Subliminal food images compromise superior working memory performance in women with restricting anorexia nervosa

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A R T I C L E   I N F O

A B S T R A C T

Prefrontal cortex (PFC) is dysregulated in women with restricting anorexia nervosa (RAN). It is not known whether appetitive non-conscious stimuli bias cognitive responses in those with RAN. Thirteen women with RAN and 20 healthy controls (HC) completed a dorsolateral PFC (DLPFC) working memory task and an anterior cingulate cortex (ACC) conflict task, while masked subliminal food, aversive and neutral images were presented. During the DLPFC task, accuracy was higher in the RAN compared to the HC group, but superior performance was compromised when subliminal food stimuli were presented: errors positively correlated with self-reported trait anxiety in the RAN group. These effects were not observed in the ACC task. Appetitive activation is intact and anxiogenic in women with RAN, and non-consciously interacts with working memory processes associated with the DLPFC. This interaction mechanism may underlie cognitive inhibition of appetitive processes that are anxiety inducing, in people with AN.

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1. Introduction

Individuals with anorexia nervosa (AN) are severely emaciated (less than 85% normal body weight), are excessively fearful of weight gain and avoid food intake (American Psychiatric Association, 2002; World_Health_Organisation, 2007). The DSM-IV diagnoses two subtypes: restricting AN (RAN) is defined by deliberate food restriction whereas binge-purging AN (BPAN) also has sporadic episodes of food consumption (followed by purging behavior). Dysfunctional eating behavior is strongly associated with cognitive traits that may underpin the control of appetite. For example, people with AN are ascetic and extremely self-disciplined (Fassino et al., 2006), strive for perfection and are obsessional (Davies, Liao, Campbell, & Tchanturia, 2009), attend excessively to detail (Lopez, Tchanturia, Stahl, & Treasure, 2009), may have a genetic predisposition to be cognitively inflexible (Holliday, Tchanturia, Landau, Collier, & Treasure, 2005), are preoccupied with food and have anxiogenic concerns about shape, weight and eating (Fairburn & Harrison, 2003). Furthermore, obsessive–compulsive disorder (OCD) is commonly comorbid in people with AN, especially RAN (Steinglass & Walsh, 2006).

It is unclear, however, how these traits are linked to brain processes, although these cognitive traits are likely to be associated with PFC functions underlying attention, planning, self-reference and working memory. Subcortical activation interacts with executive functions (e.g. cognitive inhibition, working memory, and conflict monitoring) to regulate attention and

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modulate the saliency of a stimulus (Gazzaley et al., 2007). Dysregulation between the PFC, visual cortex and appetitive activation associated with the striatum may underlie dysfunctional cognitive traits in those with eating disorders and could modulate the reward value of a food stimulus. Additionally, excessive PFC function in people with RAN may compensate for reduced or aberrant activation in mesolimbic appetite regions or conversely, the cognitive inhibition of an otherwise intact but anxiogenic subcortical response (Kaye, Fudge, & Paulus, 2009).

Whilst the anterior cingulate cortex (ACC), a monitor for conflict and prediction error, may be associated with eating disorders particularly in response to body dissatisfaction (Friederich et al., 2010), it is potentially the DLPFC that is most strongly implicated in aberrant appetitive responses in those with RAN. For example, functional magnetic resonance imaging (fMRI) studies of women with eating disorders shows aberrant activation in the DLPFC (Brooks et al., 2011, 2012b; Brooks, Savov, Allzén, Fredriksson, & Schiöth, 2012a; Uher et al., 2003). Furthermore, activation of the DLPFC is associated with successful appetite suppression (Hollmann et al., 2011). Artificial stimulation of the DLPFC using repetitive Transcranial Magnetic Stimulation (rTMS) reduces food-induced craving (Van den Eynde et al., 2010), and improves working memory performance (Andrews, Hoy, Enticott, Daskalakis, & Fitzgerald, 2011; Zanto, Rubens, Thangavel, & Gazzaley, 2011) Thus, increased DLPFC activation in females with AN could reflect cognitive inhibition of appetitive responses, for example, associated with working memory ruminations about how to control one's eating.

Subliminal stimuli activate subcortical responses that are independent of PFC processes, and such stimuli can influence cognitive processes (Banse, Seise, & Zerbes, 2001; Fazio & Olson, 2003; Gray, 2001; Hartikainen, Ogawa, & Knight, 2000; Murphy & Zajonc, 1993). (LeDoux, 1996) suggests that a 'quick and dirty' neural pathway relays sensory information from the retina directly to the visual cortex and subcortical regions without engaging conscious processes associated with the PFC. Baars, in his Global Workspace Theory (GWT) suggests that unconscious processes, particularly derived from visual stimuli, interact with cognitive processes like working memory (Baars & Franklin, 2003) to set the context for a consciously-perceived self-relevant goal. This theory seems to fit well with the behavior of a person with RAN, where eating patterns and life events in general are rigidly controlled by self-imposed strategies that ultimately maintain a suppressing effect on appetitive processes, whereby the origin is largely unconsciously derived.

Against this background, subliminally presented anxiety-provoking stimuli (e.g. ego-threats and body image cues) affect action choices in people with eating disorders (Meyer & Waller, 1999; Waller & Barnes, 2002) although evidence for an unconsciously-derived cognitive bias towards food in AN patients is inconclusive. In one study using a Startle Eyeblink Modulation (SEM) task, bias towards appetitive stimuli is independent of conscious cognitive control (Friederich et al., 2006). However, apart from a study examining distractibility in AN, which found no interference effect (e.g. increased errors and/or response times) when subliminal stimuli were presented (Dickson et al., 2008), there are no other studies directly measuring unconsciously-derived cognitive bias to food stimuli in people with AN. One recent study used the subliminal presentation of fearful faces to show that automatic anxiety responses increase the negative evaluation of food images in women with AN (Soussignan, Jiang, Rigaud, Royet, & Schaal, 2010).

This study has used two cognitive tasks (N-back and Go/No-Go tasks) associated with the PFC with subliminal food, aversive and neutral images embedded in the tasks. The rationale was to examine whether interference is a general function, or specific to the ACC or DLPFC. The N-back task measures working memory (Kane, Conway, Miura, & Colflesh, 2007), and the delay-sensitive neuronal circuitry of the DLPFC is believed to subserve working memory performance (Petrides, 2005). Additionally, the N-back task includes different levels of difficulty, such that greater engagement of DLPFC processing can be achieved. A greater level of cognitive engagement may prevent bottom-up appetitive activation from being fully processed, or, conversely, provide a greater platform for non-consciously perceived mesolimbic appetitive responses to interfere with cognitions. The Go/No-Go task measures cognitive inhibition of pre-potent responses, and activates the behavioral inhibition and activation systems in the brain (Amodio, Master, Yee, & Taylor, 2008). The inferior frontal cortices, including the ACC are associated with inhibition of pre-potent responses, especially in response to normally pleasant stimuli (Albert, Lopez-Martin, & Carretie, 2010). Additionally, subliminal primes are robustly shown to activate responses in the striatum, followed by a PFC-related inhibition of this response tendency. Thus, it is plausible that one or both of these PFC regions may interact with subliminal stimulation in those with RAN, although it is predicted that DLPFC function, which is most associated with cognitive inhibition of appetite, represented by the N-back task, will be most disturbed in those with RAN.

