NK₁ receptor antagonism and the neural processing of emotional information in healthy volunteers

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Abstract

The neuropeptide substance P and its receptor NK₁ have been implicated in emotion, anxiety and stress in preclinical studies. However, the role of NK₁ receptors in human brain function is less clear and there have been inconsistent reports of the value of NK₁ receptor antagonists in the treatment of clinical depression. The present study therefore aimed to investigate effects of NK₁ antagonism on the neural processing of emotional information in healthy volunteers. Twenty-four participants were randomized to receive a single dose of aprepitant (125 mg) or placebo. Approximately 4 h later, neural responses during facial expression processing and an emotional counting Stroop word task were assessed using fMRI. Mood and subjective experience were also measured using self-report scales. As expected a single dose of aprepitant did not affect mood and subjective state in the healthy volunteers. However, NK₁ antagonism increased responses specifically during the presentation of happy facial expressions in both the rostral anterior cingulate and the right amygdala. In the emotional counting Stroop task the aprepitant group had increased activation in both the medial orbitofrontal cortex and the precuneus cortex to positive vs. neutral words. These results suggest consistent effects of NK₁ antagonism on neural responses to positive affective information in two different paradigms. Such findings confirm animal studies which support a role for NK₁ receptors in emotion. Such an approach may be useful in understanding the effects of novel drug treatments prior to full-scale clinical trials.

Received 24 March 2009; Reviewed 24 April 2009; Revised 20 May 2009; Accepted 22 May 2009; First published online 23 June 2009

Key words: Antidepressants, aprepitant, depression, emotion, fMRI, NK₁.

Introduction

Preclinical investigations into Substance P and its neurokinin 1 (NK₁) receptor have suggested reliable effects on emotion, stress and anxiety (Ebner & Singewald, 2006). Substance P has been found in areas of the brain that are involved in emotional responses such as the cingulate cortex, the hippocampus and the amygdala and brain tissue measurements have revealed increases in substance P in these areas during stress or pain (Brodin et al. 1994; Rosen et al. 1992; Siegel et al. 1984). Consistent with this, centrally administered substance P induces responses similar to those produced by stressful stimuli (Culman & Unger, 1995; Unger et al. 1988) while antagonism of substance P via NK₁ receptor blockade reduces anxiety and depression-like behaviours across a number of paradigms including the chronic mild stress paradigm, the forced swim test, vocalizations in guinea-pig pups, the elevated plus-maze and the social interaction test (Boyce et al. 2001; Cheeta et al. 2001; Dableh et al. 2005; Kramer et al. 1998; Rupniak et al. 2000; Sartori et al. 2005; Stout et al. 2001; Varty et al. 2002; Zocchi et al. 2003). Such effects led to the suggestion that NK₁ antagonism may be a new mechanism for antidepressant and anxiolytic drug action.

A recent positron emission tomography (PET) study in humans with social phobia found that 6-wk treatment with the NK₁ antagonist GR205171 alleviated social anxiety and this was accompanied by reduced amygdala activity in a small sample (Furmark et al. 2005). Furthermore, Geracioti et al. (2006) reported an acute increase in CSF substance P following...
psychological stress in patients with major depression and those with post-traumatic stress disorder. However, clinical trials of NK1 antagonists have had mixed results. An initial placebo-controlled trial suggested positive effects of the NK1 antagonist MK-869 (aprepitant, Merck, USA) in patients with major depression and anxiety (Kramer et al. 1998) and this finding was replicated with a second antagonist compound by Kramer et al. (2004). However, more recently larger scale phase III clinical trials in depressed patients reported a lack of efficacy of aprepitant (Keller et al. 2006) while in the same clinical population, the selective serotonin re-uptake inhibitor (SSRI), paroxetine, was clearly more effective than placebo. Moreover, evidence of abnormal substance P function in depression is mixed with inconsistent observations across studies and paradigms (Berrettini et al. 1985; Geracioti et al. 2006; Rimon et al. 1984). These inconsistencies within the clinical literature highlight the need to better understand the role of NK1 receptors in human emotional function prior to the use of such a drug target in clinical populations.

Recent studies suggest reliable effects of conventional antidepressant drug treatments on the processing of emotional information using both behavioural and fMRI techniques. For example, acute administration of the SSRI citalopram increased recognition of happy faces (Harmer et al. 2003a, b) and attention toward positive stimuli in a visual probe task in healthy volunteer samples (Browning et al. 2007). In fMRI studies, acute citalopram administration reduced neural responses to negative and fearful facial expressions of emotion within the amygdala (Anderson et al. 2007; Del-Ben et al. 2005; Murphy et al. 2009). Such effects would be expected to reverse enhanced negative and threat-relevant processing which are believed to play a key role in the underlying aetiology and maintenance of depression and anxiety. These studies also suggest that acute effects of antidepressants can be reliably seen in healthy volunteers and may help characterize their neuropsychological mechanism of action.

