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Acute 5-HT$_{2A}$ receptor blocking alters the processing of fearful faces in orbitofrontal cortex and amygdala

Bettina Hornboll, MSc[1,2], **Julian Macoveanu, PhD**[1,2], **James Rowe, PhD**[2,4], **Rebecca Elliott, BA, PhD**[5], **professor Olaf B. Paulson, MD**[1,2,3], **professor Hartwig R. Siebner, MD**[1,2], and **professor Gitte M. Knudsen, MD**[2,3]

[1]Danish Research Center for Magnetic Resonance, Copenhagen University Hospital Hvidovre, Hvidovre, Denmark

[2]Center for Integrated Molecular Imaging (Cimbi), Copenhagen, Denmark

[3]Neurobiology Research Unit, Rigshospitalet and University of Copenhagen, Copenhagen, Denmark

[4]Cambridge University Department of Clinical Neurosciences, Cambridge, United Kingdom

[5]Neuroscience & Psychiatry Unit, University of Manchester, Manchester, United Kingdom

Abstract

Background—The serotonin 2A (5-HT$_{2A}$) receptor has been implicated in neural-processing of emotionally salient information. To elucidate its role in processing of fear and anger, healthy individuals were studied with functional MRI (fMRI) after 5-HT$_{2A}$ receptor blockade, while judging the gender of neutral, fearful and angry faces.

Methods—5-HT$_{2A}$ receptors were blocked with ketanserin to a variable degree across subjects by adjusting the time between ketanserin-infusion and onset of the fMRI protocol. Neocortical 5-HT$_{2A}$ receptor binding in terms of the binding potential (BP$_p$) was assessed prior to fMRI with ^{18}F-altanserin positron emission tomography (PET) and subsequently integrated in the fMRI data analysis. Also functional connectivity analysis was employed to evaluate the effect of ketanserin blocking on connectivity.

Results—Compared to a control session, 5-HT$_{2A}$ receptor blockade reduced the neural response to fearful faces in medial orbitofrontal cortex (OFC), independently of 5-HT$_{2A}$ receptor occupancy or neocortical 5-HT$_{2A}$ receptor BP$_p$. The medial OFC also showed increased functional coupling with left amygdala during processing of fearful faces depending on the amount of blocked 5-HT$_{2A}$ receptors.

Conclusions—5-HT$_{2A}$ receptor mediated signaling increases the sensitivity of OFC to fearful facial expressions and regulates the strength of a negative feedback signal from OFC to amygdala during processing of fearful faces.

Corresponding Author: Bettina Hornboll, Danish Research Center for Magnetic Resonance, University Hospital Copenhagen, Hvidovre, Department 340B, Kettegaard Allé 30, 2450 Hvidovre, Copenhagen, Denmark. Phone: +[45] 38621552, Fax: +[45] 36470302, bettinah@drcmr.dk.

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Keywords

fMRI; PET; emotion; fearful faces; serotonin; 5-HT_{2A} receptors; ketanserin

Introduction

Facial expressions such as happiness, fear, sadness, anger, disgust, and surprise represent basic human feelings that are readily decoded by members of all human cultures (Ekmann, 1999). The ability to appropriately interpret emotional facial expressions is important for our social interactions and impaired emotion-related processing is associated with an increased risk for affective psychiatric illnesses (Mayberg, 2003; Phillips et al., 2003). Neuroimaging studies in healthy volunteers have identified the amygdala and prefrontal cortex as core-regions of a functional network processing facial emotions (Adolphs, 2002). Evidence indicates that the amygdala receives visual information about facial emotions via cortical projections from the ventral stream of object processing, and from a fast subcortical pathway. The latter includes the superior colliculus and pulvinar as the only relays and is critical for automatic processing of facial emotions (de Gelder et al., 2005). The medial prefrontal cortex (PFC) and orbitofrontal cortex (OFC) are involved in evaluating cognitive aspects such as integrating information about the emotional state of others, derived from face emotion (Bechara et al., 2000; Salzman and Fusi, 2010). OFC and amygdala are strongly interconnected, with OFC exerting inhibitory control over amygdala during processing of emotional faces (Stein et al., 2007). Therefore an effective integration of neuronal activity among these core-regions is likely to be critical for efficient processing of emotions (Fairhall and Ishai, 2007; Liang et al., 2009).

Serotonin (5-HT) signaling plays an important role in the processing and regulation of emotions (Cools et al., 2007). For example, regulation of 5-HT release in the mPFC in response to aversive stimuli has been identified as a crucial mechanism in rats to deal effectively with stressors and to terminate fear-related behavior (Forster et al., 2006). Previous studies have also shown that serotonergic drugs modulate the neural processing of emotional faces in healthy individuals: For instance, Harmer et al. (2003) found that acute tryptophan depletion decreases recognition of fearful facial expressions in healthy women, while Passamonti et al. (2012) found that acute tryptophan depletion modulated the interactions between PFC and amygdala while viewing emotionally salient faces. Further, a single dose of the selective serotonin reuptake inhibitor (SSRI) citalopram, a widely used antidepressant, increased the neural response of amygdala to happy but not to fearful faces in healthy individuals (Norbury et al., 2009).

Several lines of evidence indicate that the serotonin 2 (5-HT_2) receptor-family (5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}) is involved in generation and expression of anxiety. Global disruption of 5-HT_{2A} receptor signaling reduces inhibition in conflict anxiety paradigms in mice (Weisstaub et al., 2006). In humans, there is accumulating evidence that processing of emotionally salient information is modulated by 5-HT_{2A} receptors and that regional expression of the 5-HT_{2A} receptor in the brain, constitutes a trait related to anxiety (Frokjaer et al., 2008). Recently, Fisher et al. (2009) showed that inter-individual variations in 5-HT_{2A}

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receptor density in mPFC correlated inversely with the activation of right amygdala by angry or fearful faces in a face-matching task compared to a control task. They also reported a positive correlation between amygdala-prefrontal coupling and prefrontal 5-HT_{2A} receptor binding. These neuroimaging data suggest a regulation of amygdala reactivity via feedback inhibition from PFC, which is more pronounced in individuals with greater neocortical 5-HT_{2A} receptor density.

Motivated by these reports, we adopted a multimodal neuroimaging strategy to explore the relation between 5-HT_{2A} receptor signaling and emotional face processing in amygdala and OFC. Our experimental approach integrated pharmacological functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) of 5-HT_{2A} receptor binding. We performed blood oxygen level dependent (BOLD) fMRI while healthy participants made gender-judgments on photographs of male or female faces with fearful, angry, or neutral expressions. 5-HT_{2A} receptors were acutely blocked with intravenous ketanserin infusion. By varying the relative timing between drug intake and the onset of fMRI, we adjusted the relative magnitude of acute 5-HT_{2A} receptor blockage across subjects. In addition, 5-HT_{2A} receptor binding as measured with PET, was used as trait marker of neocortical 5-HT_{2A} receptor dependent neurotransmission. This novel study design enabled us for the first time to study the impact of a gradually increasing 5-HT_{2A} receptor blockage on emotional face processing and to investigate its relation to 5-HT_{2A} receptor binding and occupancy.

We hypothesized that the individuals' cerebral 5-HT_{2A} receptor binding would have differential effects on neural processing of negative face emotions and that pharmacological blocking of 5-HT_{2A} receptors would suppress neural response in OFC while enhancing neural response in amygdala. We further predicted that the pharmacologically induced activity changes during emotional face processing would depend on the 5-HT_{2A} receptor occupancy level.