The following hypotheses have been tested:

(1) Subliminal salient (food and aversive) images in comparison to subliminal neutral images will interfere with performance during both PFC cognitive tasks (by reducing accuracy and increasing response times to targets) in all participants.
(2) Subliminal food stimuli will cause more interference in women with RAN (than in HC women) relative to aversive and neutral stimuli, and the interference will correlate with measures of anxiety.
(3) Differential interference effects will be observed between the cognitive tasks in women with RAN: there will be more interference on the DLPFC task (i.e. N-back) during the presentation of subliminal food stimuli relative to the ACC task (i.e. Go/NoGo). Furthermore, greater engagement of the PFC (e.g. during the 2-back version of N-back) will show differential interference effects when interacting with subliminal stimuli (e.g. more or less errors during the presentation of food stimuli in the RAN group).

Table 1
Participant variables.

Variable	Anorexic ($n = 13$)	Controls ($n = 20$)
Age	25 (±11)	22 (±5)
Education (years)	14 (±1.3)	14.5 (±2.59)
IQ (NART)	112 (±7.5)	111 (±7.6)
BMI	15 (±1.54)[*]	22.38 (±2.66)[*]
HADS-anxiety	18.53 (±2.34)[*]	8.77 (±3.94)[*]
HADS-depression	14.26 (±2.23)[*]	5.54 (±3.45)[*]
EDI-interoceptive awareness	2.61 (±0.81)[*]	4.96 (±0.46)[*]

Abbreviations: NART = National Adult Reading Test, IQ = Intelligence Quotient, BMI = Body Mass Index, HADS = Hospital Anxiety and Depression Scale, EDI = Eating Disorder Inventory.

[*] Significant differences ($p < 0.01$) between the anorexic group, and the control group.

2. Material and methods

2.1. Participants

Female AN patients ($n = 13$) were recruited from the Bethlem Hospital of the South London and Maudsley (SLaM) NHS Trust. They fulfilled DSM-IV criteria for RAN; we chose to examine the restricting subtype as it is regarded as the most aetiologically homogeneous of the eating disorders (Herzog et al., 1996). Healthy control women (HC, $n = 20$) were recruited by public advertisement. Groups were matched for age, IQ and years of education (Table 1). Exclusion criteria were: axis I mental disorder (First, Williams, & Spitzer, 2001), neurological disease, history of head trauma with loss of consciousness and current use of psychotropic medication. All volunteers completed the tasks in the afternoon directly after their normal lunch (RAN patients ate a prescribed lunch in the hospital) so that the effects of subliminal food stimuli were not associated with hunger states. All volunteers were paid £10 for participating. The study was approved by the Ethics Committee at the Institute of Psychiatry, King's College, London (Ethics Code: 297/02). Participants were told that they might view some emotionally strong images but were not told that these would be inserted during performance of the cognitive tasks. They all gave written consent after the procedures had been explained.

After a screening interview (the SCID-R, see below), participants took part in the N-back task of working memory and the go/no- go task of cognitive inhibition. The order of task completion was alternated between participants. The RAN and HC groups were exposed to masked neutral and emotional (food and aversive) stimuli during the tasks, and these were introduced in a pseudo-random block pattern, so that the influence of each stimulus type could be assessed (see Fig. 1). Subsequent to task completion, a visibility test was used to confirm that the stimuli were subliminal (Eimer & Schlaghecken, 2002, 2003). Tasks were performed on a 15 in. LCD monitor driven by a 1.5 GHz Pentium 4 computer. Participants responded by pressing the computer mouse: the number of errors and the time taken to respond was recorded, as a measure of interference caused by the presentation of subliminal images (compared to the group not exposed to subliminal images during the tasks). After the tasks participants completed a post hoc questionnaire to assess subjective experience of performance on the tasks, the Eating Disorders Inventory (Garner & Olmstead, 1983) and the Hospital Anxiety and Depression Scale (Zigmond & Snaith, 1983).

2.2. Questionnaire measures

2.2.1. The structured clinical interview for diagnosis-researcher version (SCID-R) (First et al., 2001)

This structured interview is used to diagnose AN, for general screening, and to obtain demographic information. Duration of illness is the time between diagnosis of AN and the time of the scan. It is noted that AN symptoms are most likely present before the formal diagnosis, but this measure gives a systematic score of illness duration.

2.2.2. Eating disorders inventory (Garner & Olmstead, 1983)

A self-report questionnaire comprising of 91 items on a 6-point Likert-scale to examine 11 subscales relating to ED: drive for thinness, bulimia, body dissatisfaction, ineffectiveness, perfectionism, interpersonal distrust, interoceptive awareness, maturity fears, asceticism, impulse regulation and social insecurity. This questionnaire was used alongside the SCID to ensure that participants were either in the RAN or HC group.

2.2.3. The Hospital Anxiety and Depression Scale (HADS, Zigmond & Snaith, 1983)

This is a 14-item self-report measure with seven related to anxiety and seven related to depression. Individual questions are scored on a 4-point scale, with higher scores indicating greater anxiety or depression.

