Short-term duloxetine administration affects neural correlates of mood-congruent memory

Indira - Tendolkar, Guido A van Wingen, Maren Urner, Robbert Verkes, Guillén Fernández

Short-term duloxetine administration affects neural correlates of mood-congruent memory

Running title: Duloxetine affects mood-congruent memory

Indira Tendolkar[1,2,3], Guido van Wingen[1,,4,5] ,Maren Urner[1,6], Robbert Jan Verkes[2], Guillén Fernández[1,4]

Affiliations:

[1] Donders Institute for Brain, Cognition and Behaviour, Center for Cognitive Neuroimaging, Radboud University Nijmegen, Nijmegen, The Netherlands

[2] Department of Psychiatry, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

[3] LVR-Hospital Essen, University Duisburg-Essen, Department of Psychiatry and Psychotherapy, Essen, Germany

[4] Department for Cognitive Neuroscience, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

[5] Department of Psychiatry, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

[6] Institute of Cognitive Neuroscience, University College London, 17 Queen Square, London WC1N 3AR

Corresponding author:
Indira Tendolkar,
Radboud University Medical Centre, Department of Psychiatry
P.O. Box 9101, 6500 HB Nijmegen, The Netherlands
Telephone (+31) (0)24 3613490
Fax (+31) (0)24 3540561
Email: i.tendolkar@psy.umcn.nl

Abstract

It is unknown how antidepressants reverse a mood-congruent memory bias, a cognitive core factor causing and maintaining depression.

Using a double-blind, placebo-controlled cross-over design, we investigated the effect of a short-term treatment (14 days) with the dual reuptake inhibitor Duloxetine on neural correlates of mood-congruent and mood-incongruent memory formation and retrieval in healthy volunteers who underwent a sad mood induction procedure.

Duloxetine did not affect acute mood state or memory performance, but interacted with brain processes mediating mood-congruent memory. It decreased activity related to successful memory formation for mood-congruent and incongruent items in a set of brain regions comprising putamen, middle frontal gyrus as well and middle and anterior cingulate cortex. Duloxetine increased amygdala activity related to successful memory retrieval for mood incongruent items specifically.

Here we show that short-term administration of duloxetine affects neural correlates of emotional memory formation and retrieval in a set of brain regions whose processing is related to affective state and its regulation. While duloxetine suppressed neural correlates of emotional memory formation in general, it enhanced amygdala processes associated with mood-incongruent memory retrieval specifically. This pattern of results shows how an antidepressant may reduce emotional memory formation and reverse mood-congruent processing biases at retrieval.

Keywords

depression, duloxetine, fMRI, amygdala, emotional memory, mood congruency

Introduction

The interaction between mood during learning and the emotional valence of an event leads to a mood-specific memory enhancement (Leppänen, 2006). While this interaction may support adaptive behavior (McGaugh, 2004), persistent sad mood can lead to negative learning schemes (Teasdale, 1983). This so-called mood-congruent memory bias is one of the cognitive trait factors causing and maintaining depression (Hasler *et al.*, 2004). Negative biases leading to dysfunctional attitudes have been related to chronic reductions in extracellular serotonin (Meyer et al., 2003; Bhagwagar et al., 2006) suggesting a potential reversion by antidepressants. The question arises how antidepressants affect these cognitive processes other than attenuating negative affect.

Investigating the antidepressant effect on mood-congruent memory is sparsely restricted to the behavioral effects in acutely depressed patients whereby a single administration of the norepinephrin reuptake inhibitor reboxetine reversed an initial reduction of memory for positive faces (Harmer *et al.*, 2008). In depressed patients with euthymic mood antidepressants may even specifically affect memory formation and retrieval (Norbury *et al.*, 2009) though this study did not dissociate neural activity during learning and retrieval as a function of (subsequent) memory success.

On a broader level, earlier positron emission tomography studies have shown that a single administration of d-fenfluramine, a serotonine agonist, induced a reduction in negative interpretation bias, that was possibly related to lower levels of extracellular serotonin as measured by the 5-HT_2 binding potential in prefrontal cortex (Meyer et al., 2003) and also parietal and occipital cortex (Bhagwagar et al., 2006). FMRI studies in depressed patients

have shown that antidepressants acting via serotonin reuptake inhibition reverse increased neural processing of negative stimuli (Fu *et al.*, 2004) and decreased processing of positive stimuli (Fu *et al.*, 2007) in brain regions mediating affective regulation or higher order visual processing. While antidepressant effects in a treatment study of patients can be a mere consequence of elevated mood, fMRI studies in healthy individuals have shown that single-dose and short-term administration of an antidepressant alter affective processing and its underlying neural circuitry (Harmer *et al.*, 2006; Murphy *et al.*, 2009; Norbury *et al.*, 2007). Finally, a study by Lopez-Solá and colleagues (2010) showed that the antidepressant duloxetine altered the neural correlates of pain processing in depressed patients already after 1 week when clinical effects were still modest. Hence, while different types of antidepressants appear to modulate affective processing directly, the effect of the current mood state on these modulatory effects particularly during mood-congruent memory is unknown.

Mood-congruent memory studies in acutely depressed patients (Hamilton and Gotlib, 2008; van Wingen *et al.*, 2010) or recovered patients under sad mood induction (Ramel *et al.*, 2007) point to the amygdala as an important mediator. The amygdala plays a central role in various aspects of affect processing and modulates the hippocampus during emotional memory (Dolcos, *et al.*, 2005). The hippocampus plays an important role in the pathogenesis of depression (Frodl and Mac Queen, 2011) and is a major target of antidepressant action (for example Warner Schmidt and Dunan, 2006). Hence, amygdala and hippocampal activity should be specifically investigated when tackling the effects of antidepressants on mood-congruent memory.

