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Disentangling the web of fear: Amygdala reactivity and functional connectivity in spider and snake phobia

Fredrik Åhs[a,*], Anna Pissiota[b], Åsa Michelgård[c], Örjan Frans[a], Tomas Furmark[a], Lieuwe Appel[d], Mats Fredrikson[a]

[a]Department of Psychology, Uppsala University, Uppsala, Sweden
[b]Unit for Transcultural Psychiatry, Uppsala University, Uppsala, Sweden
[c]Department of Clinical Neuroscience, Uppsala University, Uppsala, Sweden
[d]Uppsala Imanet, GE Healthcare, Uppsala, Sweden

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ABSTRACT

The objective was to study effects of fear on brain activity, functional connectivity and brain-behavior relationships during symptom provocation in subjects with specific phobia. Positron emission tomography (PET) and ^{15}O water was used to measure regional cerebral blood flow (rCBF) in 16 women phobic of either snakes or spiders but not both. Subjects watched pictures of snakes and spiders serving either as phobic or fear-relevant, but non-phobic, control stimuli depending on phobia type. Presentation of phobic as compared with non-phobic cues was associated with increased activation of the right amygdala and cerebellum as well as the left visual cortex and circumscribed frontal areas. Activity decreased in the prefrontal, orbitofrontal and ventromedial cortices as well as in the primary somatosensory cortex and auditory cortices. Furthermore, amygdala activation correlated positively with the subjective experience of distress. Connectivity analyses of activity in the phobic state revealed increased functional couplings between voxels in the right amygdala and the periamygdaloid area, fusiform gyrus and motor cortex. During non-phobic stimulation, prefrontal activity correlated negatively with amygdala rCBF, suggesting a phobia-related functional decoupling. These results suggest that visually elicited phobic reactions activate object recognition areas and deactivate prefrontal areas involved in cognitive control over emotion-triggering areas like the amygdala, resulting in motor readiness to support fight or flight.

1. Introduction

Specific animal phobia as, for example, spider and snake phobia is characterized by excessive and unreasonable fear, escape and avoidance behaviors (American Psychiatric Association, 1994). Recently, the lifetime prevalence of specific phobia was reported to be 12.5% in the USA (Kessler et al., 2005). The first wave of neuroimaging studies explored patterns of brain activity during phobic states using symptom provocation designs with visual (Fredrikson et al., 1993, 1995; Wik et al., 1993; Johanson et al., 1998), tactile (Mountz et al., 1989; Rauch et al., 1995) or auditory phobic cues (O'Carroll et al., 1993). The initial hypothesis was that the amygdala would display increased activity during fear provocation based on its established role in vigilance and negative affect (cf. Mountz et al., 1989; Fredrikson et al., 1993; Rauch et al., 1995). However, most early imaging studies and also some more recent (cf. Paquette et al., 2003) failed to show enhanced amygdala activity during presentations of fear-related cues. Even the reverse pattern, with enhanced amygdala activity to phobic cues in non-fearful controls but not in phobic patients, has been reported (Straube et al., 2006a). Study design and brain imaging methodology may have affected study outcomes. For example, it has been suggested that there is a rapid habituation of amygdala activity with more sustained stimulus presentations of the type that generally characterized initial positron emission tomography studies. More recent functional magnetic imaging trials have often used intermittent and repeated presentations of feared (cf. Straube et al., 2006a,b). Most studies have used visual stimulation but have rarely controlled for the visual content of the stimuli objects (cf. Paquette et al., 2003). This leaves the possibility open that activation patterns not only reflect emotional factors but in part could be confounded with differences in visual input.

Based on the findings above, one aim of the present study was to use neuroimaging to measure regional cerebral blood flow (rCBF) during the presentation of phobic cues matched for visual content. In order to minimize habituation effects, different pictures were presented intermittently. We predicted that an amygdala response would occur. We also aimed at correlating subjective distress ratings with amygdala activity because several previous studies have reported linear relationships between the subjective experience of negative

* Corresponding author. Department of Psychology, Uppsala University, Box 1225, SE-751 42 Uppsala, Sweden. Tel.: +46 18 471 78 43; fax: +46 18 471 21 23.
E-mail address: Fredrik.Ahs@psyk.uu.se (F. Åhs).

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affect and amygdala activity (cf. Tillfors et al., 2001; Pissiota et al., 2002; Fredrikson and Furmark, 2003; Shin et al., 2006; Goosens et al., 2007a; Michelgård et al., 2007).