2.2.4. Post-Hoc questionnaire of subjective experience of performance (devised by the authors)

This is a questionnaire devised in the Eating Disorders Unit to question participants about how they perceived their performance, and whether they had seen any images during the tasks. There are three sections: (a) After N-back Task, (b) After
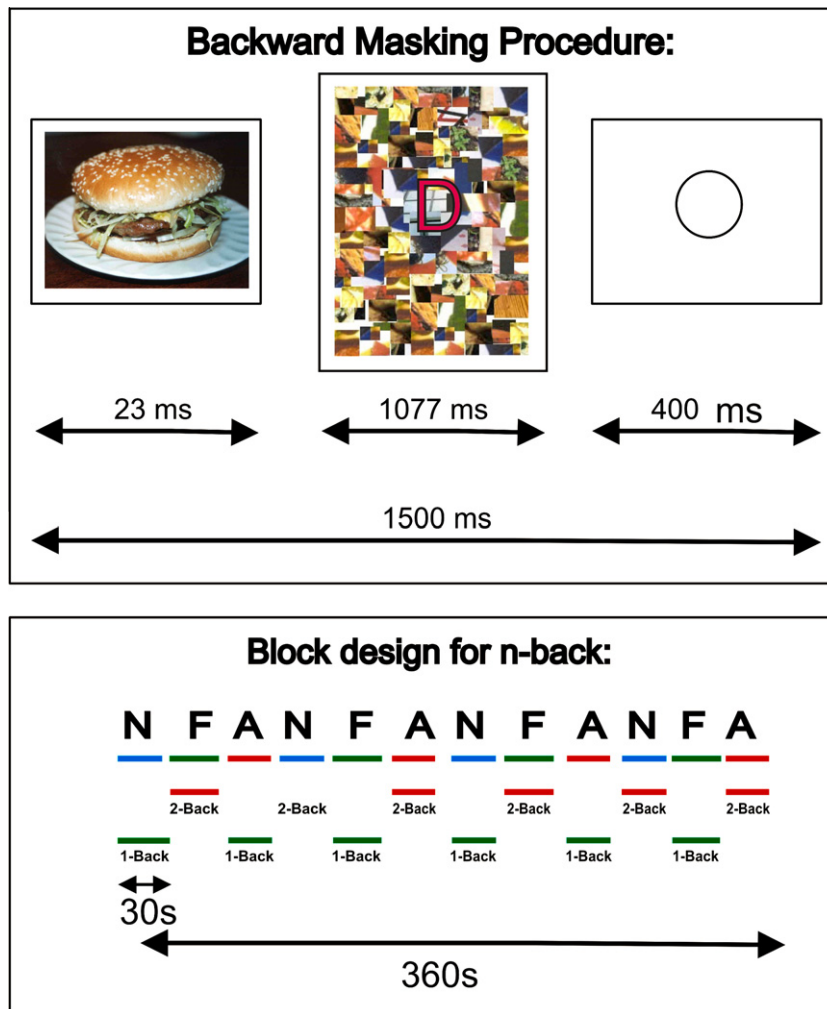
Fig. 1. Subliminal task design. *Top panel:* Backward masking procedure. An image of either food, aversive or neutral stimuli was presented for 23 milliseconds, followed by a masked image, rendering the first image subliminal. The mask consisted of a mosaic created by small non-recognisable portions of the subliminal images to maintain visual consistency. A target or non-target letter was presented in red font on the mosaic mask, participants responded if the current letter on the mask was the same as 'one previously' (1-Back) or 'two previously' (2-Back). Circle was used as a visual centring stimulus before the presentation of the next image. *Bottom panel:* Block design for the N-Back task. 20 repetitions of the backward masking procedure, for each category of subliminal stimulus (N = Neutral, F = Food, A = Aversive). Each block was 30 s, and each block alternated between 1-Back and 2-Back versions of the N-Back task. The total experiment for N-Back was 6 minutes. The Go-No-Go version was identical in design (but did not alternate between two levels of difficulty).

Go/No-Go Task, (c) After the Experiment. The questions in each section ask the participant to rate their own performance on the tasks, whether they felt they 'gave up' or abandoned the tasks at any time, and whether they saw any of the images presented subliminally (and to provide a brief written description). The aim of this questionnaire was to exclude any participants who gave a sub-optimal performance or who accurately reported seeing any of the images presented subliminally.

2.3. Stimuli

Food, aversive and neutral visual stimuli to be used were selected in two steps. In the first step, 56 color photographs were preselected from the International Affective Picture System (IAPS): (Lang, Bradley, & Cuthbert, 1996) and 126 from a database of images created by the authors. Images were selected to provide diversity of content and minimize overlap between categories (e.g. neutral images related to food, such as kitchen utensils, were excluded). See Table 2 for a list of image details and IAPS numbers where relevant. As the images were to be presented subliminally, attention was paid to clarity and recognisability: pictures with ambiguous content were excluded. We also aimed to minimize cultural dependency of content and meaning so that most pictures were recognizable and understandable for most subjects. In the second step, 5 volunteers (independent from the experiment) rated all 182 preselected photographs on five categories: pleasantness, aversiveness, salience, visual complexity and recognisability, using computerized visual analogue scales (0–100). Based on these ratings, 20

Table 2
List of images.

Neutral stimuli	Food stimuli	Aversive stimuli
Armchair	Chocolate cake (7200)	Snakes (1111)
Yellow leaf	Ice-cream (7330)	Spider (1120)
Child figures	Ice-cream (7340)	Dog (1300)
Mushroom	Chocolate bar (7430)	Shark (1930)
Tree	Burger (7450)	Bloody face (3010)
Houses	Pecan pie	Dead body (3015)
Phone	Toffee cake	Bloody face (3051)
Pencils	Burger	Dead body (3063)
Hat	Chocolate bar	Dead body (3061)
Fountain	Chicken	Disfigured face (3168)
Traffic cone	Strawberries	Injured face (3181)
Country house	Tomato and mozzarella	Disfigured face (3266)
Green leafs	Banana	Severed hand (3400)
Dog figure	Cream roles	Gun attack (3500)
Boat on lake	Sandwich and salad	Prison (6010)
Blond hair	Lasagne	Pointed gun (6230)
Dear	Fried fish	Street gang (6242)
Flower in pot	Gammon steak	Knife attack (6550)
Red car	Grilled chicken	Dirty toilet (9300)
Brushes	Chocolate	Syringe with blood (9592)

Numbers in parenthesis represent codes from the International Affective Picture System (IAPS).

pictures from each category were selected according to the following criteria: maximum recognisability, maximum aversiveness in the aversive category and maximum pleasantness in the food category. For each of these categories, we averaged the score from the 5 independent raters and chose the 20 average top-rated images, for each stimulus type, to be included in the experiment. All categories were matched for visual complexity and color.

For the aversive stimuli, color photographs were selected from the IAPS, and included scenes of violence, injury, dirt, etc. Average ratings of the 20 aversive images were: salience 75 (±17), pleasantness 30 (±19), aversion 68 (±8), complexity 48 (±23), recognisability 73 (±15). For the food stimuli, color photographs of sweet (e.g. chocolate bar, cake, ice-cream) and savory (e.g. sandwich, hamburger, chicken, pasta) foods were selected from the IAPS and additional photographs were created by the authors and matched to the neutral pictures for color and visual complexity. The order of sub-category of foods was randomly presented throughout the experiment. Average ratings of these 20 food images were: salience 61 (±13), pleasantness 71 (±10), aversion 19 (±11), complexity 34 (±17), recognisability 82 (±13). Finally, neutral stimuli were color photographs of neutral inanimate objects (household objects, vehicles, outdoor scenes) created by the authors. Average ratings of these 20 neutral images were: salience 43 (±23), pleasantness 56 (±9), aversion 24 (±9), complexity 35 (±17), recognisability 85 (±11).