Methods

Participants

Twenty-three right-handed adults (9 females), mean age 31.8 ± 6.5, were recruited from a larger cohort of healthy volunteers who have previously undergone ^{18}F-altanserin PET (Erritzoe et al., 2009). All subjects were re-interviewed prior to inclusion of the fMRI study. None of the participants reported a history of stimulant abuse or other psychiatric or neurological disorders. All participants were naïve to antipsychotics and antidepressants. They had a normal neurological examination, heart rate and electrocardiogram. Participants completed a modified Danish version of the Profile of Mood States (POMS) questionnaire (McNair et al., 1971) to assess current mood. On each fMRI session, participants completed the mood questionnaire twice, prior to the start of the fMRI scan (and drug infusion) and immediately after the fMRI scan. Written informed consent was obtained prior to both MRI and PET scanning according to the declaration of Helsinki II. The study was approved by the Ethics Committee of Copenhagen and Frederiksberg, Denmark (KF 01-2006-20).

Behavioral task

During fMRI, participants performed a gender-judgment task on face stimuli taken from the Karolinska Directed Emotional Faces database (Lundqvist et al., 1998). Unmasked colored photographs of a male or female face were presented in the middle of the screen for 1800ms, with an inter-trial-interval (ITI) of 200ms. Faces were shown from a frontal perspective and had a neutral, fearful or angry expression. Subjects responded by pressing as quickly as possible one of two buttons with their right index or middle finger.

We employed a mixed fMRI design with alternating blocks showing neutral or aversive male and female faces in equal proportion (NEUTRAL-ANGRY-NEUTRAL-FEARFUL-NEUTRAL…). Each block comprised six events; three to five face stimuli (average of four), and one to three (average of two) null events (fixation cross), which were pseudo-randomly intermixed. In total, 32 blocks of neutral, 16 blocks of fear and 16 blocks of angry faces were presented over two fMRI runs, separated by a short break. All neutral faces were presented twice in total, whereas aversive faces were only presented once. Stimulus presentation and response recordings were performed using E-prime (Psychological Software Tools, Pittsburgh, PA, USA).

Acute blockage of 5-HT_{2A} receptors

Subjects took part in four fMRI sessions. These sessions included ketanserin as below, a control condition with no pharmacological intervention (referred to as the control session throughout), and two other pharmacological interventions; intravenous treatment with the selective serotonin reuptake inhibitor (SSRI) citalopram as well as acute tryptophan depletion (ATD). The order of the drugs were fully counter-balanced across subjects. Apart from the IV line and drug infusion administered while in the scanner, the scanning protocol was identical for the control and ketanserin sessions. To test for drug-related changes in neural response that depended on the receptor occupancy, we systematically varied the interval between the onset of ketanserin administration and the onset of fMRI measurements across subjects. The time interval ranged from 5 to 75 min, leading to a blockade of 5-HT_{2A} receptors of variable degree across subjects. (Fig. 1). Ketanserin was administered intravenously as a 10 mg bolus (time 0) followed by 6 mg/h for the duration of the fMRI scan. This infusion schedule results in a gradual increase in 5-HT_{2A} receptor occupancy (O_{KET}) reaching ~100% occupancy within an hour (Pinborg et al., 2003). O_{KET} is defined as the fraction (%) of a receptor population that is occupied during treatment with an unlabelled drug. The time-dependent estimation of O_{KET} was based on data from our previous PET study with acute ketanserin infusion (Pinborg et al., 2003). First, ketanserin enters the brain from the blood stream and diffuses to the receptor to which the drug then binds, thereby liberating radioligand, which then diffuses back into the bloodstream. To describe this process, we generated a model with two exponentials; one representing the ketanserin binding and liberation of the radioligand from the receptor with a half time of $T_{k½}$, the other representing the diffusion of free radioligand out of the brain tissue into the blood with a half time of $T_{r½}$. By applying this model to experimental data of time dependent 5-HT_{2A} receptor occupancy following ketanserin injection (Pinborg et al. 2003), an excellent fit was obtained when the following two conditions were met: $T_{k½}$ and $T_{r½}$ values both were in the range of 5-10 min and the sum of $T_{k½}$ and $T_{r½}$ amounted to roughly

15 min. This enabled us to estimate the minimum and maximum $T_{k½}$ values corresponding to two O_{KET} outcomes termed O_{KET5} and O_{KET10}. In the absence of actual single subject occupancy measurements we tested the robustness of any observed relation between occupancy and fMRI data using both the estimated maximum and minimum values, O_{KET5} and O_{KET10}.

The study reported here was designed to investigate the effect of 5-HT_{2A} receptor blockade on emotion processing, and not to investigate the effects of increasing or decreasing overall serotonin levels in the brain. Results from the ATD and SSRI sessions that address global serotonin changes have been reported elsewhere (Grady et al. 2012). The fMRI sessions were performed on four different scanning days at least one week apart to ensure a proper wash-out period, with session order counterbalanced across subjects. Apart from the pharmacological manipulation, the experimental procedure was the same for all sessions. The study design with four different serotonergic challenges did not make full placebo controlling of the control session practical. We therefore controlled for nonspecific pharmacological effects of ketanserin administration (e.g. IV line present during scan) and indirect effects of drug (e.g. via induced side effects) by directly contrasting the ketanserin behavior and functional data with the behavior and functional data acquired during the SSRI session, which had a similar administration protocol with intravenous administration during the entire MRI session (at a rate of 8 mg/h). The expected neurophysiological effect of citalopram is increased general serotonergic transmission compared with ketanserin that specifically reduces 5-HT_{2A} receptor transmission. Further, the subjects were unaware of the expected effects of the 5-HT manipulations, the differences in probabilities of side effects between the different drug interventions and the degree of 5-HT_{2A} blockade during ketanserin administration.

Measurements of cerebral 5-HT_{2A} receptor binding

^{18}F-altanserin PET was undertaken as described by Pinborg et al. (2003). In short, ^{18}F-altanserin was administered as a combination of a bolus injection followed by continuous infusion to obtain steady state of the tracer in blood and tissue resulting in a maximum dose of 3.7 MBq/kg bodyweight. PET studies were conducted between noon and 6 pm. Individual ^{18}F-altanserin PET had been acquired on average 3.2±1.7 years before the fMRI experiment. Test-retest studies have shown that in healthy individuals, cerebral 5-HT_{2A} receptor binding remains relatively stable over 2 years (Marner et al., 2009), showing that ^{18}F-altanserin PET can be considered a stable trait marker for neocortical 5-HT_{2A} receptor binding.

