, the role of the thalamus in these complex networks should be furtherly studied to fully understand cortical/subcortical interactions. In this sense, the decreased connectivity that our GAD patients are showing could be implying a potential mechanism of their cognitive dysregulation.

Importantly, even though our sample did not have comorbidities, we cannot ensure that these differences are exclusively due to GAD trait because of the existent symptomatic manifestations of stress and depression. It would be interesting to compare these results with other types of anxiety disorders and stress, depression, or obsessive-compulsive disorder (OCD) groups, looking for differences or similarities. Interestingly, in one of our previous works with the same fMRI task in OCD participants, we observed similar valuation score results after regulation (Ferreira et al., 2021).

The main limitation of this study is the reduced number of participants, and that sample size assessment was not performed. We are aware that similar paradigms that study cognitive regulation and reward processes with a greater number of participants should be performed to have more robust results. In this line, during cognitive regulation it is expected, as documented before, to detect differences like decreasing dlPFC and increasing vmPFC activations (Hutcherson et al., 2012). We did not find such differences in our study, and this could be due to a lack of statistical power because of our small sample size. Nevertheless, we believe that our findings regarding connectivity among key areas of the prefrontal cortex and thalamus are a relevant addition to the

existing literature, due to the reasons discussed earlier. Moreover, the selected task is specific to cognitive regulation of food craving. Therefore, our outcomes cannot be generalized to other forms of cognitive regulation or to other disorders. Another important limitation was the 1.5T MRI scan used, as we are aware that stronger magnetic fields are currently recommended for this type of behavioral studies. Lastly, our sample had a significant difference among groups regarding level of education. We used this variable as a covariate in analyses, but we are aware it might have not been enough.

Our results showed that GAD patients might experience difficulties in the cortical regulation of subcortical structures, specifically the thalamus, which might be causing them additional emotional distress and dysregulation; which could explain their behavioral inflexibility observed during the food valuation task. As cognitive reward control and emotional regulation systems seem to be highly interrelated, further studies trying to identify their differences and commonalities in specific clinical populations are needed to better understand the decision making process.

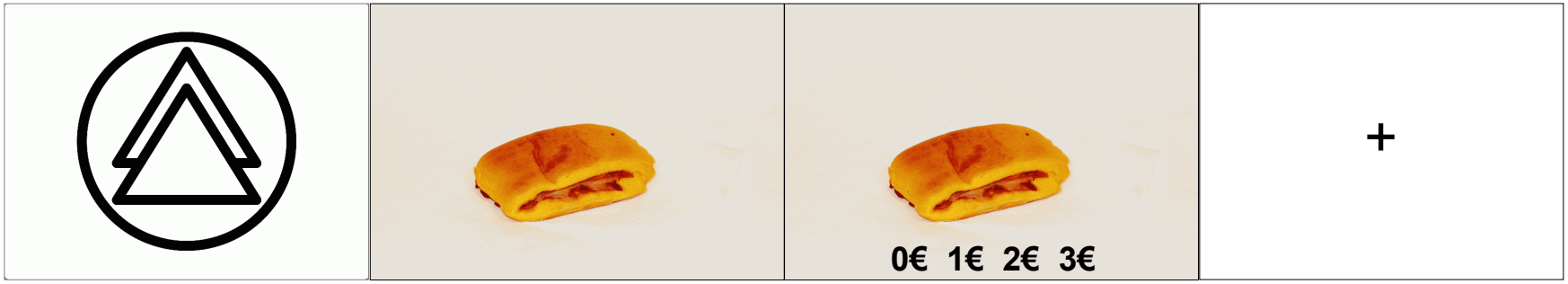

Figure 1 Representation of the functional magnetic resonance task. The instruction was presented with a cue. After, a food item was displayed, and participants had to cognitively regulate their craving accordingly to the cue (distance - downregulation; natural - no regulation; indulge - upregulation). Participants were asked to give a monetary value to the food item in accordance to their craving (from 0 to 3 €) after cognitive regulation.