Experimental sad mood induction allows assessing neural correlates of emotional memory while aligning subjects in a reduced emotional state (Lewis et al., 2005; Fitzgerald et al., 2011). It can be combined with investigation of antidepressant effects recruiting healthy subjects in a within subject crossover design which would not be feasible in a patient sample. Therefore, we combined sad mood induction with event-related fMRI to probe antidepressant effects on emotional memory processes in subjects who feel sad. Dissociating the antidepressant effects on successful and unsuccessful memory processes during encoding and retrieval further elucidates how such an antidepressant can remediate cognitive biases by affecting specific mnemonic processes. We specifically assessed neural correlates of memory formation and retrieval of emotionally positive and negative stimuli allowing us to dissociate antidepressant effects on mood-congruent (i.e. effects related to negative stimuli) and mood-incongruent (effects related to positive stimuli) memory.

Both serotonergic and noradrenergic antidepressants have been shown to remediate cognitive biases suggesting a final common pathway for this effect (for further discussion of this issue see also Harmer et al., 2009). We also did not focus on specific antidepressant effects of either serotonin reuptake or noradrenalin reuptake inhibitors on mood-congruent emotional memory processing and therefore used the dual reuptake inhibitor duloxetine (Frampton and Plosker, 2007; Gupta et al., 2007). In a setting more informative for treatment (Katz et al., 2004) we applied Duloxetine a short-term administration (two weeks) in a randomized double-blind, placebo-controlled cross-over design.

Materials and Methods

Subjects

Eighteen healthy subjects (8 male) with a Body Mass Index between 18.5 and 25 and between 18 and 50 years of age (mean age 26 ± 7 years) participated in this randomized double-blind placebo-controlled, cross-over study approved by the local ethics committee (CMO region Arnhem-Nijmegen, The Netherlands). Participants were recruited via advertisement and screened approximately one week before entering the trial.

Screening

Before screening, subjects were informed about all procedures and risks and then signed informed consent. Subjects underwent a general physical examination (including neurological assessment) including evaluation of medical history to exclude subjects with a neurological illness or general medical condition that potentially could affect the outcome of the trial. Screening for current or lifetime psychiatric illnesses was done with the Mini International Neuropsychiatric Interview (MINI (Sheehan et al., 1998)) to exclude subjects fulfilling any of the diagnoses. Medically relevant abnormalities in the ECG or laboratory parameters (general hematology and blood chemistry) taken at screening were regarded as exclusion criteria. Positive drug/alcohol and pregnancy screenings, taken at each measurement point, were additional exclusion criteria. Further exclusion criteria were a known hypersensitivity to duloxetine or contra-indications for duloxetine (hepatic impairment, severe renal impairment with a GFR < 30ml/min) as well as a history of prescribed medication within three months prior to the start of this trial except for oral contraceptives and paracetamol.

Drug intervention

After screening subjects were randomly assigned to two groups either starting with a morning dose of a capsule containing placebo or 60 mg duloxetine for 14 consecutive days. Treatment periods were separated by a washout period of at least 14 days (range: 14-42 days; mean ± SD: 21 ± 10 days). Functional MRI measurements were taken on the final day of each drug or placebo period. Serum duloxetine levels were assessed by a 10 ml venous blood sample collected in an ethylenediamine-tetraacetic acid (EDTA) anticoagulant tube around 11 AM on each scanning day. Samples were centrifuged for 10 min at 3500 rpm at room temperature. The separated plasma was kept in a labeled plastic tube in the freezer at a temperature of -20 C° until the end of the study. After study deblinding, the samples from the duloxetine session were selected for serum analysis. High performance liquid chromatography (HPLC) was used to measure duloxetine levels (10-100 µg/l) with prothiaden (100 µg/l) as internal standard. The HPLC system consisted of a Waters 1515 Isocratic Pump, delivering the mobile phase at a flow rate of 1.1 ml/min and an Inertsil ODS-3 5µ, 50 x 4.6 mm (Alltech: GL815ODS346) separation column, heated to 40 C°. Duloxetine plasma levels ranged between 7 and 247 µg/l (mean 48 µg/l).

Experimental procedure

Assessment of emotional state: The assessment of the emotional state was done before and after scanning as well as before and after mood induction as described below. Before scanning we assessed state anxiety[21] (Dutch version of the STAI) and depressive symptoms (Dutch version of the BDI (Van der Does, 2002)) as well as an overall mood rating by means of the Dutch version of the shortened Profile of Mood States (Wald and Mellenbergh, 1990) before and after scanning.

Negative mood induction procedure: Based on a previous study (Kernis *et al.*, 1997), we induced negative mood by asking subjects to watch movie fragments that were taken from the American drama film "Sophie's Choice" (Pakula 1982). Prior to the first encoding session, subjects watched an initial film segment of 12 minutes. Subjects were told that they would be watching a sad film clip, and instructed to use the situation and emotions seen in the movie to put themselves in as strong mood as possible. Thereafter, they underwent two study phases, each lasting 15 minutes. Both encoding sessions were followed by two further movie fragments (lasting ~5min) of the same movie to boost the sad mood using the same instructions. Film fragments of equal length were interspersed between the four consecutive retrieval sessions each lasting 15 minutes. Subjects rated their current mood on a computer-based rating visual analog scale (ranging from -10 to 10) before and after each of the film fragments

Memory paradigm: For an overview of the experimental design, see figure 1. During scanning participants completed a memory task, which was divided in two encoding and four test phases as mentioned before. Stimuli consisted in total of 240 emotional scenes displaying one or more humans and were taken from a pool of positive and negative pictures which had been rated during a behavioral pilot study (five-point scale ranging from 'emotionally positive' to 'emotionally negative'; mean valence rating of negative pictures was ≤ 2 and mean valence rating of positive pictures was ≥ 4). Half of the pictures were taken as study items (including 120 positive and 120 negative pictures) and the other half as lures during test. This assignment was counterbalanced across the factors test phase (i.e. which half was taken as study items and which half as lures for the test phase) and gender. The content of positive and negative pictures (i.e. individual or group, child or adult, male or female person(s)) was pseudorandomly distributed across stimulus sets. Pictures during study and test were presented for 0.5 s with a jittered interstimulus interval

of 3.7 – 4.7 s. At study, participants were instructed to memorize the 120 positive and 120 negative photographs, which were presented sequentially and randomly intermixed in two encoding sessions separated by mood-induction as outlined above. To assure that subjects were processing the stimuli conceptually, they had to make perform an emotional valence decision task during the encoding phase.