The present study therefore investigated the effects of an acute dose of the NK1 antagonist aprepitant on the neural processing of emotional information in healthy volunteers. Two tasks designed to probe different aspects of emotional processing were used, the facial expression processing task and the emotional counting Stroop task. The facial expression task involved the presentation of both happy and fearful facial expressions with 3 degrees of emotional intensity for each valence. This task allows for the dynamic range of brain responses to affective stimuli to be modelled as well as the overall capacity of response to affective trials and has been shown to be sensitive to depression and antidepressant drug treatment (Chen et al. 2007; Fu et al. 2004). The emotional counting Stroop task measures the neural response to the presentation of neutral, positive and threatening words and the interference to the counting response caused by the emotional valence of the words presented. This task is designed to detect brain responses to the emotional interference caused by the words and recruits areas of the brain believed to be involved in the higher level cognitive control functions such as conflict monitoring and decision making (Bush et al. 2000; Whalen et al. 1998a).

A previous study suggested that aprepitant increased the behavioural recognition and attention to positive affective information without affecting responses to negative and threatening information (Chandra et al. in press). It was therefore predicted that aprepitant would particularly modulate neural responses to happy facial expressions and positive word stimuli in relevant neural circuitry including the medial prefrontal cortex, the fusiform gyrus, amygdala and the anterior cingulate cortex.

Methods and materials

Subjects

Ethical approval for the study was obtained from the Oxfordshire Research Ethics Committee. Healthy subjects aged between 18 and 36 yr were screened through a medical examination and with the Structured Clinical Interview for DSM – Clinical Version (SCID-IV; Spitzer et al. 1992). Exclusion criteria were: current or past history of psychiatric disorder, pregnancy, current medication (including the contraceptive pill), or seizure disorders. Study participants’ medical family history was assessed in the screening session and those with a first-degree family relative with depression were also excluded. fMRI scanning also required the following exclusion criteria: cardiac pacemaker, mechanical heart valve, or any mechanical implants, potential pregnancy, and claustrophobia. After complete description of the study to the subjects, written informed consent was obtained.

Experimental design

Twenty-four healthy volunteers were randomly allocated to receive aprepitant (125 mg capsule) or placebo (capsule) in a double-blind, between-groups design. Groups were matched for: gender (drug, 7 males; placebo, 6 males) and age (drug: mean = 26.4 yr,
Subjects attended the laboratory at 09:00 hours having fasted after a light breakfast. Medication was administered and testing started 4 h later at which time peak levels of aprepitant in plasma would be expected. Studies with PET have indicated that a dose of 125 mg aprepitant should occupy between 80% and 90% of brain NK₃ receptors (Hargreaves, 2002). Subjects’ blood pressure, well-being, mood and subjective state were monitored for 4 h after taking the placebo/aprepitant. Four hours after taking the placebo/aprepitant, volunteers took part in the fMRI scan during which two tasks were given: facial expression processing and emotional counting word Stroop. fMRI scan stimuli were presented on a personal computer using E-Prime (version 1.0; Psychology Software Tools Inc., USA) and projected onto an opaque screen at the foot of the scanner bore, which subjects viewed using angled mirrors. Behavioural responses were recorded using a MRI-compatible keypad. Accuracy and reaction times were recorded by E-Prime.

Subjective ratings

On the day of drug administration, baseline mood and subjective state were assessed with the Beck Depression Inventory (BDI; Beck et al. 1961), Dysfunctional Attitudes Scale (DAS; Weissman, 1979) State-Trait Anxiety Inventory (STAI; Spielberger, 1983), Befindlichkeit Scale (BFS; von Zerssen et al. 1974), and visual analogue scales (VAS) of happiness, sadness, anger, disgust, alertness, and anxiety. Additionally, transient subjective state was assessed with BFS and VAS at times +1 h and +6 h after drug administration.