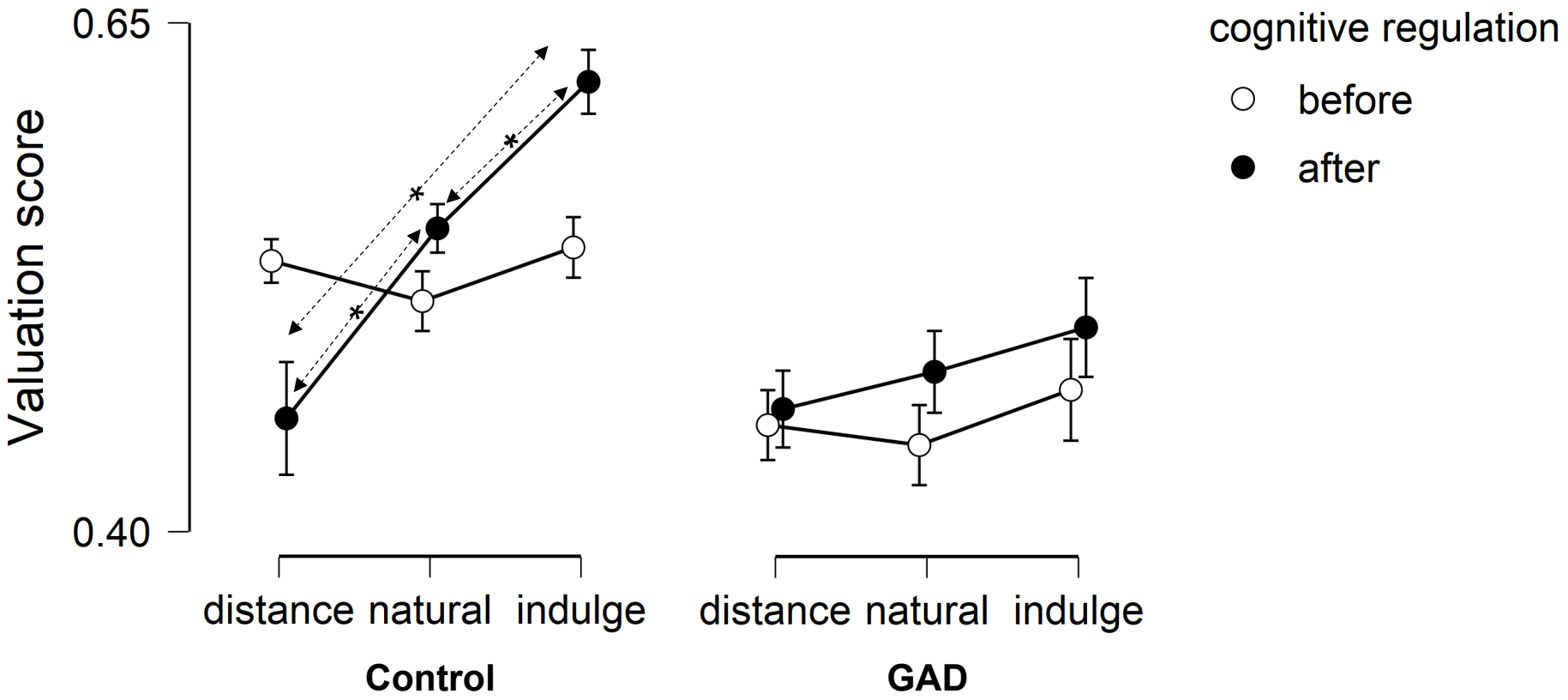

Figure 2 Representation of the valuation score before (pre-task) and after (during the task) cognitive regulation. The scores were normalized by dividing by the maximum value. The control group modulated the valuation scores accordingly to the regulation condition (*statistically significant differences) while the generalized anxiety disorder (GAD) group presented fixed valuation scores after cognitive regulation. The graphs represent the mean and standard error values.