At test, subjects were required to recognize the old and reject the same amount of randomly intermixed new photographs (i.e. 480 pictures in total). The retrieval phase was subdivided into four sessions to allow for continuous mood induction as described above. In keeping with previous studies and reducing the number of guesses during old/new recognition (Tendolkar *et al.*, 2008; Weis *et al.*, 2004), participants were encouraged to make a decision between old and new, but also had the option to make an unsure decision.

Image acquisition: MRI scans were collected using a Siemens (Erlangen, Germany) Avanto 1.5 Tesla MRI scanner equipped with a CP head coil. We obtained 326 T2* weighted BOLD images during the task for each scan session (gradient echo EPI, TE/TR: 35/2340 ms, flip angle: 90°, FOV: 212 mm, matrix size: 64*64, 3.5 mm slice thickness, 0.35 mm slice gap, 32 ascending slices). High-resolution T1-weighted structural MR images were acquired for spatial normalization procedures (MP-RAGE, 176 images, TE/TR:2.95/2250 ms, 1.0 mm slice thickness, matrix size: 256*256, FOV: 256 mm, flip angle: 15°).

Image analysis: We used SPM5 (Wellcome Department of Imaging Neuroscience, London, UK) for MRI data analysis. The first five EPI-volumes were discarded to allow for T1 equilibration, and the remaining images were realigned to the first volume. Images were

then corrected for differences in slice acquisition time, spatially normalized to the Montreal Neurological Institute (MNI) T1 template, super-sampled into 2 x 2 x 2 mm^3 voxels, and spatially smoothed with a Gaussian kernel of 8 mm FWHM.

Statistical analysis of the event-related data was performed within the framework of the general linear model (Friston et al., 1995) whereby predictor variables were defined for each subject in the first level analysis separately for the encoding and the retrieval session. During encoding, regressors for trials reflecting either later remembered or later forgotten trials were modeled as were positive and negative stimuli. With respect to the retrieval phase, trials reflecting recognized (hits) and unrecognized stimuli (misses) were modeled as were positive and negative stimuli. Each of the explanatory variables forming the above mentioned factors were modeled separately.

The explanatory variables (0.5 s) were temporally convolved with the hemodynamic response function of SPM5. In addition, the realignment parameters were included to model potential movement artifacts, as was a high-pass filter (cut-off at 1/128 Hz). Data were proportionally scaled accounting for various global effects, and temporal autocorrelation was modeled with an AR(1) process. Relevant parameter images contrasting each condition were entered in a random-effects repeated measures ANOVA with a nonsphericity correction.
In keeping with the hypotheses outlined in the introduction exploratory analyses were performed across the entire brain with appropriate correction. Additionally, specific analyses were performed in the amygdala and hippocampus using anatomically generated masks for conducting small volume corrections. Statistical tests were family-wise error rate corrected for multiple comparisons at the cluster level across the entire brain ($p < 0.05$) for the exploratory analyses, or the search volumes of interest using a small volume correction

(Worsley et al., 1996), both using an initial height threshold of $p < 0.005$ uncorrected. Amygdala and hippocampus masks were constructed based on macroscopic anatomical parcellation of a canonical T1-weighted MRI scan in MNI space (Maldjian et al., 2003; Tzourio-Mazoyer et al., 2002). Peak voxels of activated clusters are reported in MNI coordinates.

Results

Adverse events

Duloxetine plasma levels ranged between 7 and 247 µg/l (mean ± SD: 50 ± 54), which confirmed compliance with drug intake. Of the 18 participants who participated in the study 5 participants reported nauseousness, fatigue and insomnia. All reported side effects were limited to the first few days of drug intake, indicating that our results do not reflect the experience of adverse effects as the neuroimaging sessions took place only after two weeks of intake. Moreover, no serious adverse events were reported.

Mood and behavior

Mood, as measured prior to scanning by the BDI and STAI state, was not significantly affected by duloxetine (maximum t= 1.5 p= .2). Pre- and post scan measures of the POMS were compared with a repeated-measures ANOVA using the factors of time (before/after scanning), drug (duloxetine/placebo) and POMS-subscale (5 levels). There was an interaction between the factors of time and subscale ($F(4, 68)=33$, $p<.001$). Post-hoc pairwise comparisons for each subscale before and after scanning showed that depressive mood, fatigue, vigor and tension was increased by the fMRI procedure including the negative mood induction (minimum $t=3.65$, $p<.005$) except for the subscale anger. Moreover, we found no interaction between the factors of drug and subscale.

Mood ratings before and throughout the experiments were analyzed with an ANOVA using the factors of drug (duloxetine/placebo) and mood rating (before/after each video). While there was no significant effect of duloxetine on mood ratings, there was an expected difference between mood ratings before and after mood induction ($F(11,187)= 45$,

p<0.001). Post-hoc pairwise comparisons showed that mood was reduced after each mood-induction compared with baseline (minimum t=3.3, p<0.005). Moreover, mood at the end of the entire experiment was reduced compared to the start of the mood-induction (t=6.9, p<0.001). Hence, our mood induction led, as intended, to a substantial reduced, sad mood throughout the entire fMRI experiment.