2.4. Neuropsychological tasks

2.4.1. The N-back task

The *N*-back Task measures working memory, and the demand on working memory can be modified using a different number *n* (usually 1, 2 or 3). In our study, alternating blocks of 1-back (low demand on working memory) and 2-back (higher demand on working memory) were used to investigate the relationship between interference of subliminal stimuli and task difficulty. To prevent an inherent 'set-shifting' component to this design, that would add an unnecessary level of complexity to the *N*-back task, we included a break of 5 s between the end of the 1-back and beginning of the 2-back, and vice versa. We also indicated, with text on the screen ("1-BACK"/"2-BACK"), which task the participants were about to commence. Participants were presented with a sequence of cards on a computer screen with capital and lower case letters and asked to press the mouse button if the same letter (independent of case) was shown as the one preceding it by 'n' positions. Blocks of 1-back and 2-back tasks (20 cards per block) were separated by 4-s breaks when the inscription '1-BACK' or '2-BACK' was presented on the screen. There were six blocks of 1-back and six blocks of 2-back tasks. In both variants, 20% of the letters were targets (four in each block) – a percentage chosen to avoid pseudo-pattern recognition in the task. Cross-targets were avoided, so that there were no 1-back targets in the 2-back task and vice versa. A training trial of 20 cards was given at the start to ensure the participants understood the instructions. See Fig. 1.

2.4.2. The Go/No-Go task

The Go/No-Go task examines cognitive inhibition by giving participants the instruction to stop responding when a predetermined target appears on the screen. Participants were presented with a sequence of cards in the same style as the *N*-back task (e.g. identical mask, subliminal images and color of targets) depicting capital or lower case letters. Participants were instructed to press the mouse button at the presentation of a letter, but were told to withhold a response if one of

the pre-instructed 'no-go' letters (D, K, X) appeared on the screen. During this task, 240 letters were presented – 48 (20%) of which were 'no-go' letters. A training run was given at the beginning to ensure the participants understood the instructions.

In an attempt to lessen the extra element of "set-shifting" being introduced into the experiment via alternating presentations of N-back and go/no-go tasks, we added a 5 s pause at the end of each task, and provided a written indication on the screen of what the next task would be.

2.5. Block design and backward masking methodology

Using a script in the computer program 'Visual Basic' supported by Microsoft Windows (written by Dr. R. Uher), food, aversive and neutral stimuli were introduced with a backward mask, and thus subliminally in blocks of 20 during the cognitive tasks. Each picture stimulus block was used four times (twice in each task), totaling 12 blocks in the total run. In each task, the order of the blocks was counterbalanced between participants to control for order effects and differential transfer. In the N-back task, task difficulty was included in the counterbalancing procedure (12 different permutations of the stimulus category and task difficulty were used). In addition, target letter sequences were counterbalanced between conditions.

Individual stimuli, consisting of a color photograph (12×8 cm) were presented on the screen for 23 ms, immediately preceding each letter during the cognitive tasks. The target background (high contrast mosaic) served as a mask for backward masking of the stimuli, and the target/non-target letters were placed in the middle of the mosaic printed in bright red. Our pilot data indicated that a Stimulus Onset Asyncrony (SOA) of 23 ms is ideal to reliably present each full picture on the screen (stimuli were visible in the absence of the mask). Each trial (subliminal stimulus and mosaic with target/non-target letter) was presented for 1500 ms. In the N-back task, 20 trials were presented for a block of stimuli; the total time for each block was 30 s in length. 12 blocks were presented in the N-back task, alternating between subliminal stimulus type and task difficulty. The total length of the N-back task, with breaks between the commencement of 1-back and 2-back tasks was approximately 7 min. Following a short interval, participants continued with the go/no-go task. This task does not have varying levels of difficulty and so the continuous run consisted of 12 alternating blocks of 20 trials with the presentation of different subliminal stimuli. Each block was 30 s, and so the duration of the go/no-go task was 6 min (illustration of methodology available on request). See Fig. 1.

2.6. The 'interference effect' on the N-back and Go/No-Go tasks

In order to gauge the extent to which blocks of different types of subliminal visual stimuli (e.g. food, aversive, neutral) interfered with performance during the cognitive tasks, we measured total number of errors (both omission and commission errors) and response times in milliseconds. This was done by a script written in Visual Basic that recorded response times, via a press of the space bar on a keyboard (during correct responses) and the number/type of errors per subject, to a text file supported by Microsoft Windows (available from Dr. R Uher). Omission errors were when a response to a target was expected but not received, whereas commission errors were when a response was given but not expected. We chose not to distinguish between the type of errors, due to the differences in responding to each of the tasks. Furthermore, to account for differences in performance between participants, independent of subliminal stimulus, we calculated average response times and error rates across the total number of blocks for each subliminal stimulus, and for final comparisons between subliminal stimulus types and groups, we averaged the data within group and within stimulus type. All data was subsequently manually inputted into SPSS and checked by two independent researchers.

2.7. The visibility test

On completion of the tasks, participants were asked, during the 'Post-hoc Questionnaire' whether they had seen any screen images during the tasks: if 'yes', they were asked to provide a written description and were excluded if they identified any of the pictures presented subliminally. Participants who were not excluded were then told about the masked pictures and shown all 'primed' stimuli explicitly, paired with novel stimuli matched to the content category. They were asked to choose one of the two photographs they thought were presented during the tasks, and to guess in cases of uncertainty. Photographs (12×8 cm) were presented adjacent to each other on the screen (position was counterbalanced between stimuli and novel pictures) until the participant responded, by pressing the mouse. Participants who performed above chance on the visibility test task were excluded from further analysis. The participants included in this study: females with RAN ($n = 13$), and healthy controls ($n = 20$) all performed at or below chance level.

2.8. Statistical analysis

The assumptions of homogeneity of variance (Levene's Test) and normal distribution (Kolmogorov–Smirnov/Shapiro–Wilk) in all groups were not met (for response times and accuracy in performance). Therefore, non-parametric tests were chosen to examine the main effects of group and condition on accuracy (total number of errors) and response time (in milliseconds). Where appropriate, Bonferroni corrections were applied to correct for false positives (Type I errors) arising from multiple comparisons. To examine the independent factor of group, the Kruskal–Wallis test was used, with post hoc analysis using the Mann Whitney test to demonstrate the direction of the differences. To examine the within subject factor of

stimulus type the Friedman test was used, and post hoc analysis with the Wilcoxon test to demonstrate the direction of any differences. Post-hoc correlation analysis was applied to anxiety scores on the HADS and total number of errors during the subliminal presentation of food stimuli using bivariate non-parametric Spearman's Rho correlation.