Facial expression processing

Each volunteer participated in a single 16-min experiment employing rapid event-related fMRI. Eight faces (4 male, 4 female) displaying prototypical expressions of fear and happiness were taken from a standardized series of facial expressions (Ekman & Friesen, 1976). In addition to the prototypic or high-intensity (100%) facial expression, low (30%) and medium (60%) intensity expressions were created using morphing software. Previous work has suggested that linear modelling of the neural response to different intensities of positive and negative emotions is sensitive both to identifying biases in depression and neural modulation by antidepressant treatment (Chen et al. 2007; Fu et al. 2004; Kendler et al. 2006). Thus, there were six facial stimuli representing each of the following categories: high fearful (fear-H), medium fearful (fear-M), low fearful (fear-L), high happy (happy-H), medium happy (happy-M), and low happy (happy-L). Each of these faces was presented four times and 24 presentations of a fixation cross were included as baseline, giving a total of 168 trials. Stimuli were presented in a random order for 500 ms each, and the inter-trial interval varied accordingly about a Poisson distribution with a mean inter-trial interval of 5000 ms. Subjects were asked to indicate the gender of each face by pressing one of two keys on an MRI-compatible keypad. No motor response was required for baseline trials of fixation cross.

Emotional counting Stroop task

Participants were scanned while performing a modified version of the emotional counting Stroop called the ‘name the number of words’ task (Whalen et al. 1998a). Word stimuli were a subset drawn from a larger pool used in previous research (Mathews et al. 1989) examining depression and anxiety, and selected to be either neutral (e.g. mileage, molecule), physically threatening (e.g. fatal, accident), socially threatening (e.g. worthless, inferior) or positive (e.g. generous, achievement). Physically threatening and socially threatening words were combined to generate a negative word category. Words were matched for word length, frequency and imageability [see MRC psycholinguistic database (www.psy.uwa.edu.au/mrcdatabase/uwa_mrc.htm)].

Participants completed one run of the task with a total of 160 words being presented across 16 blocks. Four 20-word blocks of each stimulus type were presented in a pseudo-randomized order and interspersed with 20-s blocks of fixation, free of stimulus (no motor response) as baseline. Presentation of the four conditions was counter-balanced across participants and between the two groups. Participants completed 10 trials during each presentation block (stimulus presentation 1500 ms, inter-trial interval 500 ms). For each trial, participants viewed between one and four identical words and were instructed to report (via keypad response) the number of words presented in each trial.

Visual stimulation paradigm

A control visual stimulation paradigm was used to assess whether drug-related effects observed during facial expression processing might reflect global effects of aprepitant on baseline cerebral blood flow. A flashing checkerboard (frequency 8 Hz) was presented in blocks of 15 s alternating with 15 s of a fixation cross for a total of 20 cycles. Subjects were instructed to lie with their eyes open during this control task.
fMRI data acquisition

Imaging data were collected using a Siemens Sonata scanner operating at 1.5 T located at the Oxford Centre for Clinical Magnetic Resonance Research (OCMR). Functional imaging consisted of 40 T2*-weighted echo-planar image slices [repetition time (TR) = 4000 ms, echo time (TE) = 50 ms, matrix = 64 x 64], 3 mm x 3 mm x 4 mm isotropic voxels for the emotional faces task which was acquired coronally to enhance the signal from the frontal cortex and 35 and 30 T2*-weighted echo-planar image slices (TR = 3000 ms, TE = 50 ms, matrix = 64 x 64), 3 mm³ isotropic voxels for the Stroop task and visual control task, respectively, acquired axially. To facilitate later coregistration of the fMRI data into standard space, we also acquired a Turbo FLASH sequence (TR = 12 ms, TE = 5.65 ms) voxel size = 1 mm³. A total of 210, 218 and 105 volumes were acquired during the emotional faces task, the emotional counting Stroop task and the control visual stimulation paradigm, respectively.

fMRI data analysis

fMRI data were pre-processed and analysed using FEAT (FMRIB Expert Analysis Tool) version 4.0, part of FSL (FMRIB Software Library) (www.fmrib.ox.ac.uk/fsl). Pre-processing included within-subject image realignment, non-brain removal, spatial normalization to a standard template [Montreal Neurological Institute (MNI) 152 stereotactic template] using an affine procedure and spatial smoothing using a Gaussian kernel (5 mm full-width-half-maximum). The time series in each session was high pass-filtered (Gaussian-weighted least-squares fit straight-line fitting, with sigma = 40 s). FSL was used to compute individual subject analyses in which the time series were pre-whitened to remove temporal autocorrelation (Woolrich et al. 2001). An event-related design was employed to explore the rapid blood oxygen level-dependent (BOLD) responses triggered by the emotional faces task. There were seven explanatory variables modelled, including each intensity (low, medium, high) of fear and happy as well as fixation.