Memory performance, as measured by the difference between hit and false alarm rates for all valence and drug conditions separately, was significantly above chance level (minimum t= 6.9, p< 0.001). There was no significant difference in memory performance between emotionally negative pictures and positive pictures. Duloxetine had no significant effect on memory performance. There was also no significant effect of duloxetine or valence on the reaction time data. Reaction times, however differed as a function of memory condition ($F(3, 2.7)=23.2$, $p<0.001$) whereby reaction times were fastest for hits (1785ms), followed by correct rejections (1945 ms) and almost equally slow for misses and false alarms (2118 and 2112ms respectively). A Spearman-Rho correlation was performed between the behavioral outcome measures of the memory test but revealed no significant relationship neither for the recognition- scores (hits minus false alarms) nor for the reaction times (minimum p > = 0.11)

Imaging results

Encoding: First, we conducted an exploratory whole brain analysis looking at main effects. In line with earlier studies, a main effect of memory (i.e. larger activity for subsequently remembered compared to subsequently forgotten stimuli) gave rise to clusters in parahippocampal gyrus extending into fusiform gyrus bilaterally (local maximum at 46, -50, -18 and -40, -46, -18 respectively, minimum pcorr<.001), and large clusters in bilateral

amygdala extending into the hippocampus (local maximum at 22, -4, -18 and -20, -6, -16 respectively, minimum pcorr <.001; see Fig.2). In the right posterior cortex an activation was found in a cluster ranging from occipital cortex into temporal cortex (local maximum at 48, -62, -14, pcorr <.001; see Fig. 2)

Successful memory formation was associated with an activity increase in left (local maximum at -52, 30, 0, pcorr<0.01) and right inferior frontal gyrus (54, 32, 6, pcorr<0.01).

To probe the main effect of drug, we compared larger activity for the duloxetine compared to the placebo condition. This analysis did not give rise to any significant activation, indicating that duloxetine did not generally affect BOLD signal in this experimental setup.

Most important with respect to our experimental question, we probed the effect of duloxetine on neural correlates of successful memory formation by comparing the subsequent memory effect between the drug and the placebo condition. The subsequent memory effect was larger in the placebo condition than in the duloxetine condition in the right hemisphere in the putamen (local maximum at 30, -12, 0, p_{corr}<0.01), anterior (local maximum at 2, 32, 12, p_{corr}<0.05) and middle cingulate cortex (local maximum at 12, -24, 38, p_{corr}<0.05) as well as middle frontal gyrus (local maximum at 26 52 6, p_{corr}<0.02; see Fig. 3). This memory by drug interaction was not significantly affected by stimulus valence. *For a summary of the significant effects see table 1.*

Retrieval: The brain regions involved in successful recognition memory were identified by comparing responses for hits and misses. This whole-brain analysis gave rise to a significant cluster in the right middle cingulate cortex (local maximum at 4, -36, 36; p_{corr}<0.05). The

ROI analysis focusing in on the amygdala and hippocampus revealed significant effects within left (local maximum at -22 2 -18, $p_{corr}<0.02$) and right amygdala (local maximum at 24 -2 -18, $p_{corr}<0.05$) as well as right hippocampus (local maximum at 22 -4 -22, $p_{corr}<0.05$). In line with the data on memory formation, there was no evidence for a significant main effect of drug in the retrieval data. Additionally, we did not find a main effect of valence.

Importantly, we observed a significant three-way-interaction between the factors of drug, valence and memory in the ROI analysis of the right amygdala (local maximum at 26 0 -24, $p_{corr}<0.05$, see figure 4). As is evident from the contrast estimates shown in figure 4, this interaction seems to arise due to a larger recognition memory effect (i.e. contrast between hits and misses) for happy stimuli only in the duloxetine compared to the placebo condition. We therefore performed post-hoc tests within the anatomically defined ROI of the right amygdala on the difference in recognition memory effects between the drug and placebo conditions separately for happy and sad pictures. Indeed, we found a significant drug by memory interaction only for positive scenes ($p_{corr}<0.001$). In other words, our data show that under sad mood induction duloxetine specifically enhances the recognition memory effects for positive stimuli in the right amygdala. For a summary of the significant effects see table 1.

To perform a spearman´s rank correlation analysis between the neural correlates of memory and Duloxetine plasma levels, beta-weights per subjects were extracted from the regions of interest that gave rise to a significant interaction with the factor drug during encoding or during retrieval. For encoding, we correlated the drug-induced change in the subsequent memory effect (placebo condition - drug condition) with Duloxetine plasma levels in right putamen, right anterior and middle cingulate and right inferior frontal gyrus.

These analyses only gave rise to a significant negative correlation between the drug-induced change in successful memory encoding in the right putamen(p=-0,007).

For retrieval, we correlated the drug-induced change in the successful memory retrieval effect (drug condition - placebo condition) in the amygdala with the Duloxetine plasma levels but did not find any significant result.

Discussion

This is the first study that investigated the effect of a short-term administration of the dual serotonin and noradrenaline reuptake inhibitor duloxetine on the neural correlates of mood-congruent and mood-incongruent memory formation and retrieval in healthy volunteers. The paradigm used was designed to mimic the effect of mood on memory during depression by employing relatively long series of explicit sad mood induction using emotional movie clips during memory formation and retrieval. The experimental set-up allowed us to dissociate the effect of duloxetine on successful and unsuccessful memory formation and retrieval and not just a global effect on memory processes.