PET data were acquired in 3D using an eighteen-ring GE-Advance scanner (GE, Milwaukee, WI, USA). ^{18}F-altanserin PET images and the structural T1-weighted MR images were co-registered and the PET images were then normalized to the same anatomical template as that used for MR images (Pinborg et al., 2003). Volumes of Interest (VOI's) were automatically delineated on each individual transaxial MRI slices in a strictly user-independent fashion (Svarer et al., 2005). Given the extensive co-variation of neocortical 5-HT_{2A} receptor binding across neocortical areas a global neocortical region was defined for each participant as described in Erritzoe et al. (2010). PET images were partial volume

corrected using the segmented MRI. A two-tissue model based on gray matter, white matter, and cerebrospinal fluid was used (Muller-Gartner et al., 1992, Quarantelli et al., 2004). The binding potential of specific binding relative to plasma was calculated as:

$$BP_P = V_T - V_{ND} = \frac{C_T - C_{ND}}{C_P} \quad (1)$$

C_T and C_{ND} being the radioactive concentration in each region of interest and in the reference region, V_T and V_{ND} being the distribution volumes in each regions of interest and in the reference region, and C_P being the metabolite corrected plasma [^{18}F]altanserin. Cerebellum was used as reference region, as it represents non-displaceable uptake only (Pinborg et al. 2003).

Magnetic resonance imaging

As for the PET scannings, all MRI measurements were carried out between noon and 6 pm. Images were acquired on a 3T Trio scanner with an eight-channel head array coil (Siemens, Erlangen, Germany). Blood oxygen level dependent (BOLD) fMRI uses a T2*-weighted gradient echo spiral echo-planar (EPI) sequence with a repetition time of 2.5s, echo time of 26ms, flip angle of 90°, and 41 slices with a slice thickness of 3 mm and 25% gap between slices.

The EPI sequence was optimized for signal recovery in orbitofrontal cortex by tilting slice orientation from a transverse toward a coronal orientation by about 30° and the use of a preparation gradient pulse (Deichmann et al., 2003). A total of 128 whole-brain volumes were acquired in each of the two sessions (total 12.8 min). We additionally acquired a high-resolution 3D structural brain scan using a T1-weighted spin echo sequence (TI/TE/TR =800/3.93/1540 ms, flip angle 9°, 1 × 1 ×1 mm isotropic resolution).

Analysis of the fMRI data

Data were preprocessed and analyzed using SPM5 (Wellcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm/software/spm5). Images were realigned and normalized to MNI (Montreal Neurological Institute) stereotactic space using transformation parameters derived from segmentation of the structural MRI. The normalized images were smoothed using a symmetric 8-mm Gaussian kernel. None of the subjects had at any time head motions that exceeded 3mm (voxel size) in any direction. We tested for differences in head movement between the drug sessions by calculating the root mean square of the movement parameters in x, y and z direction and included the individual values in a repeated measures ANOVA with drug session and movement direction as within-subject factors.

The paradigm was analyzed in an event-related fashion with three event types defined at subject level, corresponding to presentation of neutral, angry, or fearful faces. Each event was modeled as a delta function with onset coinciding with the appearance of the cue. Covariates were then convolved with a canonical hemodynamic response function. A two-stage random effects model was created for each subject, modeling the fMRI runs of the ketanserin and control sessions. Each fMRI run was modeled with the three covariates described above together with a mean (constant) term over scans for each run in order to

model the main effects of runs. The within-subject model also included 40 nuisance regressors to account for variance caused by physiological noise, including heart beat (10 regressors), respiration (6 regressors), and head movements (24 regressors) (Glover et al., 2000; Lund et al., 2006).

Parameter estimates for each covariate were calculated and statistical parametric maps of the t-statistic (SPM{t}) resulting from linear contrasts of covariates were generated for each subject. Thus, we generated contrast images for the relative increase in BOLD signal induced by the emotional faces relative to neutral faces in both the control and ketanserin sessions.

At the group level, individual contrast images were entered into separate paired t-test models testing the difference in BOLD response of emotional faces relative to neutral faces in the ketanserin relative to the control session. Additional analog group level models were set up by substituting the control contrasts with the equivalent contrasts of the SSRI session.

We also computed one-sample t-tests for the emotion contrast images from the ketanserin session only, including average neocortical BP_P, time-dependent O_{KET} (O_{KET5} and O_{KET10}), and in order to look at the linear relationship between the two covariates we calculated the product (i.e., BP_P times O_{KET}). BP_P values were time corrected to comply with the delay between PET and fMRI scannings according to Erritzoe et al. (2009) This model enabled us to identify brain regions where ketanserin-induced changes in emotional face processing depending on the neocortical BP_p, O_{KET}, or the product of the two, and was performed for both O_{KET} covariates. In order to test for correlations with mood state, a separate analysis was performed using POMS factor scores (anger/hostility, vigor/activity, and fatigue/inertia) as covariates.

We used the psychophysiological interaction (PPI) method described by Friston et al. (1997) to identify ketanserin-induced changes in OFC connectivity during the processing of fearful faces that can be explained by O_{KET} , BP_p or an interaction between the two factors. In the first stage, we defined a spherical region of interest (ROI), 8mm in diameter and centered in the peak OFC region (MNI x,y,z = 4,38,−24) showing an attenuation of the BOLD response in the ketanserin session vs. control during perception of fearful faces and we extracted time-course of the BOLD response from this region. A PPI term was calculated by multiplying the estimated deconvolved time-course from the OFC ROI with the fear vs. neutral contrast. We then computed new subject-specific SPMs where the calculated PPI term and the time-course of the seed region were added as regressors to the initial first level subject model which included the three task regressors (neutral, angry and fearful faces). Individual contrasts based on the PPI term were entered in a second level multiple regression model with BP_p, O_{KET} (O_{KET5} and O_{KET10}), and the product of the two (i.e., BP_p times O_{KET}) as covariates. Linear contrasts were specified and SPMs based on one-tailed t-statistics were generated.

As general significance level, we used a threshold of $p<0.01$ on a voxel-wise level and considered clusters significant at $p<0.05$ after Family-Wise Error (FWE) correction for multiple non-independent comparisons. All imaging results are reported by the Z score and

stereotactic MNI coordinates of the regional maxima. We expected amygdala's neuronal activity to change directly in response to aversive faces. To define amygdala, we delineated spherical regions of interest (ROI) with a radius of 8 mm (the size of the smoothing kernel) centered in the maximum activation likelihood estimations from the Fusar-poli et al. (2009) meta-analysis for fearful faces. We first converted the estimated voxels for fear vs. neutral contrasts from Talairach to MNI space according to (Lancaster et al., 2007) and then used the resulting coordinates ([−23 −4 −15] and [22, −4, −20]) to perform FWE correction for the voxels using small volume correction (SVC) for fear contrasts as well as angry and aversive.

Analysis of task performance

Behavioral data were analyzed using SPSS (version 18, Chicago, Illinois, USA). Individual scores on mood questionnaires were analyzed using a three-way repeated measures ANOVA with the within-subject factors *session* (ketanserin versus control), *mood factors* of the POMS (6 levels), and *time* of assessment relative to fMRI (before versus after). Reaction time differences were assessed using a two-way repeated measures ANOVA with within-subject factors *session* (ketanserin versus control, or ketanserin versus SSRI) and *emotion* of the face stimuli (neutral, anger, fear). The Greenhouse-Geisser method was used to correct for non-sphericity if appropriate. Conditional on significant F-values in the ANOVA, post-hoc paired t-tests were performed. Error rates were analyzed using nonparametric Wilcoxon signed-rank tests, comparing each facial expression from the control session with the same facial expression from the ketanserin session. Behavioral data are given as mean ± standard deviation.