The main contrasts of interest were fear vs. happy expressions (and vice versa) for each intensity level, i.e. fear-H vs. happy-H; fear-M vs. happy-M, fear-L vs. happy-L. In addition, each individual activation map was analysed by fitting linear trends at each voxel at the three intensity levels of fear and happy, separately, with orthogonal polynomial trend analysis.

The emotional counting Stroop task had a block design and had three explanatory variables that were modelled: ‘neutral’, ‘positive’ and ‘threatening’ words. All variables were convolved in each block with a haemodynamic response function, using a variant of a gamma function (i.e. a normalization of the probability density function of the gamma function) with a standard deviation of 3 s and a mean lag of 6 s. Temporal derivatives were included as covariates of no interest to increase statistical sensitivity. All analyses at the group level employed a full mixed-effects approach (Woolrich et al. 2004). Z (Gaussian T) statistical images were thresholded using clusters determined by Z > 2.3 and a family-wise error (FWE) corrected spatial extent threshold of p < 0.05. Foci of activation were localized with the aid of a standard anatomical atlas (Talairach & Tournoux, 1988). For regions where a significant drug group x task interaction was observed (e.g. fear vs. baseline), % BOLD signal change was extracted with FSL Featquery and examined with analysis of variance (ANOVA). Significant interactions were explored further with simple main effect analyses to identify the profile of drug effect. Due to the strong a-priori evidence implicating the amygdala in the processing of facial expressions (Harmer et al. 2006), we also performed a region-of-interest (ROI) analysis. The ROIs for the left and right amygdala in standard space were obtained with mri3dX (http://www.aston.ac.uk/lhs/staff/singhk/d/mri3dX/mri3dX.jsp), which uses a stored representation of the Talairach Daemon Database (Lancaster et al. 2000). Mean % BOLD signal change during the processing of fearful and happy expressions in these ROIs was computed and compared between antidepressant and placebo groups. A ROI analysis was also carried out in the emotional faces task using a fusiform gyrus 20-mm sphere (−44, −56, −20) created with the WFU Pickatlas (Maldjian et al. 2003, 2004) based on the coordinates that have been shown to be involved in face-processing in previous experiments (Vuilleumier & Schwartz, 2001). The fusiform gyrus has also been shown to have reduced activation to positive faces in depressed patients vs. healthy controls in a previous study (Surguladze et al. 2005) and antidepressant treatments such as reboxetine have been shown to increase activity in this area for happy vs. neutral faces (Norbury et al. 2007). For the control stimulation paradigm, a whole brain analysis was carried out for the apreptitant group vs. the placebo group for visual stimulation on vs. visual stimulation off. We also compared mean % BOLD signal change between groups in the occipital (calcarine) cortex activated by photic stimuli across all subjects, a structural mask of the calcarine cortex was created with the FSLView structural atlas toolbox, Harvard-Oxford structural atlas (http://www.fmrib.ox.ac.uk/fsl/).
Statistical analysis of behavioural and mood data

Behavioural data and mood ratings were analysed with repeated-measures ANOVA with group as the between-subjects factor and time as the within-subjects factor. Significant interactions were analysed further with simple main effect analyses. All statistical analyses were performed with the Statistical Package for Social Sciences (SPSS Inc., USA).

Results

Mood and subjective state

The two groups were well matched in terms of general mood, personality, and subjective state, indicated by the absence of significant baseline differences in BDI, DAS, STAI and VAS scores (all p > 0.1). There were no differences in VAS scores +1 h and +6 h after drug administration between the groups (all p > 0.3) and there was no difference in BFS scores +1 h and +6 h after drug administration between the groups (all p > 0.1). Absence of mood effects is also seen after short-term administration of antidepressant drugs to healthy volunteers and allows us to examine changes in the neural processing of emotional information unconfounded by changes in subjective state.

Facial expression processing

Main effect of task

There were no significant differences between the placebo and the aprepitant groups in their reaction times in deciding if the emotional faces were male or female (all p < 0.1) or in accuracy to correctly respond over all of the different emotional valences (all p < 0.08).

Processing of fearful and happy facial expressions activated a largely overlapping neural network including fusiform gyri, occipital cortex, frontal pole, superior frontal gyrus and paracingulate gyrus in placebo-treated volunteers, consistent with previous reports (Morris et al. 1996; Vuilleumier et al. 2003; Vuilleumier & Schwartz, 2001). Fearful vs. happy faces activated the fusiform gyrus, the frontal pole and the superior frontal gyrus. For peak cluster activation in regions activated during processing of faces (see Table 1).