Previous investigations of the effect of antidepressants in healthy controls often revealed changes in emotional processing in the absence of significant differences in ratings of mood and anxiety (Harmer *et al.*, 2009; Harmer *et al.*, 2004). Also in the present study, Duloxetine did not affect significantly subjective mood or anxiety ratings, nor our behavioral outcome measures. This limits the generalizability of our findings to the clinical state of depression at first sight. However, mood changes are not anticipated in a healthy subject population and the number of subjects included in the present study was designed to fulfill the power needs of a neuroimaging study, which appear more favorable than for behavioral studies. We designed the study to investigate fMRI but not behavioral differences. In as such, we appreciate the fact that we did not find a behavioral difference, because those may complicate the interpretation of the imaging results as well (Price and Friston, 2002). We found a valence unspecific effect of duloxetine on neural correlates of emotional memory formation and a valence-specific effect on the neural correlates of emotional recognition memory. In the absence of direct effects of duloxetine on mood state, these neural effects

are therefore more likely caused directly by the drug than by an indirect effect of increased mood.

Another recent fMRI study by Norbury and colleagues also showed that a selective noradrenergic antidepressant (the noradrenaline reuptake inhibitor reboxetine) directly modulated neural processing of emotional material in an emotional memory task in the absence of effects on mood or anxiety ratings (Norbury, et al., 2008). During a study phase consisting of a categorization task, reboxetine was associated with greater activation to positive words, relative to negative words, in left precuneus and right inferior frontal gyrus. However, during the test phase recognition memory under reboxetine was associated with reduced responses to positive words in left precuneus, anterior cingulate and medial frontal gyrus. It is important to note that results from that study are not directly comparable to our results, because Norbury and colleagues (2008) did not investigate neural activity related to memory formation by dissociating trials as to whether they were subsequently remembered or forgotten (for a review see (Fernandez and Tendolkar, 2001)). Likewise they did not investigate the neural correlates of recognition by analyzing the contrast between correctly recognized items and missed or new stimuli. By these means, Norbury and colleagues did not directly measure the effect of antidepressants on successful memory processes, which however is crucial to understand the cognitive effects of antidepressants. Here, we provide first evidence that duloxetine affects successful memory for biological salient stimuli by acting on successful memory formation in a valence-unspecific manner and on successful memory retrieval in a valence-specific manner.

In line with previous experiments testing emotional memory, we found significant activations in amygdala and hippocampus both during memory formation (cf (Richardson et

al., 2004)) and retrieval (Dolcos et al., 2005) next to the replication of an often shown left inferior frontal activation related to successful memory formation. During successful memory formation, duloxetine decreases activation in a set of brain regions comprising the putamen, middle frontal gyrus as well middle and anterior cingulate cortex. These regions have been implicated to play an important role in a so-called anterior emotional system known to be involved in emotion regulation (Phillips et al., 2003). A recent study (Lopez-Solá *et al.*, 2010),investigating the effect of duloxetine in acutely depressed patients also found a significant reduction of activation in subgenual anterior cinngulate, extended medial prefrontal cortex and basal ganglia including putamen. With respect to specific effects related to mood-congruent memory bias, an overactive interaction between caudate/putamen and hippocampus seems to account for mood-congruent memory in depression (Hamilton and Gotlib, 2008). Our data are in line with these findings and suggest that even in healthy controls the negative mood induction leads to an increased activity of this network during emotional memory formation, an effect, which can be reversed by duloxetine. It has long been debated whether antidepressants can affect fundamental cognitive processes apart from their role in attenuating pervasive negative affect (see Harmer et al. 2009). We now show that duloxetine targets the same affective neurocircuitry that is implicated in depression (Phillips et al., 2003), but without the improvement in mood that normally accompanies the drug-induced down-regulation of this circuitry in depressed patients. We thus provide indirect evidence that the working mechanism of this type of antidepressants is a direct attenuation of bottom-up processing in (para)limbic affective neurocircuitry rather than a more indirect effect on mood.

Moreover, part of the above mentioned brain regions also fulfill other functions when the brain is at rest. The anterior cingulate cortex, ventromedial prefrontal cortex, and dorsal

medial prefrontal cortex belong to the so-called default mode network, a set of brain regions, which may fulfill self-referential tasks involved in the evaluation of potentially survival-salient information from the body and the world (Buckner *et al.*, 2008; Raichle *et al.*, 2001). Evidence is accumulating that there seems to be a dysregulation of the default mode network in depression (Greicius *et al.*, 2007; Sheline *et al.*, 2009). Whereas activity within this network is decreased in healthy controls in order to allow a shift to goal-oriented behavior, default-mode network activity is not equally decreased in depressed patients during cognitive tasks. Sheline and colleagues (2009) suggested that these abnormalities might contribute to deficits in "automatic" and controlled processing of affective stimuli. Our data suggest that sad mood induction leads to increased activity of the default mode network in the placebo condition comparable to what is found in depression and that this effect is reversed by duloxetine.

During retrieval, duloxetine specifically enhanced mood incongruent neural activity in the amygdala related to the successful recognition of positive scenes. The amygdala plays a central role in various aspects of affect processing and mood regulation and has been shown to have a modulatory effect on the hippocampus during emotional memory (Dolcos, *et al.*, 2005). Harmer and colleagues (Harmer, *et al.*, 2009) proposed that antidepressants reverse affective biases in depression and anxiety. Our data add to this hypothesis by suggesting that at least during memory retrieval a mood-congruent memory bias reversal can be related to a processing enhancement for positive stimuli rather than an attenuation of processing for negative stimuli. Though the consequence of such effects on behavior needs to be tested in larger samples, an enhancement of positive memories seems more favorable than a mere decrease of negative memories. By these means, antidepressants do not only serve to decrease negative memories but improve a positive memory bias, which is usually

found in healthy subjects. In terms of clinical impact, this could mean that depressed patients under antidepressant treatment are allowed to gain again from positive aspects of their environment.