Results

Mood assessment

The effect of ketanserin on mood was evaluated by comparing POMS scores collected before ketanserin was given as well as right after completion of the fMRI session. Compared to the control session, acute ketanserin challenge had a specific effect ($F(1.7; 30.9)=44.1$; $p<0.001$). In the ketanserin session, participants reported significant decreases in vigor/activity (1.79±0.62 versus 1.36±0.80, $t(21)=4.6$; $p<0.001$), and increases in fatigue/inertia (0.5±0.51 versus 0.91±0.61, $t(21)=-3.7$; $p=0.001$) compared to the responses prior to ketanserin infusion. Conversely, in the control sessions the scores for anger/hostility were significantly lower at the end of the session relative to scores at the beginning of the session (0.26±0.23 versus 0.20±0.13), $t(18)=2.6$; $p=0.02$). None of these mood changes correlated significantly with the fMRI activation patterns.

Task performance

Mean RT was longer when subjects judged the gender of a fearful or angry face relative to a neutral face, showing that gender-judgment was delayed when faces showed an aversive emotion ($F(1.8; 38.7) = 16.53$; $p<0.001$). The delay in RT induced by an aversive facial emotion was comparable in size with or without ketanserin treatment (Figure 1). Mean RT was longer for fearful than for neutral faces in both the ketanserin session ($t(23)=2.80$; $p=0.011$) and control session ($t(23)=6.35$; $p<0.001$). The same was true for RT's when

comparing trials with angry faces versus neutral faces for both ketanserin session (t(23)=2.47; p=0.022) and control session (t(23)=3.,53; p=0.006). In the control session, mean RT's were also longer in trials with fearful compared to angry faces (t(23)=−2.77; p=0.011).

Ketanserin treatment prolonged mean RT, with an overall increase of approximately 5% compared to the control session (ketanserin session: 774ms±118.6, control session: 741ms ±105.5, F(1;23)=18.10; p=0.001). Post-hoc paired t-tests showed this increase was consistent across all facial expressions (p<0.001). Ketanserin had the same effect on RT responses to neutral and aversive faces (Fig. 2) also the ANOVA revealed no interaction between facial emotion and intervention. The relative increase in RT in the ketanserin session correlated with the ketanserin-induced decrease in self-report on vigor (Pearson's r=0.387, p=0.037), whereas no correlation was found with the reported increase in fatigue (Pearson's r=0.05, p=0.411).

Error rates did not differ between control and ketanserin session for any of the three facial expressions (Neutral faces: p=0.186. Angry faces: p=0.903. Fearful faces: p=0.613). This shows that the overall slowing of RT found during the ketanserin session was not paralleled by a change in accuracy.

fMRI results

Judging the gender of angry or fearful faces, relative to neutral faces, consistently activated the expected set of brain regions **involved in face and emotional processing** (Fig. 3, Table 1). Amygdala showed a bilateral increase in neural activity when responses to angry and fearful faces were pooled together (Fig. 3A) or considered separately (Fig. 3B and 3C). Additional bilateral clusters in the fusiform gyrus and visual cortex displayed increases in activity when angry or fearful faces were presented relative to neutral faces (Fig. 3A-C).

Effect of ketanserin on face processing

Orbitofrontal cortex—Ketanserin attenuated the regional neuronal response to aversive facial emotions relative to the control session in medial OFC (Fig. 4; peak reduction at x,y,z=4,40,−18, Z=4.12, p_{FWE}=0.005). This attenuation was mainly driven by a reduced responsiveness of the OFC to fearful faces (peak reduction at x,y,z=4,38,−24; Z=4.03, p_{FWE}<0.001). Inspection of the regional response profile in the OFC revealed an interaction with ketanserin having an opposite effect on OFC activity depending on the emotional content. Ketanserin attenuated the OFC response to aversive faces, especially fearful faces (Fig.4D). The ketanserin-related effects on OFC activity were not correlated with inter-individual variations in neocortical BP_P. The region in OFC where ketanserin reduced the response to fearful faces also had a stronger influence on the coupling with left amygdala, when the interaction of the two covariates BP_P and O_{KET} (i.e. BP_P times O_{KET}) was considered. The strength of OFC-to-amygdala connectivity correlated positively with BP_P times O_{KET}, meaning that the higher number of 5-HT_{2A} receptors blocked by ketanserin, the stronger the coupling between OFC and left amygdala for both O_{KET5} and O_{KET10} (peaked at MNI coordinates; O_{KET5}: x,y,z=−22,4,−18, Z=3.92, p_{FWE}=0.007. O_{KET10}: x,y,z= −20,4,−18, Z=4.38, p_{FWE}=0.002,, corrected within the amygdala ROI, Fig. 5A and B). This

also held true when excluding the two extreme values from the analysis. Fig 5B shows the correlation analysis between the BOLD response in left amygdala and the BP_p times O_{KET10} interaction. There was no significant difference in the magnitude of head movements during the fMRI acquisitions between the control and ketanserin sessions (F(1;22)=0.388;p=0.540).

Amygdala—Ketanserin did not change the overall amygdala response to aversive faces, and this was also the case when considering inter-individual variations in either neocortical BP_P or any of the O_{KET} covariates (O_{KET5} and O_{KET10}). When looking at the linear relationship between the two covariates BP_P and O_{KET} (i.e. BP_P times O_{KET}) we saw a trend towards an increase in activation in left amygdala when processing fearful or aversive faces when using the O_{KET5} covariates (peak modulation for fearful faces at −24,−6,−8, Z=2.76, p_{FWE} = 0.06, for aversive faces at −26,−6,−10, Z=2.71, p=0.06).

Comparison between the ketanserin and SSRI sessions—In order to control for non-specific effects that could have been induced by administering ketanserin we did a post hoc validation of the observed changes by contrasting the ketanserin session with the SSRI session acquired in the same subjects following a similar IV administration protocol.

Mean RTs from the SSRI session did not differ significantly to the control session (Control session: 741ms±105.46, SSRI session: 745±110.9, F(1.0; 21)=16.622; p=0.760) thus the longer mean RTs found in the ketanserin session compared to control session were also found in the SSRI session (Ketanserin session: 774ms±118.6, SSRI session: 745±110.9, F(1.0; 21)=4.078; p=0.056).

Compared to the ketanserin session, SSRI data confirmed our initial results showing decreased BOLD response in OFC during aversive (peak reduction at x,y,z=2,34,−26; Z=4.30, p_{FWE}<0.001) and fearful face presentation (peak reduction at x,y,z=2,34,−26; Z=3.70, p_{FWE}<0.001).

Discussion

Acute 5-HT_{2A} receptor blockade with ketanserin modulated emotional face processing in the medial orbitofrontal cortex and in amygdala, leading to two main findings. First, ketanserin suppressed the neural response to fearful faces in medial OFC. Second, the more 5-HT_{2A} receptors that were blocked, the stronger the functional coupling between medial OFC and left amygdala in response to fearful faces.

Effect of 5-HT_{2A} blockade on face processing in orbitofrontal cortex

Acute pharmacological 5-HT_{2A} receptor blocking reduced the regional response of the OFC to fearful and to a lesser degree angry faces (Fig. 4) supporting that 5-HT_{2A} receptor signaling is involved in cortical processing of fearful facial expressions. This result corroborates the notion that OFC provides an interface between cognitive and emotional functions (Paulmann et al., 2010). The ability to change behavior based on facial expressions relies partly on OFC (Kringelbach and Rolls, 2003). Patients with uni- or bilateral OFC lesions show an inability to respond appropriately to other people's emotions and an impaired recognition of emotional features in face and voice (Hornak et al., 1996,

2003). This is probably not related to a failure to recognize facial expressions *per se* but is rather caused by a deficit in using this social information to guide appropriate actions or decisions (Willis et al., 2010).