More importantly though, connectivity analyses are of interest because in addition to amygdala function, activity in the prefrontal cortex appears to be aberrant in several anxiety disorders (cf. Tillfors et al., 2001; Etkin and Wager, 2007). In specific phobia, a number of studies have reported attenuated prefrontal activity during symptom provocation (cf. O'Carroll et al., 1993; Wik et al., 1993; Fredrikson et al., 1995; Johanson et al., 1998; Carlsson et al., 2004; Schienle et al., 2007). The prefrontal cortex is held to exert an inhibitory or modulatory influence on amygdala activity (Kim et al., 2003; Meyer-Lindenberg et al., 2005; Pezawas et al., 2005; Stein et al., 2007), and it has been speculated that the irrational nature of phobia, i.e. the inability to control fear with reason, in part is due to compromised prefrontal control of the amygdala (cf. Fredrikson and Furmark, 2003). However, the hypothesis that there is a functional decoupling between prefrontal activity and amygdala activation in individuals with specific phobia has not been tested directly. To study functional connectivity between amygdala activity and other brain territory, connectivity analyses using whole brain voxelwise correlative approaches were performed. We specifically predicted that activity in the anterior cingulate cortex (ACC), which has been suggested to exercise a inhibitory control over the amygdala, would be negatively correlated with amygdala activity during fear-relevant but not phobic conditions (Pezawas et al., 2005; Meyer-Lindenberg et al., 2005; Kim et al., 2003; Stein et al., 2007).

The visual cortex seems to increase its activity both in response to generally arousing events as well as during negative affect (Sabatinelli et al., 2005, 2007) including phobic conditions (Fredrikson et al., 1995; Dilger et al., 2003; Paquette et al., 2003). Especially the fusiform gyrus and its connectivity to the amygdala has been the focus of studies on visual processing of fear (see Vuilleumier and Driver, 2007 for a review). We therefore predicted that activity in associative visual brain areas would increase as a function of fear together with increased functional connectivity to the amygdala. Because several symptom-provocation studies on specific phobia have reported activation of the insula cortex (cf. Dilger et al., 2003; Straube et al., 2006a; Goosens et al., 2007b), we also predicted exaggerated insula activity during the phobic condition.

Thus, the objective was to induce fear in snake and spider phobic individuals using identical visual input in order to study effects on brain activity, functional connectivity and brain-behavior relationships. To accomplish this, phobic subjects fearful of snakes or spiders, but not both, were exposed to pictures of snakes and spiders while regional cerebral blood flow (rCBF) was measured.

2. Materials and methods

2.1. Subjects

Sixteen right-handed female volunteers (mean age = 22.8, S.D. = 4.12) who fulfilled the *DSM-IV* (American Psychiatric Association, 1994) criteria for specific spider ($n = 8$) or snake ($n = 8$) phobia were included in the study. For a detailed description of the recruitment procedure, see Pissiota et al. (2003). Briefly, subjects were included if they were phobic of one but not the other class of stimuli. Thus, for snake phobic individuals snake pictures induced negative affect and spider pictures acted as fear-relevant control stimuli. The reverse was true for spider phobics. Screening included Swedish versions (Fredrikson, 1983) of the Snake Anxiety Questionnaire (SNAQ, range 0–30), the Spider Phobia Questionnaire (SPQ, range 0–31) (Klorman et al., 1974) and a clinical interview. Spider-phobic participants had a mean SPQ score of 23.3 and a mean SNAQ score of 2.4 corresponding to the >95 and <25 percentiles, and snake -hobic subjects had scores of 23.9 and 3.2 for the SNAQ and SPQ corresponding to >95 and <50 percentiles, respectively. Subjects could not meet any of the following criteria: current psychiatric disorder (other than specific snake or spider phobia), organic brain disorder, prescribed medication against those conditions, somatic disease, left-handedness, substance abuse or pregnancy. The study was approved by the local ethics and radiation safety committees. Written informed consent was obtained from all subjects.

2.2. Stimuli and procedure

Subjects were presented slides of snakes and spiders on a computer screen approximately 40 cm in front of their face during scanning. Each presentation contained 25 pictures of either snakes or spiders, randomly presented for 2–4 s with an interval of 1–3 s. Phobic and fear-relevant non-phobic pictures were shown on two separate days with the order of conditions counterbalanced between subjects. After each presentation block, subjects rated the distress experienced during picture presentations on a visual analogue scale (0 = min, 100 = max). In addition, one 60-min [^{11}C]GR205171 PET scan was performed with symptom provocation on the same days as the [^{15}O] water scans in order to measure effects on the substance P Neurokinin-1 receptor system (Michelgård et al., 2007). The [^{11}C] GR205171 PET scans were always conducted after the scans with [^{15}O] water.