Antidepressants acting via serotonergic reuptake inhibition appear to have a more general blunting effect whereby emotional reactivity to both negative and positive experiences can be reduced (Opbroek *et al.*, 2002; Price *et al.*, 2009). The more general effect of Duloxetine decreasing neural activity in brain regions known to be involved in emotion regulation could therefore rely on a more "inhibitory" serotonergic effect during emotional memory formation where decreased effects of mood are likely to normalize biased processing. However, previous studies have also shown that SSRIs can increase the neural processing related to happy faces in depressed patients in the course of eight weeks of treatment (Fu et al., 2007), which potentially could explain the effect we found during retrieval. It has been postulated that the amygdala contributes to emotional memory via noradrenergic activation (Cahill *et al.* 1994; McGaugh , 2004; Strange and Dolan 2004). While this at first sight could account for the significantly larger recognition effect under duloxetine during retrieval, it has also been suggested that the effect of noradrenergic activation is more likely to affect memory formation than retrieval. A recent study by McCabe and colleagues (2009) directly compared the effect of an SSRI and an NRI on the neural correlates of reward processing and found that the SSRI had reduced activation in the ventral striatum and the ventral medial/orbitofrontal cortex while the NRI increased neural responses within medial orbitofrontal cortex to reward. Finally, on the one hand Harmer and colleagues in a recent review suggested that at least in healthy volunteers a common overlapping mechanism may account for the effects of conventional antidepressant drugs with different neurochemical actions on emotional processing. In the light of these aforementioned findings it is certainly relevant to disentangle the specific effects of noradrenergic and serotonergic treatment on

remediating mood-congruent memory bias. On the other hand knowledge of common antidepressant effects on cognitive biases are important when attuning the combination of antidepressant and cognitive behavioral therapy.

A couple of limitations have to be taken into account. Firstly, we choose to investigate the effect of duloxetine on mood-congruent and mood-incongruent memory processes with the largest contrast possible (negatively versus positively valenced stimuli). Thus, we did not include neutral stimuli to keep an efficient design. However, that decision precludes to conclude that duloxetine affects emotional memory specifically. Though the sample size appears quite optimal for a within-subject design and in balance with sample sizes of other fMRI studies with similar questions (Norbury *et al.*, 2007; McCabe *et al.*, 2009; Murphy *et al.*, 2009), we cannot rule out that the number of subjects included in the present study prohibited us from finding higher order interactions such as a triple interaction with the factors of drug, memory and valence not only during retrieval but also during encoding. Given that no previous fMRI study has combined pharmacological challenge with such an extensive emotional memory set-up, certainly further studies are needed to support our conclusion also with respect to negative findings namely that there is a more general effect of Duloxetine during encoding but not retrieval. For now we can only compare our results with a previous study of our own group (Urner *et al.*, 2011) that used the same experimental set-up except for the fact that that study investigated the effect of a genetic variation instead of a drug on the neural correlates of emotional memory. In that study also no interaction with both memory and valence was found during encoding, but during retrieval. Regardless, the present results might not generalize to euthymic or postive mood, but the experimental procedure was set-up as mimicking acutely reduced mood as it may occur in depression. Finally, although each subject showed an increased duloxetine level, we observed a relative large variation in plasma duloxetine levels . Higher plasma levels could

be caused by late drug intake so that the intake-test delay got shortened. Also some of the subjects might have been poor metabolizers (Cyp1A2 en Cyp2D6). In turn, lower plasma levels than expected were observed rarely in the present sample. They might have been the consequence of longer intake-test delay or rapid metabolization.

Taken together, our data show that the antidepressant duloxetine has specific effects on memory formation and retrieval when subjects are sad. While during emotional memory formation, it seems to down regulate more globally a network involved in affect regulation; during retrieval it specifically acts upon a brain region known to modulate emotional memory in a valence-specific way. Though of course this finding has to be replicated with other antidepressants, we suggest that these effects can be seen as final common pathways of antidepressants reversing mood-congruent processing biases.

Acknowledgements

This work was supported in part by a grant from Eli Lilly Nederland B.V. (who also provided the study medication) to Dr. Tendolkar, Prof. Fernandez and Dr. Verkes. We would like to thank Sabine Kooijman for her assistance in recruting and monitoring the participants

Financial disclosures

The authors declare that over the past three years RJV has served on the advisory boards of Eli Lilly, Servier and Organon, has received grants from Astra Zeneca, Eli Lilly, Pfizer, Wyeth and Organon, and honoraria and/or speaker's fees from Astra Zeneca, Eli Lilly, Lundbeck, Wyeth and Organon. All other authors report no biomedical financial interests or potential conflicts of interest.

References

Bhagwagar Z, Hinz R, Taylor M, Fancy S, Cowen P, Grasby P (2006). Increased 5-HT(2A) receptor binding in euthymic, medication-free patients recovered from depression: a positron emission study with [(11)C]MDL. *Am J Psychiatry*, **163**: 1580–1587

Beck A, Steer R, Brown G (1996): Manual for the Beck Depression Inventory-II. Psychological Corporation: San Antonio.

Buckner RL, Andrews-Hanna JR, Schacter DL (2008). The brain's default network: anatomy, function, and relevance to disease. *Ann N Y Acad Sci, **1124***: 1-38.

Cahill L, Prins B, Weber M, McGaugh JL (1994). Beta-adrenergic activation and memory for emotional events. *Nature*, ***371***(6499): 702-704.Dolcos F, LaBar KS, Cabeza R (2005). Remembering one year later: role of the amygdala and the medial temporal lobe memory system in retrieving emotional memories. *Proc Natl Acad Sci U S A, **102***(7): 2626-2631.