The OFC did not express an emotion-specific response pattern in the control session, when participants judged the gender of neutral, angry or fearful faces. The lack of a specific response to aversive as opposed to neutral faces suggests that OFC automatically processes a wealth of face features relevant to social interaction including face identity, gender, and emotional state. Blocking the 5-HT_{2A} receptors attenuated the OFC response to fearful faces and enhanced the response to neutral faces (Fig. 4). This differential effect of 5-HT_{2A} receptor blockade indicates a shift in preferential processing towards non-threat related face features in OFC.

The distribution of the 5-HT_{2A} receptors in the cerebral cortex would allow for such a shift in the relative weight of complementary processing routes within OFC: Immunocytochemical studies in the cortex of macaques have shown that excitatory 5-HT_{2A} receptors are not only expressed in the apical dendritic field proximal to the pyramidal cell soma, but also in GABAergic interneurons known to specialize in the perisomatic inhibition of pyramidal cells (Jakab and Goldman-Rakic, 1998, 2000). A possible scenario is that acute 5-HT_{2A} blockade reduced excitatory signaling in OFC circuits which compute fear related information while increasing neural processing in circuits processing other social stimuli features.

The individual change in emotion-related activity in OFC during acute 5-HT_{2A} receptor blocking was not correlated with individual BP_p, O_{KET} or the product of the two. These findings suggest a non-linear relationship between 5-HT_{2A} receptor related signaling and the neural responsiveness of OFC to fearful facial expressions, with a rapid attenuation of the response to threatening facial features as a result of even a relatively small reduction of 5-HT_{2A} receptor signaling.

Effect of 5-HT_{2A} blockade on OFC-amygdala connectivity

The same OFC region in which ketanserin modified facial expression-related neuronal activity also showed a stronger correlation with neural activity in left amygdala during the processing of fearful faces. The ketanserin-induced increase in functional connectivity between medial OFC and left amygdala was related to how many 5-HT_{2A} receptors had been blocked. The more 5-HT_{2A} receptors were blocked, the stronger was the increase in functional connectivity between OFC and left amygdala. Since Stein et al (2007) showed that OFC exerts inhibitory control over amygdala, we now propose that the OFC-to-amygdala projections are under control of orbitofrontal 5-HT_{2A} related neurotransmission and blocking of 5-HT_{2A} receptors enhances the impact of OFC on amygdala responsiveness to fearful faces. Experimental evidence from animal and human studies supports our hypothesis. Forster et al. (2006) showed that in rats, fear correlated negatively with 5-HT levels in OFC. Moreover, Fisher et al (2009) showed an inverse relationship between a greater level of 5-HT_{2A} receptors in OFC, and reduced amygdala activity, as well as a functional coupling between the habituation of amygdala responses with prefrontal regulatory regions, supported by Passamonti et al. (2012) who found an altered connectivity

between amygdala and PFC during acute tryptophan depletion. We infer that the efficiency of 5-HT_{2A} receptor blocking (as indexed by the proportion of blocked receptors) had the strongest impact in individuals with a high density of neocortical binding sites as the ketanserin-induced effect on OFC-to-amygdala coupling only became evident when the product between the magnitude of receptors (BP_P), and the relative proportion of 5-HT_{2A} receptors blocked by ketanserin (O_{KET}) was considered. These results suggest an important general implication showing that individual variations in regional receptor binding might determine individual susceptibility to drug-induced manipulation of receptor function. If this observation can be replicated in future studies, it will have a large impact on the current view of assessment of receptor drug occupancy as the single most important measure for prediction of drug efficacy.

Methodological considerations

Ketanserin caused a general slowing in RT in the gender-judgment task. This effect on RT accords with the known effects of ketanserin. When given orally, 20 mg of ketanserin may reduce sustained attention (Wingen et al., 2007) or alertness (Koudas et al., 2009), although the clinical effect of ketanserin on arousal is not profound (Herrmann and Baumgartner, 1986). Further, at this dose, ketanserin does not significantly effect cerebral blood flow (Olsen et al., 1992). Participants reported a decrease in vigor and increased fatigue, confirming the known effects of the drug. Importantly, the relative RT cost associated with the gender-judgment of angry or fearful faces relative to neutral faces was not altered by ketanserin. Moreover, individual changes in RT or mood state did not correlate with drug-induced changes in task-related activation or connectivity as revealed by fMRI. Therefore, we argue that effects of ketanserin on task performance and mood state did not account for the observed changes in activation patterns.

Receptor occupancy was not continuously monitored in each individual. Rather, the receptor occupancy was estimated on the basis of time elapsed from the beginning of ketanserin infusion. However, even if the absolute occupancy levels were not precise, the occupancy term nonetheless approximates the normal distribution required for statistical parametric mapping. Further, the robustness of the estimates was supported by the use of two different estimates for the time-dependent occupancy. Another potential limitation of the study was that the pharmacological challenge was not double-blinded. A placebo control would be advantageous in several respects, and prevent the need to consider placebo effects or effects of IV versus no manipulations. However, when the study was designed, and approved by the ethics committee, it was felt that a full placebo control of the oral ATD solution and the IV infusion for ketanserin and SSRI sessions, would be too excessive for a within-subject design. Given the heterogeneity of 5-HT_{2A}, and other genetic or personality factors relevant to inhibition, a between subjects design might have been compromised differently, by uncertainty over the cause of differences between groups and imperfect matching. The no-drug condition without blinded placebo IV/oral solutions was seen as an acceptable choice. Although subjects were made aware of potential side effects within the study, they were not made aware of the specific differences between the interventions. However, the drug effects on neural activity were specific to fearful relative to neutral faces and the changes in amygdala activity depended on the magnitude of 5-HT_{2A} receptor blockade and the

individual 5-HT_{2A} receptor density. Given that the volunteers had no prior information about expected effects of the drug given, nor the degree of their individual blocking, these specific effects cannot be accounted for by a simple placebo effect or a lack of blinding. Furthermore, we confirmed the effects of ketanserin by contrasting against both the control session and the SSRI session, the latter sharing the intravenous infusion.

One must also consider potential effects of ketanserin arising from receptors other than 5-HT_{2A}. Ketanserin has some affinity for the 5-HT_{2C}, α1 adrenergic and histamine receptors (Korstanje et al., 1986). However, the affinity of ketanserin is approximately 14-fold higher for the 5-HT_{2A} relative to the 5-HT_{2C} receptor (Glennon et al., 2002). Our hypothesis was entirely based on ketanserin's modulation of the serotonergic system by blocking the 5-HT_{2A} receptors. We therefore specifically studied the interaction between the estimated 5-HT_{2A} receptor blockade and 5-HT_{2A} receptor density as measured by PET. We consider therefore unlikely that α1 adrenergic, histamine, or 5-HT_{2C} receptor pharmacological effects could have a significant impact on our observations.