2.3. Image acquisition and analysis

Scans were performed using an ECAT EXACT HR+ PET scanner (CTI/Siemens, Knoxville, TN, USA) operated in 3D mode with a 155-mm axial field of view and an axial resolution of approximately 5 mm, producing 63 planes with 2.5-mm plane spacing. Subjects were positioned in the scanner with their head gently fixated. Then, a 10-min transmission scan was performed. Approximately 10 MBq/kg bodyweight of [^{15}O]-water was injected during the emission scans and data were collected in three 30-s frames, starting at bolus arrival to the brain. Data were corrected for attenuation and scatter and reconstructed to a 128×128 matrix using back projection and an 8-mm Hanning filter. The three 30-s frames for each scan were summed to produce a 90-s image for each individual and condition to obtain a better statistical reference for realignment and subsequent analyses.

PET images were realigned and normalized to the Montreal Neurological Institute's (MNI) stereotactic template ICBM152, using SPM2 (Wellcome Department of Cognitive Neurology, London, UK). Images were smoothed using a 12-mm Gaussian kernel and scaled to give all scans the same global value. Data were statistically evaluated using within-group comparisons in SPM2 with rCBF data fitted to the general linear model (Friston et al., 1994). The generated *t*-maps were converted to *z*-scores, and the MNI co-ordinates (*x*,*y*,*z*) were transformed into Talairach space (Talairach and Tournoux, 1988) using a non-linear transformation proposed by Matthew Brett (http://imaging.mrc-cbu.cam.ac.uk/imaging/MniTalairach).

Effects on rCBF were evaluated at the voxel level (1 voxel = 2×2×2 mm) by examining statistically significant changes ($P<0.05$) corrected for multiple comparisons using the false discovery rate (FDR) (Genovese et al., 2002). Anatomical localization was guided by the Talairach atlas (Talairach and Tournoux, 1988), the Talairach Daemon (Lancaster et al., 2000) and the detailed medial temporal lobe atlas of Mai (Mai et al., 2004).

For areas where we predicted an altered activity, we performed region of interest (ROI) analyses using small volume correction. However, if significant changes in these areas were observed in the whole brain analyses, the latter statistics were reported. The ROI volumes were defined by the WFU PickAtlas Toolbox, an automated method to generate ROI masks implemented within the SPM2 software (Maldjian et al., 2003).

Functional connectivity was analysed by using the mean right amygdala rCBF as a seed that was entered as a co-variate in SPM for fear-relevant and phobic conditions separately. Only the right amygdala was used as seed since phobic cues did not activate the left amygdala (see Section 3). We examined negative functional connectivity between the amygdala and the anterior cingulate cortex using small volume correction. Criteria for statistical significance were again set to $P<0.05$ FDR-corrected for multiple comparisons. In addition to SPM2 evaluations, statistical analyses were performed using SPSS 12.1 (SPSS Inc., Chicago, Il., USA).

3. Results

3.1. Behavioral data

Distress ratings were higher during the phobic than the non-phobic condition: $t\,(15) = 9.13$; $P<0.001$. See Fig. 1.

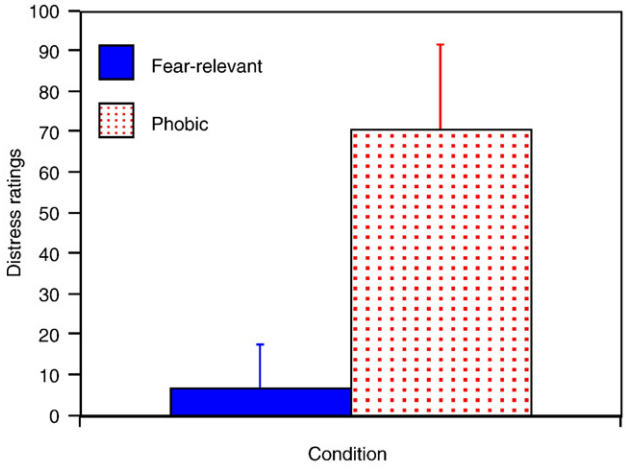

Fig. 1. Mean (±S.D.) distress ratings during phobic and fear-relevant but non-phobic conditions in individuals with specific phobia.