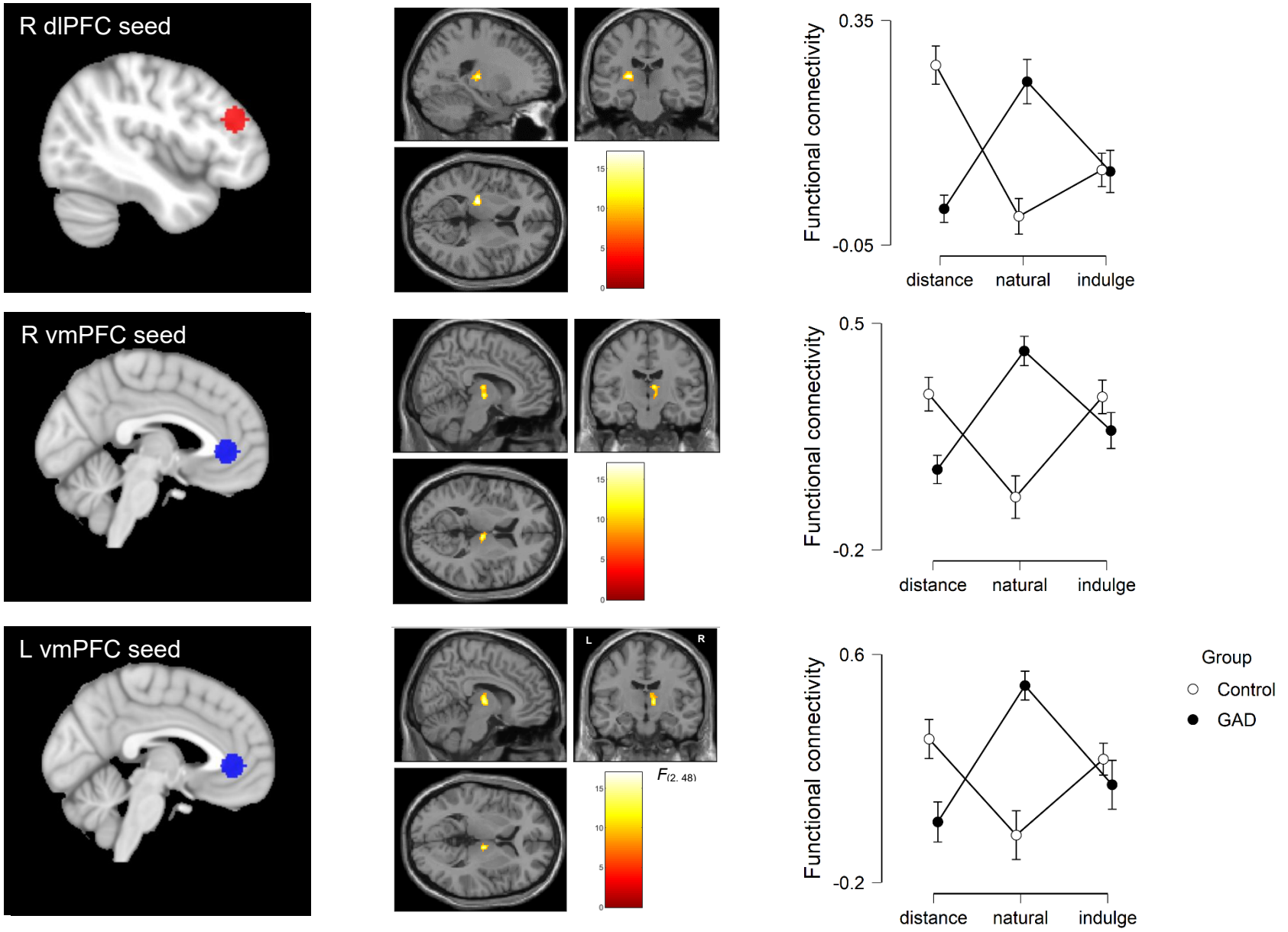

Figure 3 Representation of statistically significant clusters of functional connectivity resulting from cognitive regulation during the task. We observed an interaction between group and condition (distance, natural and indulge) in all clusters. Post-hoc tests demonstrated that the functional connectivity during the regulated conditions was lower than the non-regulated condition in the generalized anxiety disorder group (GAD) while the opposite was observed for the control group (more information on Table 3 and 4). The graphs represent the mean and standard error values. L - left; R - right; dlPFC - dorsolateral prefrontal cortex; vmPFC - ventromedial prefrontal cortex.

Table 1 Results for statistical tests on demographic information and psychometric scales.