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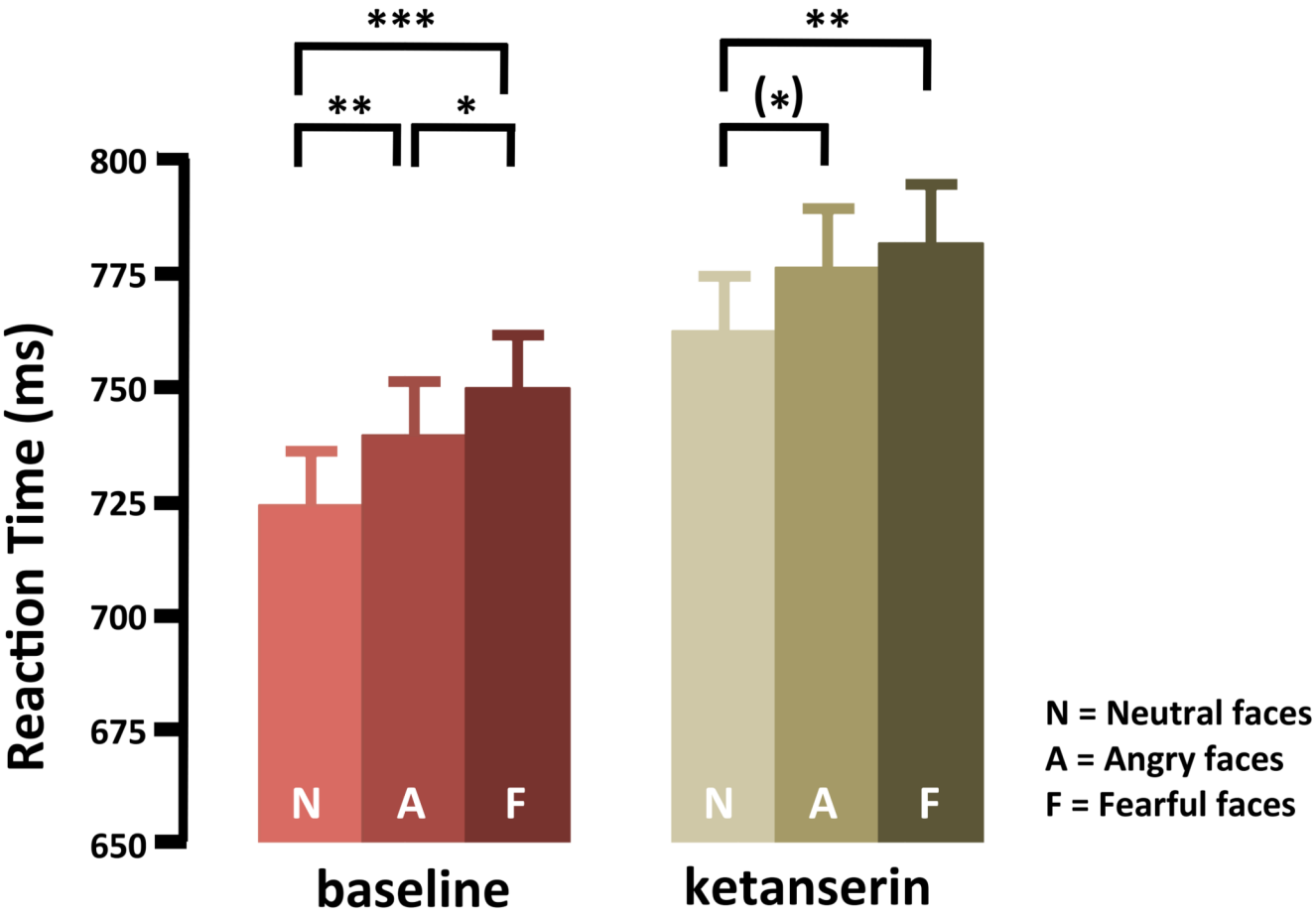

Figure 1.

Estimated levels of 5-HT_{2A} receptor blocking over time for each subject shown for both $Oket_5$ (triangles) and $Oket_{10}$ (squares).

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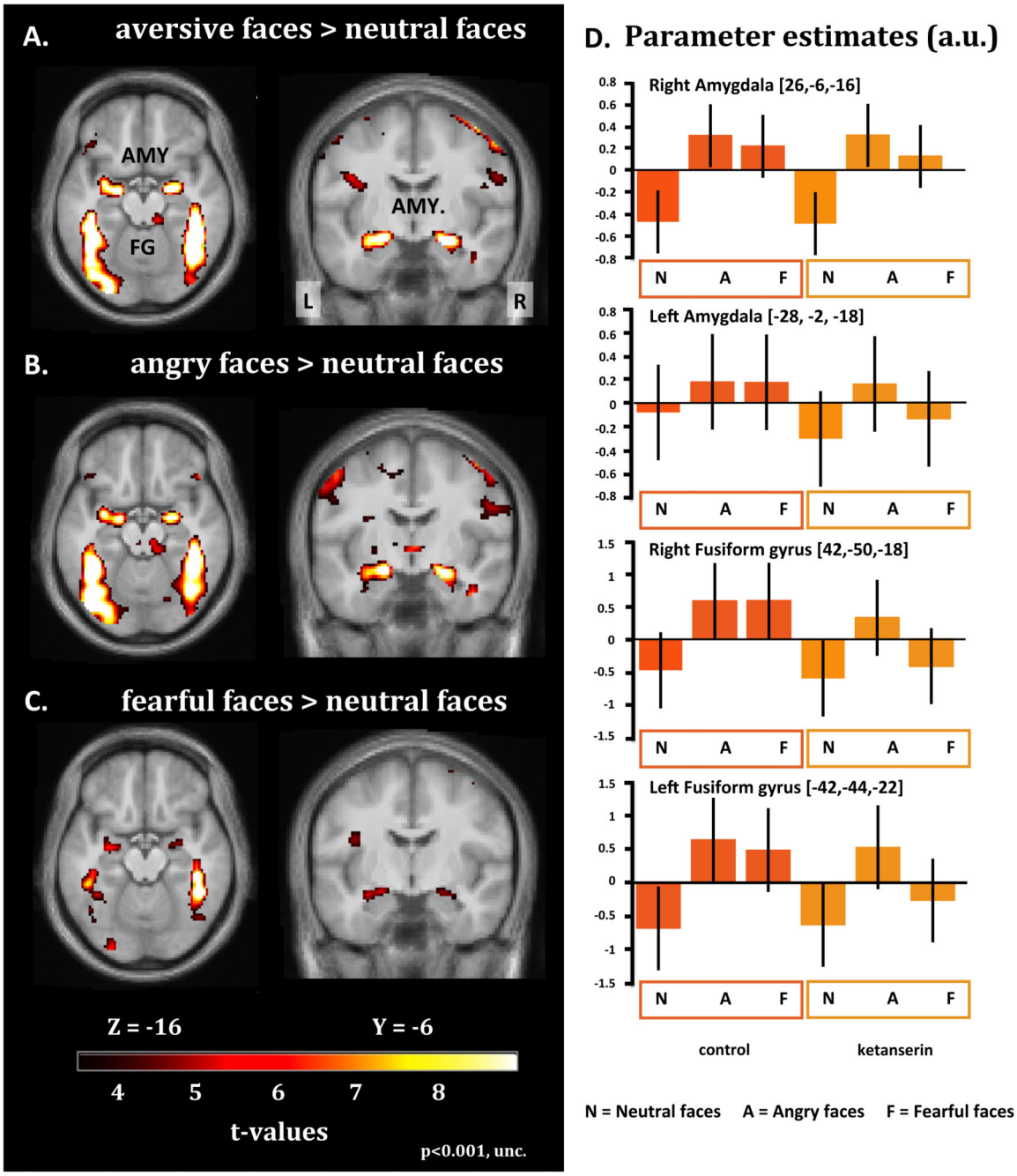

Figure 2.