Aprepitant × task interactions

Whole brain analysis. During presentations of happy vs. fearful expressions, there was a significant interaction between drug group and emotion in the anterior cingulate cortex (Table 1, Fig. 1a) with peak cluster activation (MNI coordinates: x = 2, y = 46, z = 0). Analysis of BOLD signal change in this area revealed an overall group × emotion × intensity interaction [ANOVA: F(1, 22) = 4.49, p < 0.017] and a main effect of group [F(1, 22) = 9.39, p = 0.006]. This was driven by an interaction between group × intensity interaction specifically for the happy facial expressions [happy: F(1, 22) = 3.347, p = 0.04; fear: F(1, 22) = 1.22, p = 0.3] and a main effect of group for the happy faces [happy: F(1, 22) = 14.36, p < 0.001]. Overall volunteers receiving aprepitant showed larger responses to the happy facial expressions of emotion in the anterior cingulate at high and medium intensities (see Fig. 1b). There was also no main effect of group for fear although the trend suggests that aprepitant may also increase the responses to fear in the anterior cingulate [fear: F(1, 22) = 3.8, p = 0.063].

Fusiform gyrus ROI. This revealed no significant differences in the fusiform gyrus to the processing of emotional faces between the groups (all p < 0.1).

Amygdala ROI. ANOVA revealed an overall group × emotion × intensity interaction [ANOVA: F(1, 22) = 3.16, p = 0.05] in the right amygdala (see Fig. 2a). Further analysis of happy faces revealed a group × intensity interaction [F(1, 22) = 5.44, p = 0.008] and a main effect of group [F(1, 22) = 4.25, p = 0.05] but no interaction or main effect of group for fearful faces [F(1, 22) = 1.02, p = 0.36; F(1, 22) = 0.3, p = 0.58]. Hence, volunteers receiving aprepitant showed larger responses within the right amygdala to the happy facial expressions at high intensity levels (see Fig. 2b). There was no effect of drug on the processing of emotional faces in the left amygdala (p > 0.1 all conditions).

Emotional counting Stroop task

Main effect of task

There were no significant differences between the placebo and the aprepitant groups in their reaction times to count the number of words on the screen (all p < 0.7) or in accuracy to correctly respond over all of the different emotional valences (all p < 0.6, see Table 2).

Processing of emotional words, consistent with previous reports (Mannie et al. 2008; Strakowski et al. 2005; Whalen et al. 1998a) activated neural networks including the putamen, occipital cortex and thalamus in placebo-treated volunteers. For peak cluster activation in regions activated during processing of emotional words see Table 1.
Aprepitant task interactions

Whole brain analysis. During presentations of positive vs. neutral words, there was a significant interaction between drug group and emotion in the medial orbitofrontal cortex with peak cluster activation (MNI coordinates: \( x = 14, y = 26, z = -18 \)) and in the precuneus with peak cluster activation (MNI coordinates: \( x = 2, y = -72, z = 30 \)) (Table 1, Fig. 3a). Analysis of BOLD signal change in the medial orbitofrontal cortex

Table 1. Peak cluster activation in brain regions of significantly increased BOLD response during facial expression processing and emotional counting Stroop task in placebo-treated volunteers (main effect of task) and in drug group vs. placebo group

<table>
<thead>
<tr>
<th>Task and region</th>
<th>Cluster size</th>
<th>Z value</th>
<th>Coordinates*</th>
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<td><strong>Main effect of faces task</strong></td>
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<td>Placebo</td>
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<td>Fearful faces (all fear)</td>
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<tr>
<td>Temporal occipital fusiform gyrus</td>
<td>224</td>
<td>3.49</td>
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<tr>
<td>Precentral gyrus</td>
<td>60</td>
<td>3.46</td>
<td>-38</td>
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<tr>
<td>Happy faces (all happy)</td>
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<tr>
<td>Temporal occipital fusiform gyrus</td>
<td>240</td>
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<td>Fearful faces (all fear)</td>
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<td>Happy faces (all happy)</td>
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<td>Happy (high) vs. fear (high)</td>
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<td>Premotor cortex</td>
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<td><strong>Main effect of emotional counting word Stroop task</strong></td>
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<td>Positive words</td>
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<tr>
<td>Putamen</td>
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<td>Neutral words</td>
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<tr>
<td>Precuneus cortex</td>
<td>272</td>
<td>3.68</td>
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BOLD, Blood oxygen level-dependent.