3. Results

3.1. Subjective threshold

Participants who accurately described any of the subliminal images were excluded from the analysis. This led to 10 healthy controls being excluded from the analysis but no RAN patients. A Chi-Squared test revealed that this was a significant association between the RAN and control group in terms of level of awareness ($\chi^2 = 8.04$, $df = 1$, $p = .005$). In the end, 20 controls were tested and 13 people with restricting AN.

3.2. Objective threshold

The 13 RAN patients completed the visibility test task at the end of the experiment and their mean score for correctly choosing subliminal 'primed' stimuli was 28 (±2.1)/60, whereas for the 20 controls the mean score was 27 (±1.8)/60. Both
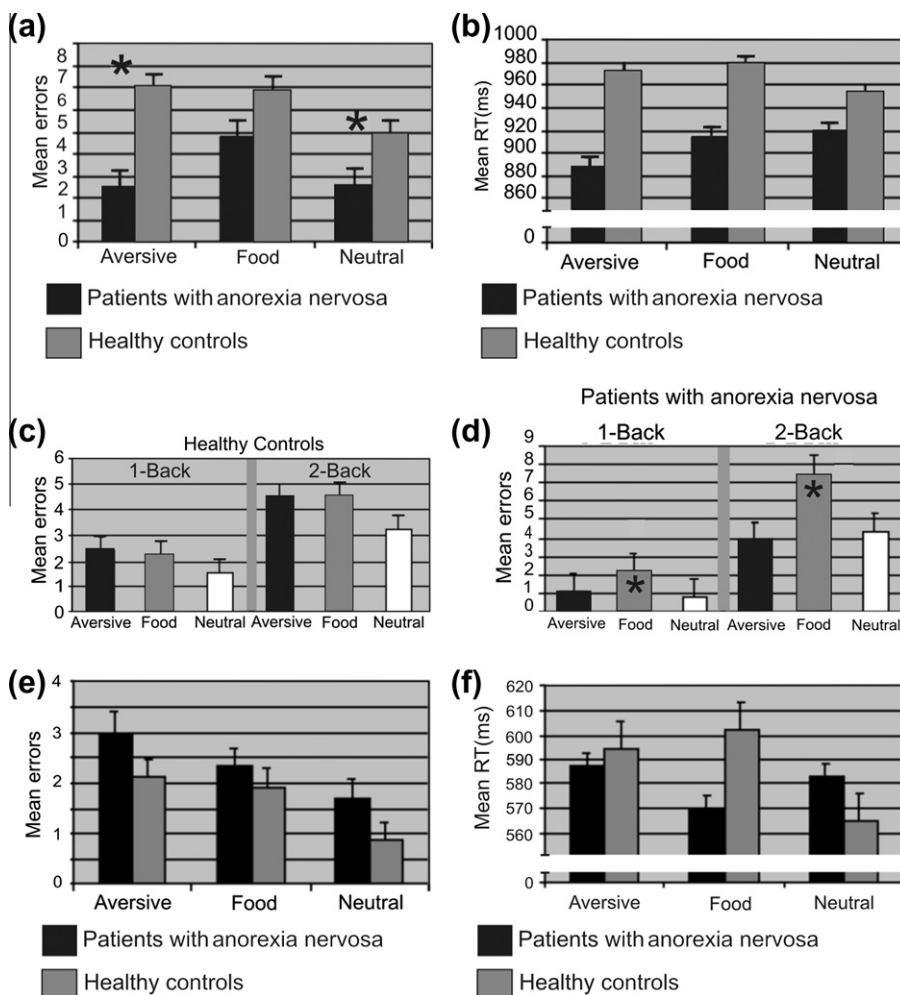

Fig. 2. Error rates and response times during the subliminal tasks. (a) Mean errors for N-Back task (both 1- and 2-back). Asterisks= significant difference $p < 0.01$. (b) Mean response times in milliseconds for N-Back task (both 1- and 2-back). No significant differences. (c) Mean errors for 1-back and 2-back separately in healthy controls, during the presentation of different subliminal stimuli. No significant differences. (d) Mean errors for 1-back and 2-back separately in patients with anorexia nervosa, during the presentation of different subliminal stimuli. Asterisks = significant difference $p < 0.01$. (e) Mean errors during the Go/No-Go task between patients with anorexia and healthy controls during the presentation of different subliminal stimuli. No significant differences. (f) Mean response times in milliseconds during the Go/No-GO task between patients with anorexia and healthy controls during the presentation of different subliminal stimuli. No significant differences.

groups performed at chance level (with no significant difference) and therefore the subliminal images were deemed not consciously perceived by either group during the experiment. The next objective was to show that there are differences in the way the subliminal stimuli were perceived between groups – by demonstrating interference effects during two unrelated cognitive tasks. Before inferences can be made about the effects of subliminal stimuli, it must be demonstrated that the images were processed subliminally by both groups. This was achieved by showing that there was a greater interference effect in the 'subliminal (RAN and HC)' versus 'non-subliminal exposure' (HC)' groups. See Fig. 2 for all results described below.

3.3. Anorexic patients versus controls

3.3.1. N-back (working memory, DLPFC) task: accuracy rates and Response Times

Comparison of the anorexic and control groups revealed that there was a significant difference in total errors made ($\chi^2(1) = 4.828$, $p = .027$), as healthy controls made significantly more errors during all N-back tasks than patients with RAN ($U = 70.5$, $p = .022$, $r = -.38$). This was unlikely to be due to the subliminal images being more salient for the control group as no significant difference was found between the groups in the number of primed images chosen during the visibility test task. The difference in accuracy between anorexic and control groups occurred irrespective of task difficulty – the difference remained in both the 1-back and 2-back tasks. The 2-back task was significantly more difficult than the 1-back task because both patients with RAN ($T = -2.911$, $p = .001$, $r = -.51$) and healthy controls ($T = -3.708$, $p < .01$, $r = -.51$) made more errors overall during the 2-back task than in the 1-back task (Fig. 1e.)

An examination of the interfering effects of specific subliminal stimuli on accuracy during performance of the N-back task revealed a group difference ($\chi^2(1) = 6.782$, $p = .008$) during the presentation of aversive ($U = 62.5$, $p = .006$, $r = -.453$) and a group difference ($\chi^2(1) = 5.233$, $p = .022$) during neutral subliminal stimuli presentation ($U = 73$, $p = .025$, $r = -.4$). Specifically, controls made significantly more errors than RAN patients during subliminal aversive and neutral stimulus presentation, but there was no significant difference between RAN patients and controls during subliminal food presentation. Thus, it seems that the superior performance of the RAN patients during the N-back task was only compromised when food images were presented subliminally (e.g. they made significantly less errors during the subliminal presentation of aversive and neutral images).