Mean reaction time recorded during the gender-judgment task based on facial expressions in the control and ketanserin fMRI sessions. Control session; neutral faces: 724 ±23.55, angry faces: 739±22.23, fearful faces: 750±23.20. Ketanserin session; neutral faces: 762±23.99, angry faces: 776±23.99, fearful faces: 781±25.70, data are presented as mean ± SEM. (*)=p<0.1, *=p<0.05, **=p<0.01, ***=p<0.001. Faces with an aversive emotion delayed the gender-judgment in both sessions relative to neutral faces. Compared to the control session without medication, ketanserin treatment was associated with longer RT.

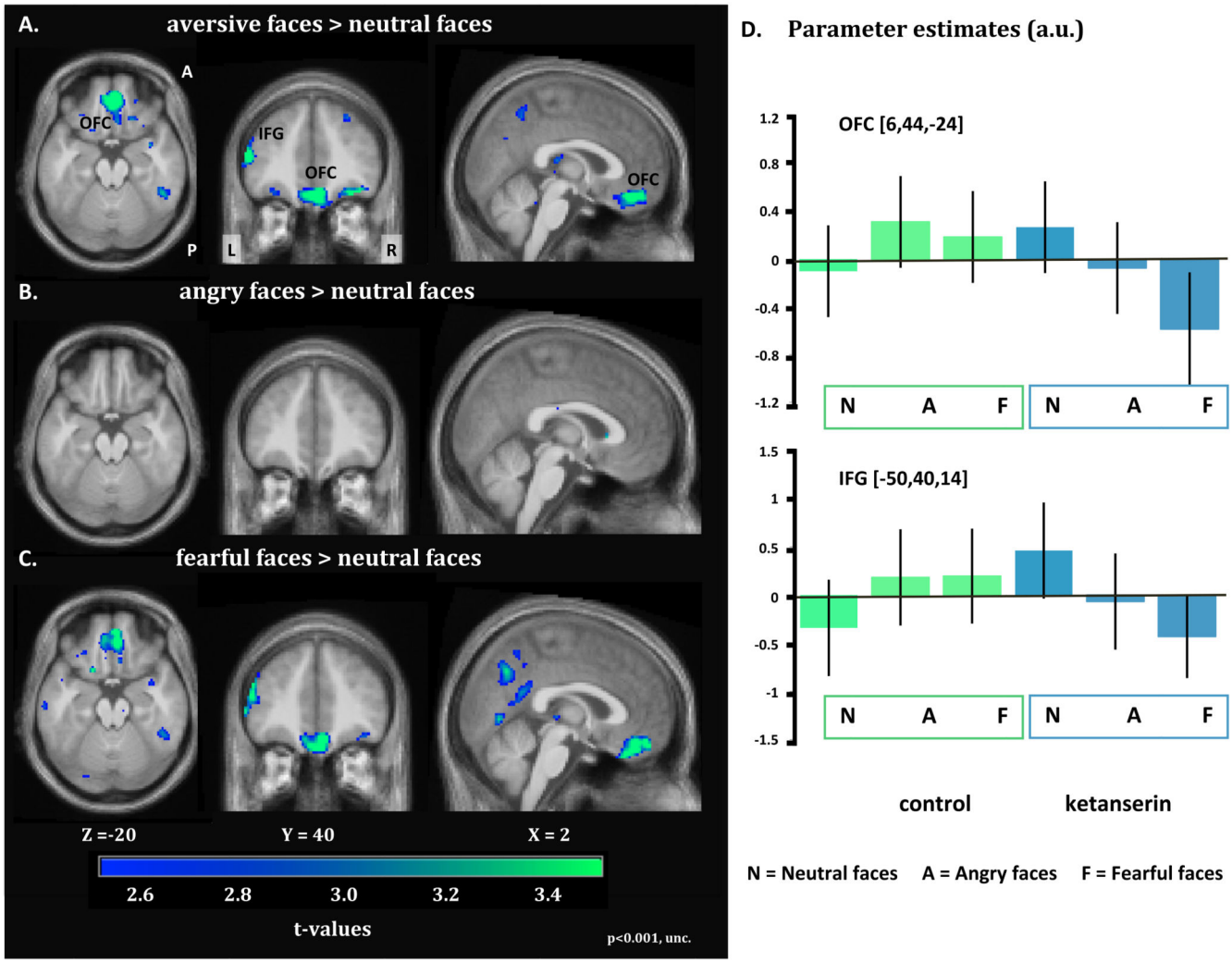

Figure 3.

Statistical parametric maps (SPMs) showing brain areas, which are activated by aversive facial expressions relative to neutral faces, as reflected by an increase in BOLD signal. The SPMs are color coded in yellow and red indicating increases in activity, and are thresholded at $p<0.001$ (uncorrected). The upper left panel gives the activation maps for the contrast aversive faces > neutral faces (A). The middle and lower panel on the left display the activation maps for the two facial emotions separately. Angry faces > neutral faces (B); fearful faces > neutral faces (C). The bar graphs presented in the right panel give statistical estimates (arbitrary units) of face related activity levels in the amygdala and fusiform gyrus for the control session (left column) and ketanserin session (right column) (D). The parameter estimates are taken from the regional maxima showing the strongest increase in regional activity for aversive faces relative to neutral faces. The error bars represent the 90% confidence intervals of the mean.