*Montreal Neurological Institute (MNI) coordinates (x, y, z) refer to peak activation within each cluster identified, thresholded at \( Z = 2.3 \) and \( p < 0.05 \) corrected.
with repeated-measures ANOVA revealed a group (aprepitant, placebo) x emotion (positive, neutral) interaction [ANOVA: F(1,22) = 18.8, p < 0.001] and further one-way ANOVA revealed that aprepitant increased the neural response to positive words compared to placebo [ANOVA: F(1,23) = 7.294, p = 0.01] (Fig. 3b). We found no significant difference in this part of the orbitofrontal cortex to the threatening word stimuli of the drug group compared to placebo [F(1,46) = 2.409, p = 0.127] (Fig. 3b).

Analysis of BOLD signal change in the precuneus with repeated-measures ANOVA revealed a group (aprepitant, placebo) x emotion (positive, neutral) interaction [ANOVA: F(1,22) = 20.3, p < 0.001] and further one-way ANOVA revealed that this interaction was driven by a trend towards aprepitant increasing the neural response to positive words compared to placebo [ANOVA: F(1,23) = 3.68, p = 0.06] and a significant increase to positive words vs. neutral words within the aprepitant group [ANOVA: F(1,23) = 15.43, p < 0.001] but not the placebo group [F(1,23) = 0.99] (Fig. 3c). We found no significant difference in this part of the precuneus cortex to the threatening stimuli between drug groups [F(1,46) = 0.494, p = 0.48].

**Visual stimulation control experiment**

A whole brain analysis of visual stimulation on vs. visual stimulation off for the aprepitant group compared to the placebo group revealed no significant differences. A region of occipital (calcarine) cortex activated by photic stimulation across all subjects was identified as a ROI. Analysis of mean % BOLD signal change in this region confirmed no differences between groups [F(20) = 0.77, p = 0.4] indicating that the observed effects of aprepitant did not result from global haemodynamic changes.

**Discussion**

The present results suggest that an acute dose of the N\(K\)\(_1\) receptor antagonist aprepitant in healthy volunteers with no current or previous history of psychiatric disorder can increase the neural activity underlying the processing of positive information in two different emotion paradigms involving to some extent different psychological processes (perception and attention), stimuli (pictorial and verbal) and neural circuitry (amygdala and different areas of the prefrontal cortex). During face emotion processing, aprepitant increased activity in the anterior cingulate cortex and amygdala to happy facial expressions compared to placebo and this was particularly evident at the high emotion intensity levels. Similarly, during presentation of affect-laden verbal material, those receiving aprepitant showed increased neural responses specifically to the positive stimuli in the medial prefrontal cortex and precuneus. These effects occurred in the absence of any difference in subjective mood between the drug and placebo groups suggesting that these effects are a direct modulation of the processing of emotional information.

The emotional faces expression task recruits visual processing areas such as the fusiform face area and the occipital cortex for identification and recognition and the emotional valence of faces has been shown to consistently activate a frontal limbic network including the amygdala (Breiter et al. 1996; Whalen, 1998; Whalen et al. 1998b) and medial frontal cortex (Fairhall & Ishai, 2007; Gobbini & Haxby, 2007; Haxby et al. 2000). Affective disorders such as depression are associated with increased BOLD responses within this
circuitry to negative vs. positive facial expressions, consistent with evidence of negative biases in affective processing (Bouhuys et al. 1999; Chen et al. 2007; Fu et al. 2004; Gur et al. 1992; Surguladze et al. 2004). In the present study, acute blockade of the NK1 receptor increased neural responses to happy vs. fearful facial expressions in both the anterior cingulate and amygdala, suggesting a potential role for this neurokinin in human emotional processing.

The amygdala has been hypothesized to act as a vigilance system by directing visual processing towards events which require further attention (Whalen, 1998). Such a hypothesis can explain why, although the amygdala is most consistently activated by threat-relevant stimuli, it is also activated in response to some positive events, particularly of relevance to the subject (Browning et al. 2007; Canli et al. 2002). The medial prefrontal cortex is intimately associated with this amygdala circuitry and is also often activated in tasks involving the processing of affective information (Bush et al. 2000; Lane et al. 1997; Reiman et al. 1997; Yoshimura et al. 2009), particularly those

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**Fig. 1.** Neural response to overtly presented fearful and happy faces and effects of aprepitant. (a) Aprepitant increased activation during processing of happy (high) vs. fearful (high) faces in the rostral anterior cingulate cortex (cluster maximum: x = 2, y = 46, z = 0, Z score = 3.28) compared to placebo. Images are thresholded at Z = 2.0, p = 0.05, corrected. (b) Plot of mean % blood oxygen level-dependent (BOLD) signal change to happy (low, medium and high) faces modelled against baseline (fixation periods) for aprepitant (■) and placebo (○) in the region of significant drug group (aprepitant, placebo) × emotion (fearful, happy) × intensity (low, medium, and high) interaction. Bars show the mean, error bars show the s.e.m. (c) Plot of mean % BOLD signal change to fearful (low, medium and high) faces modelled against baseline (fixation periods) for aprepitant (■) and placebo (○) in the region of significant drug group (aprepitant, placebo) × emotion (fearful, happy) × intensity (low, medium and high) interaction. Bars show the mean, error bars show the s.e.m.