Fernandez G, Tendolkar I (2001). Integrated brain activity in medial temporal and prefrontal areas predicts subsequent memory performance: human declarative memory formation at the system level. *Brain Res Bull, **55***(1): 1-9.

Fitzgerald DA, Arnold JF, Becker ES, Speckens AE, Rinck M, Rijpkema M, Fernández G, Tendolkar I (2011). How mood challenges emotional memory formation: An fMRI investigation. *Neuroimage* (Epub)

Frampton JE, Plosker GL (2007). Duloxetine: a review of its use in the treatment of major depressive disorder. *CNS Drugs, **21***(7): 581-609.

Friston KJ, Holmes AP, Poline JB, Grasby PJ, Williams SC, Frackowiak RS, et al. (1995). Analysis of fMRI time-series revisited. *Neuroimage, **2***(1): 45-53.

MacQueen G, Frodl T (2011).The hippocampus in major depression: evidence for the convergence of the bench and bedside in psychiatric research? *Mol Psychiatry*, **16**(3): 252-624.

Fu CH, Williams SC, Brammer MJ, Suckling J, Kim J, Cleare AJ, et al. (2007). Neural responses to happy facial expressions in major depression following antidepressant treatment. *Am J Psychiatry, **164***(4): 599-607.

Fu CH, Williams SC, Cleare AJ, Brammer MJ, Walsh ND, Kim J, et al. (2004). Attenuation of the neural response to sad faces in major depression by antidepressant treatment: a prospective, event-related functional magnetic resonance imaging study. *Arch Gen Psychiatry, **61***(9): 877-889.

Greicius MD, Flores BH, Menon V, Glover GH, Solvason HB, Kenna H, et al. (2007). Resting-state functional connectivity in major depression: abnormally increased contributions from subgenual cingulate cortex and thalamus. *Biol Psychiatry, **62***(5): 429-437.

Gupta S, Nihalani N, Masand P (2007). Duloxetine: review of its pharmacology, and therapeutic use in depression and other psychiatric disorders. *Ann Clin Psychiatry, **19***(2): 125-132.

Hamilton JP, Gotlib IH (2008). Neural substrates of increased memory sensitivity for negative stimuli in major depression. *Biol Psychiatry,* ***63***(12): 1155-1162.

Harmer CJ, Goodwin GM, Cowen PJ (2009). Why do antidepressants take so long to work? A cognitive neuropsychological model of antidepressant drug action. *Br J Psychiatry,* ***195***(2): 102-108.

Harmer CJ, Heinzen J, O'Sullivan U, Ayres RA, Cowen PJ (2008). Dissociable effects of acute antidepressant drug administration on subjective and emotional processing measures in healthy volunteers. *Psychopharmacology (Berl),* ***199***(4): 495-502.

Harmer CJ, Mackay CE, Reid CB, Cowen PJ, Goodwin GM (2006). Antidepressant drug treatment modifies the neural processing of nonconscious threat cues. *Biol Psychiatry,* ***59***(9): 816-820.

Harmer CJ, Shelley NC, Cowen PJ, Goodwin GM (2004). Increased positive versus negative affective perception and memory in healthy volunteers following selective serotonin and norepinephrine reuptake inhibition. *Am J Psychiatry,* ***161***(7): 1256-1263.

Hasler G, Drevets WC, Manji HK, Charney DS (2004). Discovering endophenotypes for major depression. *Neuropsychopharmacology,* ***29***(10): 1765-1781.

Katz MM, Tekell JL, Bowden CL, Brannan S, Houston JP, Berman N, et al. (2004). Onset and early behavioral effects of pharmacologically different antidepressants and placebo in depression. *Neuropsychopharmacology,* ***29***(3): 566-579.

Kernis M, Greenier K, Herlocker C, Whisenhunt C, Abend T (1997). Self-perceptions of reactions to doing well or poorly: The roles of stability and level of self-esteem. *Person Individ Diff,* ***22***: 845-854.

Leppänen JM (2006). Emotional information processing in mood disorders: a review of behavioral and neuroimaging findings. *Curr Opin Psychiatry,* ***19***(1): 34-39.

Lewis PA, Critchley HD, Smith AP, Dolan RJ (2005). Brain mechanisms for mood congruent memory facilitation. *Neuroimage,* ***25***(4): 1214-1223.

Lopez-Sola M, Pujol J, Hernandez-Ribas R, Harrison BJ, Contreras-Rodriguez O, Soriano-Mas C, et al. (2010). Effects of duloxetine treatment on brain response to painful stimulation in major depressive disorder. *Neuropsychopharmacology*, ***35***(11): 2305-2317.

Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH (2003). An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *Neuroimage,* ***19***(3): 1233-1239.

McCabe C, Mishor Z, Cowen PJ, Harmer CJ (2010). Diminished neural processing of aversive and rewarding stimuli during selective serotonin reuptake inhibitor treatment. *Biol Psychiatry*, ***67***(5):439-445.

McGaugh JL (2004). The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annu Rev Neurosci,* ***27***: 1-28.

Meyer JH, McMain S, Kennedy SH, Korman L, Brown GM, DaSilva JN et al. (2003). Dysfunctional attitudes and 5-HT2 receptors during depression and self-harm. *Am J Psychiatry*, ***160***: 90–99

Murphy SE, Norbury R, O'Sullivan U, Cowen PJ, Harmer CJ (2009). Effect of a single dose of citalopram on amygdala response to emotional faces. *Br J Psychiatry,* ***194***(6): 535-540.

Norbury R, Mackay CE, Cowen PJ, Goodwin GM, Harmer CJ (2007). The effects of reboxetine on emotional processing in healthy volunteers: an fMRI study. *Mol Psychiatry,* ***13***(11): 1011-1020.