Next we examined the different versions of the N-back task (1-Back and 2-Back) to take into account the effect of varying difficulty levels on the difference in errors between AN patients and controls. In the 1-back task there was a significant difference between RAN patients and controls during the presentation of aversive ($\chi^2(1) = 6.782$, $p = .008$) and neutral ($\chi^2(1) = 5.233$, $p = .02$) subliminal stimuli, but no significant difference in errors during presentation of subliminal food stimuli. Mann Whitney U tests confirmed that controls made more errors during subliminal aversive ($U = 62.5$, $p = .009$) and neutral ($U = 73$, $p = .023$) stimuli. In the 2-back task there was a significant difference between AN patients and controls during the presentation of aversive stimuli only ($\chi^2(1) = 9.572$, $p = .001$). A Mann Whitney U test confirmed that controls made more errors during subliminal aversive stimuli presentation ($U = 47$, $p = .001$). These results illustrate the difference in errors between RAN patients and Healthy Controls in the 1-back and 2-back tasks separately. Comparing these effects across the 1-back and 2-back tasks revealed no significant differences, which means that the effects of subliminal stimuli on performance was the same in both versions of the task in both groups.

To examine whether there were significant differences between the errors caused by subliminal stimuli within each group a Friedman test analysis was conducted on each group. The test confirmed a significant difference in number of errors in the RAN group ($\chi^2(5) = 24.973$, $p < .01$) and Healthy Control group ($\chi^2(5) = 42.681$, $p < .01$). Post Hoc analysis using the Wilcoxon Signed Ranks test revealed that RAN patients made more errors during subliminal food stimuli presentation compared to aversive in 1-back ($T = -2.333$, $p = .017$) and 2-back ($T = -1.792$, $p = .037$). There were no other significant differences in amount of errors. Post Hoc analysis using the Wilcoxon Signed Ranks test showed that Healthy Controls made more errors during subliminal aversive stimuli presentation compared to neutral in 1-back ($T = -2.275$, $p = .028$) and 2-back ($T = -2.293$, $p = .018$), and during subliminal food stimuli presentation compared to neutral in 2-back ($T = -2.192$, $p = .03$). (Fig. 1g and h.) However, in the previous analysis we show that the number of errors in both 1-back and 2-back during sub-

Table 3
Mean error rates and response times in *N*-back and Go/No-Go during subliminal exposure.

Number of errors	1-Back		2-Back		Go/No-Go	
	AN (*n* = 13)	HC (*n* = 20)	AN (*n* = 13)	HC (*n* = 20)	AN (*n* = 13)	HC (*n* = 20)
Subliminal stimuli exposure						
Aversive	0.54 (±0.97)	2.45 (±2.72)	1.92 (±2.12)	2.45 (±2.72)	3 (±2.12)	2 (±1.17)
Food	1.08 (±1.26)	2.25 (±2.25)	3.69 (±3.45)	4.55 (±3.19)	2.31 (±1.65)	1.75 (±1.21)
Neutral	0.38 (±0.87)	1.55 (2.19)	2.15 (±1.77)	3.25 (±2.21)	1.69 (±1.11)	0.75 (±1.11)
Response times (ms)						
Aversive	565 (±92)	617 (±83)	642 (±157)	712 (±78)	588 (±114)	594 (±84)
Food	582 (±102)	620 (±78)	64 (±162)	716 (±113)	570 (±111)	602 (±101)
Neutral	582 (±119)	615 (±69)	669 (±122)	677 (±119)	583 (±109)	565 (±77)

liminal presentation of aversive and neutral images were significantly less in the RAN group (but not significantly different during the subliminal presentation of food images).

There were no significant differences between controls and anorexic patients in the time taken to respond correctly to targets during the 1-back and 2-back tasks. Subliminal images had no significant effect on the time taken to respond correctly to targets in both groups (Fig. 1f).

3.3.2. Go/No-Go (cognitive inhibition, ACC) task: accuracy rates and response times

Comparing the anorexic and control groups there was a significant difference in the number of errors made ($\chi^2(1) = 3.868$, $p = .05$). Specifically, the Mann Whitney U Test revealed that anorexic patients made significantly more errors than the control group overall ($U = 77$, $p = .049$, $r = -.34$).

A significant difference in the amount of errors caused by a specific type of subliminal stimuli was found in the control group only ($\chi^2(2) = 8$, $p = .016$). Post-hoc analysis revealed that the controls made more errors during the subliminal presentation of aversive stimuli ($T = -2.796$, $p = .004$, $r = -.49$) and food stimuli ($T = -2.254$, $p = .02$, $r = -.49$). There was no significant difference between the amounts of errors the controls made during the subliminal presentation of these 'emotional' stimuli (Fig. 1i)

The presentation of subliminal stimuli had no significant effect on the response times to correct targets in the anorexic group. However, there was a significant effect detected in the control group ($\chi^2(2) = 11.1$, $p = .003$). Controls took significantly longer to respond correctly to targets during the subliminal presentation of aversive ($T = -2.912$, $p = .002$, $r = -.51$) and food ($T = -3.136$, $p = .001$, $r = -.55$) stimuli in comparison to neutral stimuli. (Fig. 1j). See Table 3.

3.4. Analysis of questionnaire measures and correlations

Post-hoc analyses revealed significant differences, after Bonferroni correction (significant $p = .01$), between RAN patients and healthy controls on self-report measures of anxiety collected after completion of the cognitive tasks. Self-reported trait anxiety on the HADS (Zigmond & Snaith, 1983) differed significantly between RAN and controls ($t[30] = -8.794$, $p < .01$), as women with RAN reported more anxiety. Self-reported measures of depression on the HADS also differed significantly between RAN and controls ($t[30] = -8.699$, $p < .01$), in that RAN patients were more depressed. Finally, reports of interoceptive awareness from the EDI-2 (i.e. the ability to experience bodily sensations) were significantly different between RAN and controls ($t[31] = 10.649$, $p < .01$); controls reported greater interoceptive awareness.

Using Spearman's Rho correlation analysis also Bonferroni corrected (significant $p = .01$), we ran post hoc correlations between interference scores (number of errors and response times) in both cognitive tasks and scores on the measures above, to examine whether any self-report measures acted as mediators for interference. Some of the self-report measures were significantly related to level of interference during the tasks. A significant positive correlation was found between response time to targets during subliminal presentation of food stimuli in the N-back task and HADS anxiety score in RAN patients ($R^2 = .658$, $n = 13$, $p < .001$). A positive trend was found between trait anxiety score on the HADS and total errors during subliminal presentation of food stimuli in the N-back task in RAN patients only ($R^2 = 0.21$, $n = 13$, $p = .07$). There were no significant correlations in the control group between self-report measures and the interference effects of subliminal food stimuli on performance in the N-back task.