**Fig. 2.** Amygdala response to overtly presented happy faces vs. baseline (fixation) effects of aprepitant. (a) Neural response to presentations of happy vs. fearful faces for aprepitant and placebo groups. Images are thresholded at Z = 2.0, p = 0.05, corrected. (b) Plot of mean % blood oxygen level-dependent (BOLD) signal change in this region to happy (low, medium and high) faces modelled against baseline (fixation periods) for aprepitant (■) and placebo (○) in the region of significant drug group (aprepitant, placebo) × emotion (fearful, happy) × intensity (low, medium and high) interaction. Bars show the mean, error bars show the s.e.m. (c) Plot of mean % BOLD signal change to fearful (low, medium and high) faces modelled against baseline (fixation periods) for aprepitant (■) and placebo (○). Bars show the mean, error bars show the s.e.m. There were no differences between groups on the processing of fearful faces in the right amygdala.
with a self-referential component (Fossati et al. 2003, 2004; Phan et al. 2004). The increased response to happy facial expressions of mid- to high intensity seen here within both the amygdala and medial prefrontal cortex therefore implies that these positive and socially reinforcing stimuli may be more salient or assigned a higher priority following NK₁ blockade. Such observations are consistent with a recent study which found enhanced behavioural identification of happy facial expressions following acute aprepitant administration (Chandra et al. 2009).

The emotional counting Stroop task measures the neural response to the presentation of neutral, positive and threatening words and the interference to the counting response caused by the emotional valence of the words presented. This task is assumed to recruit parts of the brain involved in cognitive control, error detection and areas with the ability to balance the processing of two competing information streams (Bush et al. 2000; Whalen et al. 1998a). Previous studies have shown that this task particularly recruits middle frontal gyrus, motor cortex, inferior temporal gyrus, and superior parietal cortex (Bush et al. 2006; Whalen et al. 2006). In the present study, NK₁ blockade led to increased responses to the positive emotional information in the medial orbitofrontal cortex and the precuneus area of the superior parietal lobe. The precuneus has been particularly associated with processing of self-referent information involving mental imagery and memory retrieval (Fossati et al. 2004; Northoff et al. 2006) and is part of the circuitry which appears to support negative affective bias in depression (Grimm et al. 2008; Mittelshaffer, 2008). By contrast, the medial orbitofrontal cortex plays a key role in the representation and hedonic value of rewarding stimuli (Blood & Zatorre, 2001; Gottfried et al. 2003; O’Doherty et al. 2001, 2003) and has been hypothesized to play a role in anhedonia in depression (Gorwood, 2008). Together, therefore, these effects suggest that aprepitant modulates neural circuitry involved in self-referent processing and the rewarding value of positive stimuli across paradigms, consistent with behavioural biases seen with the same drug manipulation (Chandra et al. 2009).

Our results confirm the involvement of limbic areas in the actions of NK₁ blockade seen in preclinical animal investigations (Dableh et al. 2005; Papp et al. 2000; Zocchi et al. 2003), though the nature of the effects appear rather different. In particular, while the present investigation found consistent and specific effects of NK₁ blockade on the processing of positive affective information, with no significant effect on threat-relevant processing, the vast majority of preclinical studies have reported effects on the processing of aversive, painful or anxiogenic material (Ebner & Singewald, 2006). A recent PET imaging study found that 4-wk treatment of the NK₁ antagonist GR205171 decreased social anxiety in patients with social phobia.
compared to placebo and this was accompanied by an attenuation of amygdala rCBF in response to aversive public speaking (Furmark et al. 2005). The absence of such effects in our study suggests that repeated administration of NK₁ blockade may be required to see these effects in humans or that they may only be apparent with more aversive stimuli than those used here.