	GAD	Control	Statistical effect	Test value	*P* value	*E*ffect size
Age	29.0 (17.0)	26.0 (21.5)	Group	$U = 84.0$	$p = 0.958$	
Sex	6 female | 5 male	8 female | 7 male	Group	$\chi^2(1) = 4.0\times10^{-3}$	$p = 0.951$	
Education	12.0 (5.0)	17.0 (2.0)	Group	$U = 129.0$	$p = 0.015$	$rc = 0.6$*
Anxiety (BAI)	26.8 ± 17.6	6.0 ± 3.2	Group	$F_{(1, 23)} = 10.0$	$p = 0.004$	$\eta^2 = 0.3$*
			Education	$F_{(1, 23)} = 5.6$	$p = 0.027$	$\eta^2 = 0.1$*
Depression (BDI)	16.4 ± 12.8	3.3 ± 2.5	Group	$F_{(1, 23)} = 7.8$	$p = 0.010$	$\eta^2 = 0.2$*
			Education	$F_{(1, 23)} = 2.1$	$p = 0.159$	
Stress (PSS)	22.8 ± 7.9	10.6 ± 5.8	Group	$F_{(1, 23)} = 10.7$	$p = 0.003$	$\eta^2 = 0.3$*
			Education	$F_{(1, 23)} = 4.0$	$p = 0.056$	

Values represent median (interquartile range) and mean ± standard deviation. *Statistical significance; GAD – general anxiety disorder; BAI - Beck Anxiety Inventory; BDI - Beck Depression Inventory; PSS - 10 items Perceived Stress Scale: *rc* - rank-biserial correlation.

Table 2 Results for statistical tests of behavioral variables associated with the functional magnetic resonance imaging task. Parameters correspond to the valuation before (pre) and after (post) cognitive regulation.

	GAD pre			GAD post			Control pre			Control post			Statistical effect	Test value	*P* value	Effect size
	Distance	Natural	Indulge	Distance	Natural	Indulge	Distance	Natural	Indulge	Distance	Natural	Indulge				
Reaction time (ms)		-		768.5 ± 189.5	703.3 ± 149.2	774.0 ± 215.4		-		758.8 ± 160.9	699.2 ± 155.3	733.5 ± 170.1	Condition (distance, natural, and indulge)	$F_{(2, 46)} = 2.3$	$p = 0.116$	
													Group × Condition	$F_{(2, 46)} = 0.3$	$p = 0.720$	
													Group	$F_{(1, 23)} = 0.6$	$p = 0.461$	
													Education	$F_{(1, 23)} = 1.1$	$p = 0.303$	
Valuation score (normalized)	0.45 ± 0.12	0.44 ± 0.13	0.47 ± 0.12	0.46 ± 0.10	0.48 ± 0.10	0.50 ± 0.07	0.53 ± 0.13	0.51 ± 0.14	0.54 ± 0.14	0.46 ± 0.08	0.55 ± 0.12	0.62 ± 0.12	Time (before and after cognitive regulation)	$F_{(1, 23)} = 0.1$	$p = 0.801$	
													Group × time	$F_{(1, 23)} = 0.3$	$p = 0.617$	
													Condition	$F_{(2, 46)} = 2.0$	$p = 0.146$	
													Group × condition	$F_{(2, 46)} = 3.0$	$p = 0.061$	
													Time × condition	$F_{(1.3, 30.0)} = 1.1$	$p = 0.324^{a}$	
													Group × time × condition	$F_{(1.3, 30.0)} = 6.4$	$p = 0.011$	$\eta^2 = 1.1\times10^{-2*a}$
													Group	$F_{(1, 23)} = 2.4$	$p = 0.135$	
													Education	$F_{(1, 23)} = 2.4\times10^{-2}$	$p = 0.879$	

Values represent mean ± standard deviation. *Statistical significance; [a]Greenhouse-Geisser correction for non-sphericity; GAD – general anxiety disorder.

Table 3 Regions with different functional connectivity during cognitive regulation between the control and generalized anxiety disorder groups using the ventromedial and dorsolateral prefrontal cortical seeds ($p < 0.001$, minimum cluster size of 88 voxels).

Brain regions	Cluster size (voxels)	Peak voxel intensity	MNI peak voxel coordinates (mm)
R dlPFC seed			
L thalamus (pulvinar, ventral posterior lateral nucleus, lateral posterior nucleus); L lentiform nucleus (putamen).	108	17.1	-22 -22 8
R vmPFC seed			
R thalamus (medial dorsal nucleus, ventral lateral nucleus); brainstem, subthalamic nucleus; mammillary body.	96	14.3	10 -14 0
L vmPFC seed			
R thalamus (medial dorsal nucleus, ventral lateral nucleus, and ventral anterior nucleus).	106	14.6	10 -14 4

MNI - Montreal Neurologic Institute; L - left; R - right; dlPFC - dorsolateral prefrontal cortex; vmPFC - ventromedial prefrontal cortex.