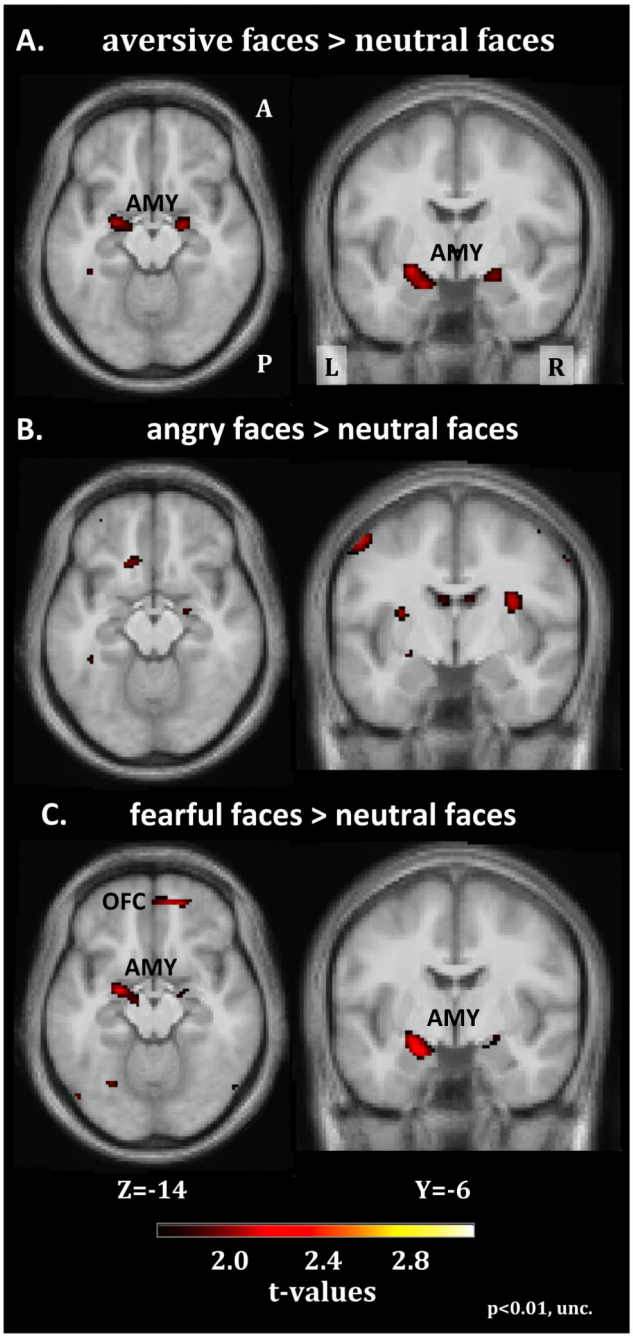

Figure 4.

Statistical parametric maps (SPMs) showing brain regions, which show a decrease in activation for aversive face expressions relative to neutral faces in the ketanserin session as opposed to baseline (control session). The SPMs are color coded in green and blue indicating decreases in BOLD signal and are thresholded at $p<0.001$ (uncorrected). The upper left panel depicts decreases in regional responsiveness to aversive (angry, fearful) faces under ketanserin treatment (A). The middle and lower panel on the left present the corresponding SPMs for angry (B) and fearful (C) faces. The bar graphs plot the statistical

estimates (arbitrary units) of face related activity levels in the amygdala and fusiform gyrus for the control session (baseline) and ketanserin session (D). The parameter estimates are taken from the regional maxima showing the strongest decrease in the regional response to aversive faces relative to neutral faces. The error bars equal the 90% confidence intervals of the mean.

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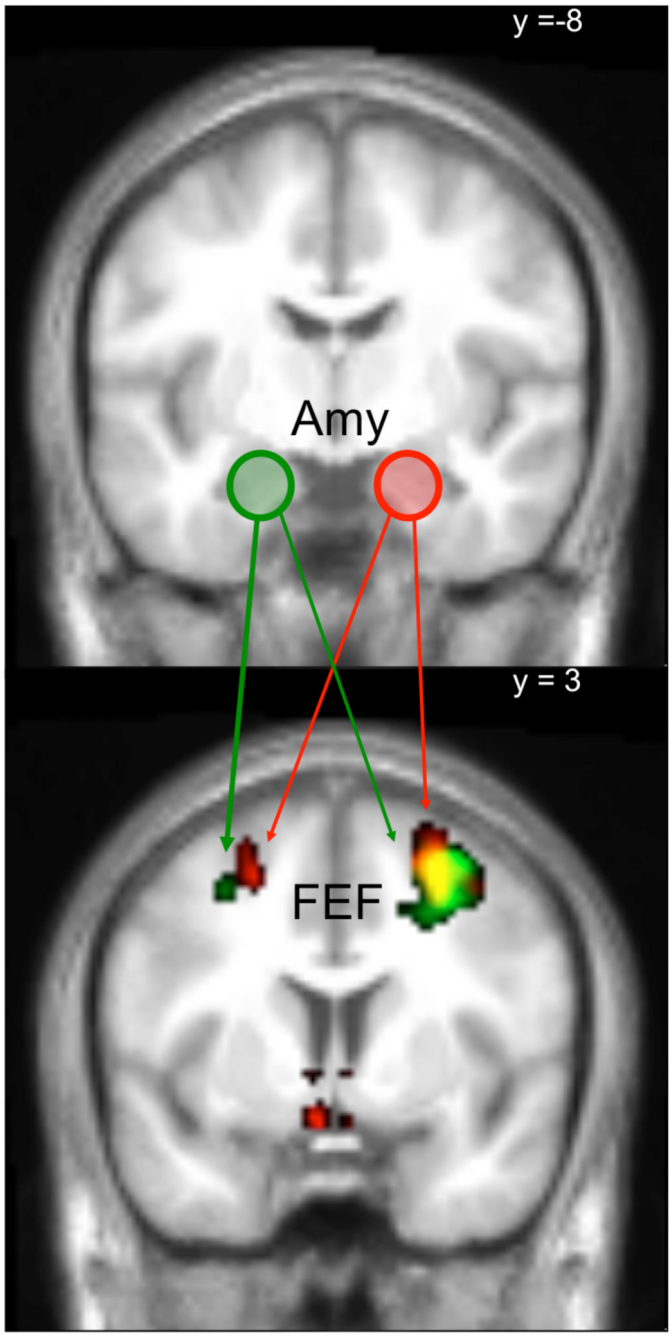

Figure 5.

The figure summarizes the results of the PPI connectivity analysis exploring ketanserin related changes in OFC-amygdala connectivity during fear events (for details see methods section). (A) Maps showing the changes in coupling between the OFC seed region and the amygdala following acute 5-HT_{2A} blockade. (B) Positive correlation between the OFC-amygdala connectivity and the interaction between neocortical 5-HT_{2A} receptor binding (BP_p) and ketanserin-induced 5-HT_{2A} receptor occupancy (O_{KET10}). The higher the O_{KET} and the higher the neocortical BP_P, the stronger was the individual increase in connectivity

between the OFC and left amygdala. Values are mean normalized. The extent threshold of the SPMs is set at $p<0.01$ (uncorrected).

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Table 1

Coordinates and Z-scores of the voxel showing a peak increase in BOLD signal when viewing aversive (angry/fearful) faces relative to neutral faces.

		Aversive > Neutral				Fear > Neutral				Angry > Neutral			
		Area		**[x,y,z]**	**Z-score**	**Area**		**[x,y,z]**	**Z-score**	**Area**		**[x,y,z]**	**Z-score**
A	**Main effect of task**												
		Amygdala	R	24, −8, −16	6.52	Amygdala	R	30, −4, −20	4.48	Amygdala	R	24, −6, −16	6.29
			L	−20, −10, −14	6.78		L	−24, −10, −14	4.65		L	−18, −10, −12	6.37
		Fusiform Gyrus	R	40, −52, −14	inf.	Fusiform Gyrus	R	44, −44, −16	6.80	Fusiform Gyrus	R	44, −38, −20	7.60
			L	−40, −66, −12	6.80		L	−32, −56, −12	4.22		L	−42, −54, −14	inf.
		Caudate Nucleus	R	12, 4, 16	5.52	Caudate Nucleus	R	8, 8, 10	4.91	Thalamus	L	−18, 28, 6	4.35
			L	−12, 0, 22	4.31		L	−6, 4, 10	5.78				
B	**Main effect of drug**												
		OFC	R	4, 40, −18	4.16	OFC	R	4, 38, −24	3.71				
		Inferior Frontal Gyrus	L	−50, 34, 22	3.38	Inferior Frontal Gyrus	L	−48, 40, 16	3.11				
C	**BP_p*KEToc**												
		Amygdala	R	20, −8, −14	2.78	Amygdala	R	26, 4, −26	2.51				
			L	−24, −6, −12	2.51		L	−20, −6, −18	3.08				
						OFC	R	10, 50, −18	3.35				