The absence of effect on the processing of these threat-relevant stimuli with NK₁ receptor blockade contrasts with antidepressant drug effects in other similar fMRI studies where acute administration of the SSRI citalopram decreased amygdala responses to negative facial expressions of emotion with both i.v. administration at 10 mg (Anderson et al. 2007; Del-Ben et al. 2005) and oral dosing at 20 mg (Murphy et al. 2009), although opposite effects have been reported at 20 mg i.v. (Bigos et al. 2008). Decreased amygdala responses to threat have also been seen following repeated (7d) dosing with both 20 mg citalopram (Harmer et al. 2006) and 8 mg reboxetine (Norbury et al. 2007) in healthy volunteer samples. The present results of increasing processing of positive information does, however, show some similarities to antidepressant drug administration using these same models in healthy volunteers. For example, acute dosing of the SSRI citalopram also increased the recognition of happy faces and increased attention to positive information in previous studies (Browning et al. 2007; Del-Ben et al. 2005) and 7-d administration of citalopram has been shown to increase the labelling of facial expressions as happy and the recall for positive personality adjectives in a memory task (Harmer et al. 2004). Furthermore neuroimaging studies reported that 7-d treatment with the noradrenergic antidepressant reboxetine increased fusiform gyrus activations to happy facial expressions, while repeated citalopram treatment also increased responses to happy cues within the amygdala (Norbury et al. 2007; R. Norbury et al. unpublished observations).

These intriguing similarities and differences to conventional antidepressant drug administration require further investigation. Such effects may be relevant to observations that NK₁ blockade is active in pre-clinical animal models (Ebner & Singewald, 2006) and possibly in the treatment of some cases of depression (Kramer et al. 2004), although this does not generalize to efficacy across large patient groups (Keller et al. 2006). Future studies are required to assess the effects of repeated administration of aprepitant in direct comparison to conventional antidepressant drug treatment on emotional processing to fully characterize these effects. Such studies will help to isolate those aspects of neuropsychological drug action which may be important in the therapeutic actions of treatments for depression. This approach may also generate hypotheses about the types of patients who may benefit from aprepitant treatment, for example, whether those who show particular deficits in the processing of positive stimuli rather than excessive processing of negative stimuli particularly respond to this drug treatment.

Emotional processing studies may therefore be useful in our attempts to understand and screen candidate agents for the treatment of depression. Difficulties with the predictive validity of pre-clinical animal models for depression highlight how important it is for us to find new ways of improving the characterization of novel drug treatments early in development. Our results suggest that using an experimental medicine approach with healthy volunteer models could provide useful information prior to the initiation of full-scale clinical trials and allow us to understand how such drug treatments affect emotion in humans. Such human experimental medicine paradigms might, therefore, allow the likely efficacy of different candidate molecules emerging from animal screens to be assessed much more quickly and cost-effectively than in a Phase II treatment study (Dawson & Goodwin, 2005).

A limitation of pharmacological fMRI is that the observed effects could be the result of more global effects of the drug on blood flow or neural coupling (Bonne et al. 1999). However, in the present study, aprepitant had no effect on neural response within the ROI in primary visual cortex consistently activated by photic stimulation. It is therefore likely that the observed effect of aprepitant on fronto-parietal activation was specific for the processing of positive information rather than a result of global haemodynamic changes. A further limitation of the study was that because significant habituation with the kinds of emotional stimuli used here is known to occur with repeated testing, a between-groups design was adopted. While it would be ideal to use individuals as their own control in a within-subjects design, future studies will be required to find a way of repeating the tasks without reducing the emotional potency of the stimuli. The observation that these kinds of effects have been well replicated for the antidepressants using between-groups designs (see Harmer, 2008) does, however, suggest that subject matching across group can be successfully used to explore the neuropsychological action of pharmacological agents.

In summary, the results of this study confirm that blockade of NK₁ receptors affects neural responses to
emotional information across key circuitry including the amygdala, medial prefrontal cortex and pre-cuneus. Such findings suggest that substance P may play a key role in human emotion particularly for positive socially relevant stimuli. These findings provide further evidence for the importance of understanding novel drug action prior to the initiation of full-scale clinical trials. Further studies are required to investigate and compare the effects of aprepitant relative to conventional antidepressants on these models with subchronic administration.

Acknowledgements

This study was funded by the Medical Research Council.

Statement of Interest

Dr Harmer has acted as a consultant for the following companies: Lundbeck, P1Vital, Merck, Sharpe and Dohme and has received grant income from Merck, Sharpe and Dohme. Professor Cowen has been a paid member of advisory boards of Eli Lilly, Servier, Wyeth and Xytis and has been a paid lecturer for Eli Lilly, Servier and GlaxoSmithKline.

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