In the Go/No-Go task, post hoc (Bonferroni corrected) Spearman's Rho correlations revealed that for people with RAN there was a significant positive relationship between errors made during subliminal presentation of aversive stimuli and self-reported anxiety ($R^2 = 0.665$, $n = 13$, $p < .001$) (HADS). In the control group, there were significant positive correlations between number of errors made during subliminal presentation of aversive stimuli, self-reported anxiety ($R^2 = 0.590$, $n = 19$, $p = .008$) and self-reported interoceptive awareness ($R^2 = 0.538$, $n = 19$, $p < .001$). Thus, on the Go/No-Go task (but not the N-back task) there was no significant difference between the AN and control groups in terms of how anxiety levels influenced the number of errors they made.

4. Discussion

We present here for the first time, novel preliminary empirical evidence regarding working memory function and unconscious processes in women with restricting anorexia nervosa (RAN), that contributes to theories on non-conscious processes in the brain e.g. Baars' Global Workspace Theory (Baars & Franklin, 2003). We show that overall women with RAN, who have dysfunctional goals regarding food and eating, are more accurate than healthy controls (HC) during the N-back task of working memory (and less accurate during the Go/No-Go task of cognitive inhibition). This complements a study by our group (Dickson et al., 2008) where women with RAN had better working memory performance than controls, and is in accord with the view that people with AN are highly proficient in working memory function (Fowler et al., 2006). Thus, combined with our findings of the effect of subliminal food stimuli on the working memory performance of women with RAN, it is plausible that greater executive functioning is a core symptom of the disorder. It could be that the *context* of working memory employed by those with RAN is modulated by unconsciously perceived visual and emotional representations of appetitive stimuli, in line with Baars' Global Workspace Theory (GWT). This is a particularly attractive explanation, given that subliminal visual food stimuli in this study interacted with working memory and not conflict monitoring in women with RAN.

We found that on the Go/No-Go task, which incorporates a conflict monitoring element, people with RAN perform worse than controls. This could also highlight a deficit in people with RAN to shift between rules, i.e. 'set-shifting' (Roberts, Tchant-uria, & Treasure, 2010). Perhaps people with RAN are unable to change their response to a no/go target because they get stuck responding to the more frequent (80%) go response during this task. An inability to set-shift during the go/no-go task could reflect response inflexibility that manifests as perseverative or stereotyped behaviors in people with AN.

This study showed that subliminal images cause interference to performance on the cognitive tasks described above. This supports studies showing that automatic processing of stimuli can interfere with conscious cognitions (Banse et al., 2001; Fazio & Olson, 2003; Murphy & Zajonc, 1993). Specifically, our data fits with the notion that subliminal appetitive stimuli at first activate approach-state motor responses in the striatum, followed by cognitive inhibition systems associated with the PFC (Eimer & Schlaghecken, 2003). Furthermore, in Baars' GWT, it is purported that multiple "back-stage" unconscious perception cycles occur in the brain, but that *seriality* is imposed when a single successful cycle emerges onto the conscious-ness stage, goaded by contextual cues. This might explain the interference effect caused to working memory performance by subliminal food stimuli, as the women with RAN were only able to consciously attend to one process at a time.

Subliminal food stimuli were shown to cause more interference to people with RAN, and this may indicate a higher sal-iency for those with RAN, and that cognitive inhibition systems, via working memory functioning, are competitively en-gaged. Although the women with RAN made significantly less working memory errors overall than the control group, their superior performance was compromised, and matched the error rate of the healthy controls when food stimuli were presented subliminally. This effect was only observed during the N-back task of working memory and not during the Go/No-Go task. Thus, it is plausible that in real life, non-consciously perceived appetitive activation in the brain of a person with RAN drives and supports working memory ruminations and cognitive strategies that maintain a stranglehold on impulsive drives. This is in line with the GWT of Baars, in that the largely conscious process of working memory is supported and some-what guided by non-consciously perceived stimuli, particularly 'visual semantics' (Baars & Franklin, 2003), e.g. subliminal images of food versus subliminal images of aversive or neutral scenes. Continual use of a working memory system to sup-press appetitive drives in everyday life might make a person with AN excessively proficient at working memory tasks, to the point where such cognition becomes counter-productive in most other life domains (e.g. excessive rumination, inflexible thought, lack of spontaneity, attention to detail etc.).

It might be argued that the interference effect of subliminally-presented food stimuli on the N-back task of working mem-ory presented here is *prima facie* in disagreement with Baars' GWT, which purports that non-consciously perceived stimuli support and guide working memory function. However, the subliminal stimuli presented here were unrelated to the cogni-tive tasks, and thus, likely to cause interference particularly for highly self-relevant stimuli (e.g. food images for women with AN). Nevertheless, we suggest that our data (working memory, and not other cognition, is disturbed by non-conscious appe-titive processes) hints that Baars' GWT might explain real-life cognitive mechanisms that drive dysfunctional eating behavior in people diagnosed with AN "...to help mobilize and guide unconscious routines that carry out working memory functions" (Baars & Franklin, 2003). It is plausible that non-consciously perceived appetitive processes in those suffering from AN drive aberrant ruminations about shape weight and eating, and are supported by DLPFC-dependent working memory processes. This explanation is particularly appealing in the context of these experiments, given that the women with RAN had signif-icantly better working memory performance than compared to the healthy subjects, until subliminal food stimuli were presented.

The Go/No-Go task examines conflict/response inhibition, which is linked to activation in the ACC (Albert et al., 2010). Cognitive inflexibility is commonly observed in AN (Roberts et al., 2010), however, we found less interference by subliminal stimuli during this task in the women with RAN, which could indicate that when the ACC is engaged it is not available to process subliminal emotional or disease-related stimuli. Conversely, we propose that it is the highly efficient (shown by sig-nificantly less errors on the N-back task) working memory system of the DLPFC in women with RAN that is usually engaged to cognitively inhibit appetitive responses.