Table 4 Results for post-hoc tests on the statistically significant clusters found for cognitive regulation during the task for the interaction Group × condition (*p*-values with Bonferroni correction).

Statistical effect	Test value	*P* value	*E*ffect size
R dlPFC seed			
GAD - Condition main effect	$\mathbf{F_{(2, 20)} = 11.4}$	$\mathbf{p = 9.876\times10^{-4}}$	$\mathbf{\eta^2 = 0.3^*}$
Distance vs. Indulge	t = -1.6	p = 0.392	
Distance vs. Natural	t = -5.2	p = 0.001	d = -1.6*
Indulge vs. Natural	t = -2.7	p = 0.070	
Control - Condition main effect	$\mathbf{F_{(2, 28)} = 18.7}$	$\mathbf{p = 1.369\times10^{-5}}$	$\mathbf{\eta^2 = 0.4^*}$
Distance vs. Indulge	t = 4.1	p = 0.003	d = 1.1*
Distance vs. Natural	t = 5.6	$p = 1.905\times10^{-4}$	d = 1.4*
Indulge vs. Natural	t = 2.0	p = 0.205	
R vmPFC seed			
GAD - Condition main effect	$\mathbf{F_{(2, 20)} = 14.9}$	$\mathbf{p = 2.182\times10^{-4}}$	$\mathbf{\eta^2 = 0.3^*}$
Distance vs. Indulge	t = -1.7	p = 0.384	
Distance vs. Natural	t = -6.4	$p = 2.192\times10^{-4}$	d = -1.9*
Indulge vs. Natural	t = -3.3	p = 0.024	d = -1.0*
Control - Condition main effect	$\mathbf{F_{(2, 28)} = 10.1}$	$\mathbf{p = 9.688\times10^{-4}}$	$\mathbf{\eta^2 = 0.1^*}$
Distance vs. Indulge	t = 0.1	p = 1.000	
Distance vs. Natural	t = 3.6	p = 0.008	d = 0.9*
Indulge vs. Natural	t = 3.6	p = 0.009	d = 0.9*
L vmPFC seed			
GAD - Condition main effect	$\mathbf{F_{(2, 20)} = 12.4}$	$\mathbf{p = 6.248\times10^{-4}}$	$\mathbf{\eta^2 = 0.4^*}$
Distance vs. Indulge	t = -1.1	p = 0.918	
Distance vs. Natural	t = -6.7	$p = 1.573\times10^{-4}$	d = -2.0*
Indulge vs. Natural	t = -3.5	p = 0.017	d = -1.1*
Control - Condition main effect	$\mathbf{F_{(2, 28)} = 6.3}$	$\mathbf{p = 0.012}$	$\mathbf{\eta^2 = 0.1^*}$
Distance vs. Indulge	t = 0.9	p = 1.000	
Distance vs. Natural	t = 2.9	p = 0.038	d = 0.7*
Indulge vs. Natural	t = 2.6	p = 0.066	

*Statistical significance. L - left; R - right; dlPFC - dorsolateral prefrontal cortex; vmPFC - ventromedial prefrontal cortex; GAD - generalized anxiety disorder.

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Data availability

The data that support the findings of this study and the brain maps of all analyses are available from the corresponding author upon reasonable request.

Conflict of interest.

P Morgado has received in the past 3 years grants, CME-related honoraria, or consulting fees from Angelini, AstraZeneca, Bial Foundation, Biogen, DGS-Portugal, FCT, FLAD, Janssen-Cilag, Gulbenkian Foundation, Lundbeck, Springer Healthcare, Tecnimede and 2CA-Braga.

Ethics statement

This study was carried out in accordance with the Declaration of Helsinki. All participants gave written informed consent. The protocol was approved by the Ethics Subcommittee for the Life and Health Sciences of University of Minho, Portugal, and by the Ethics Committee of the *Hospital de Braga*, Portugal.

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