Salient subliminal stimuli (e.g. aversive and food images versus neutral), particularly appetitive stimuli, likely activate subcortical responses in the striatum, (Berridge, Ho, Richard, & DiFeliceantonio, 2010), amygdala, insular cortex and anterior cingulate cortex (Brooks et al., 2012a) in healthy people, that impose competitive effects on PFC regions. Competitive effects on accuracy and response times were observed during this study while PFC regions were otherwise engaged in two unrelated cognitive tasks. The N-back task utilizes working memory function, which is linked to activation in the DLPFC (Petrides, 2005). People ill with RAN have reduced DLPFC activation when passively viewing food images (whereby appetitive re-sponses could be avoided), however, the DLPFC is activated when appetite is restored in women who have recovered from RAN (Uher et al., 2003), and when women currently ill with RAN are explicitly asked to think about eating food (Brooks et al., 2011, 2012a, 2012b). In women with RAN, working memory function associated with activation of the DLPFC may reflect excessive concerns about weight, shape and eating in response to appetitive subcortical responses that impose a food-related cognitive bias, or contextual flavor on working memory in everyday life. Presenting visual food stimuli subliminally when working memory function is otherwise engaged (in the case of the N-back task), causes the activation of automatic appetitive responses that are not modulated (as might be usual for a woman with RAN) by working memory-based goal strategies. Thus, non-consciously perceived appetitive responses to subliminal food stimuli are able to compete with the limited pro-cessing capacity in the DLPFC, at least for those whose concerns favor thoughts about food.

This scenario is in line with current views on 'quick and dirty' neural activations between the amygdala and non-consciously perceived visual cortical representations that initially process and prepare action in response to salient stimuli

(LeDoux, 2003). Post-hoc correlational analyses provided some support for this notion-anxiety measured by HADS in RAN correlated with increased errors and response times, but only when food stimuli (and not aversive or neutral stimuli) were presented subliminally during the *N*-back task. Additionally, an affective framework such as the *mood as information* paradigm (Schwarz & Clore, 1983) could be used to explain the relationship between interference during valence-specific presentation of subliminal stimuli and anxiety. Within this framework, it is deemed that the subliminal food stimuli likely place the women with RAN in a negative mood, which then causes performance anxiety and thus, more errors during the *N*-back task. It is an attractive possibility to consider, that momentary mood inducement, via unconscious stimulation (particularly by stimuli related to a psychiatric condition) can influence conscious cognitions, because such a paradigm could be used to gauge disorder severity. However, it is still not yet clear whether unconsciously derived mood inducement helps or hinders conscious cognitions. It is likely that people with RAN are unaware of the origin the negative affective state that becomes associated with food. However, in line with Schwarz and Clore's theory, if it is possible to alter or reduce these unpleasant affective states, disorder-maintaining misattribution of negative affect towards real-life events (e.g. relationships, outings with friends etc.) might not occur.

What are the implications of data showing that subliminally presented food stimuli potentially interfere with DLPFC processes in people with RAN? Preoccupation with food may cause overexposure to food stimuli, which become more self-relevant and preferentially processed in women with RAN, thus increasing the saliency of the stimuli and causing greater task interference. According to Monahan, Murphy, and Zajonc (2000) repeated exposure to a stimulus increases preference for that stimulus – a preference which '...increases logarithmically as a function of the number of exposures.' Furthermore, '...the seemingly simple process of repeated exposures may play a significant role in the internalization of cultural practices and the affective investment in one's cultural artifacts'. Repeated exposure to a stimulus can eventually bypass semantic processing in favor of a quick motor response (Gaillard et al., 2006). It is plausible that spontaneous repetitive thoughts that are adaptive in healthy people (Baars, 2010) become hijacked by the disease state in people who are predisposed to develop AN. These ideas suggest that in women with RAN, overexposure to food stimuli increases its emotional saliency, which influences behavior independent of PFC modulation. The automatic affective response to food stimuli may become an 'internalized' and highly repetitive aspect of the RAN person's sense of self and lead to 'competitive interference' between cortical and subcortical processing.

Another view is that, rather than preoccupation with food strengthening the saliency and processing bias for food stimuli in RAN, it is the automatic anxiogenic effects of the subliminal food stimuli that drive the interference observed in this study. We found that interference caused by subliminal food stimuli positively correlated with those RAN patients who reported higher levels of anxiety. This finding is in line with a theory (Ohman & Soares, 1994) that negative stimuli can influence behavior by triggering automatic anxiety responses. Another recent study found that anxiety sensitivity influences attentional bias for masked (e.g. subliminal) threat cues (Hunt, Keogh, & French, 2006). Thus, rather than being automatic, appetitive responses that drive the dysfunctional cognitions observed in AN, it might be secondary processes, such as the activation of anxiogenic concerns about body shape, weight and eating that trigger restrained eating behavior (via cognitive inhibition mechanisms in the PFC) in women with RAN.

There are some limitations to this preliminary study. Individual optimal thresholds for perceptual awareness were not measured, especially in relation to trait anxiety (Li, Zinbarg, & Paller, 2007) although the visibility test task and subjective measures provided assessments of lack of awareness. We did not use a sub-category of 'pleasant' stimuli, an important aspect of a general emotional response – future studies could use stimuli rated as pleasant to compare the valence effects of automatic emotional processing. Additionally, the number of participants tested within each group was relatively small. Many current studies do not compare the subliminal condition with a non-subliminal condition, only with a 'subliminal neutral' condition, as was the case here, although a previous preliminary study by our group examined subliminal vs. supraliminal effects in women with AN (Dickson et al., 2008). However, future studies could also match with a 'non-subliminal' working memory condition, to explicitly compare the interference effects of subliminal salient stimuli on working memory.

This study has therapeutic implications. Cooper and Fairburn (1994) suggest that the degree of interference on the 'Food Stroop' task is a good indicator of disease severity. Similarly, interference (errors and response times) on the working memory task during the subliminal presentation of food stimuli likely shows the severity of cognitive restriction in people with AN. It could also be used to examine different eating disorder subtypes: for example, people with BN or those who are obese might find food more salient, or anxiogenic, and this might stimulate greater interference from the subliminal processes. The value of this paradigm is that patients are unaware of the presence of the disease-related stimuli, and so are unable to alter their responses in the context of the therapy session, or get distressed when directly confronting their fears about food. Core pathology can be identified without being obscured by meta-cognitions adopted by the patient. Future studies could compare the interference of subliminal food stimuli before and after a course of therapeutic intervention, such as Cognitive Remediation Therapy (CRT) (Tchanturia, Davies, & Campbell, 2007), which tackles automatic patterns of general cognition, not just those related to food.

5. Conclusion

This study demonstrates that subliminal food stimuli interferes with working memory performance associated with DLPFC function in people with RAN, and that this interference may be driven by non-consciously perceived appetitive

processes, or related to anxiety. This was shown because the mechanism by which RAN working memory performance was superior to that of the control group was impaired when food images were presented subliminally, and this impairment correlated with anxiety measures. Further exploration of the non-consciously perceived appetitive effects on cognitions in AN is needed, particularly by examining physiological responses. The therapeutic implications are that subliminal measures could be used non-invasively to gauge severity of symptoms in AN. A reduction of automatic anxiogenic responses to food may help to improve outcome of cognitive behavioral therapy (CBT) in people with RAN.

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