Norbury R, Taylor MJ, Selvaraj S, Murphy SE, Harmer CJ, Cowen PJ (2009). Short-term antidepressant treatment modulates amygdala response to happy faces. *Psychopharmacology (Berl),* ***206***(2): 197-204.

Opbroek A, Delgado PL, Laukes C, McGahuey C, Katsanis J, Moreno FA, et

al. (2002). Emotional blunting associated with SSRI-induced sexual dysfunction.

Do SSRIs inhibit emotional responses? *Int J Neuropsychopharmaco,l* ***5***: 147–151.

Phillips ML, Drevets WC, Rauch SL, Lane R (2003). Neurobiology of emotion perception II: Implications for major psychiatric disorders. *Biol Psychiatry,* ***54***(5): 515-528.

Price CJ, Friston KJ (2002). Functional imaging studies of neuropsychological patients: applications and limitations. *Neurocase*, ***8***(5): 345-354.

Price J, Cole V, Goodwin GM (2009). Emotional side-effects of selective serotonin reuptake inhibitors: Qualitative study. *Br J Psychiatry*, ***195***: 211–217.

Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL (2001). A default mode of brain function. *Proc Natl Acad Sci U S A,* ***98***(2): 676-682.

Ramel W, Goldin PR, Eyler LT, Brown GG, Gotlib IH, McQuaid JR (2007). Amygdala reactivity and mood-congruent memory in individuals at risk for depressive relapse. *Biol Psychiatry,* ***61***(2): 231-239.

Richardson MP, Strange BA, Dolan RJ (2004). Encoding of emotional memories depends on amygdala and hippocampus and their interactions. *Nat Neurosci,* ***7***(3): 278-285.

Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry,* ***59 Suppl 20***: 22-33;quiz 34-57.

Sheline YI, Barch DM, Price JL, Rundle MM, Vaishnavi SN, Snyder AZ, et al. (2009). The default mode network and self-referential processes in depression. *Proc Natl Acad Sci U S A,* ***106***(6): 1942-1947.

Strange BA, Dolan RJ (2004).Beta-adrenergic modulation of emotional memory-evoked human amygdala and hippocampal responses. *Proc Natl Acad Sci U S A*, ***101***(31): 11454-11458.

Teasdale JD (1983). Negative thinking in depression: Cause, effect, or reciprocal relationship? *Advances Beh Res Ther* ***5***: 3-25.

Tendolkar I, Arnold J, Petersson KM, Weis S, Brockhaus-Dumke A, van Eijndhoven P, et al. (2008). Contributions of the medial temporal lobe to declarative memory retrieval: manipulating the amount of contextual retrieval. *Learn Mem,* ***15***(9): 611-617.

Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, et al. (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage,* ***15***(1): 273-289.

Urner M, van Wingen G, Franke B, Rijpkema M, Fernández G, Tendolkar I (2011).Genetic variation of the α2b-adrenoceptor affects neural correlates of successful emotional memory formation. *Hum Brain Mapp* ***Epub***.

Van der Does AJ (2002): BDI-II-NL. De Nederlandse versie van de Beck Depression Inventory-second edition. Zwets en Zeitlinger: Lisse.

van der Ploeg HM, Defares PB, Spielberger CD (1980): Handleiding bij de Zelf-Beoordelings Vragenlijst, ZBV: Een Nederlandse vertaling van de Spielberger State-Trait Anxiety Inventory. Swets & Zeitlinger: Lisse.

van Wingen GA, van Eijndhoven P, Cremers HR, Tendolkar I, Verkes RJ, Buitelaar JK, et al. (2010). Neural state and trait bases of mood-incongruent memory formation and retrieval in first-episode major depression. *J Psychiatr Res,* ***44***(8): 527-534.

Wald FDM, Mellenbergh GJ (1990). De verkorte versie van de Nederlandse vertaling van de Profile of Moods State (POMS). [The short Dutch version of the Profile of Moods State (POMS) questionnaire]. *Nederlands Tijdschrift voor de Psychologie,* ***45***: 86-90.

Warner-Schmidt JL, Duman RS (2006). Hippocampal neurogenesis: opposing effects of stress and antidepressant treatment. *Hippocampus*, ***16***(3):239-249.

Weis S, Specht K, Klaver P, Tendolkar I, Willmes K, Ruhlmann J, et al. (2004). Process dissociation between contextual retrieval and item recognition. *Neuroreport,* ***15***(18): 2729-2733.

Worsley KJ, Marrett S, Neelin P, Vandal AC, Friston KJ, Evans AC (1996). A unified statistical approach for determining significant signals in images of cerebral activation. *Hum Brain Mapp,* ***4***(1): 58-73.

Tables

Study Phase	*Effect*	*Region*	*X*	*Y*	*Z*	*p-value*
Encoding	Memory	R Parahippocampal gyrus	46	-50	18	0.000
		L Parahippocampal gyrus-	-40	-46	-18	0.000
		R Amygdala	22	-4	-18	0.001
		L Amygdala	-20	-6	-16	0.000
		L Inferior frontal gyrus	-52	30	0	0.001
		R Inferior frontal gyrus	54	32	6	0.003
		R Inferior temporal gyrus	48	-62	-14	0.000
	Drug x Memory	R Putamen	30	12	0	0.004
		R Anterior Cingulate	2	32	12	0.042
		R Middle Cingulate	12	-24	38	0.046
		R Middle frontal gyrus	26	52	6	0.012
Retrieval	Memory	R Middle cingulate	4	-36	36	0.046
		R Amygdala(ROI)	24	-2	-18	0.018
		L Amygdala(ROI)	-22	2	-18	0.046
		R Hippocampus (ROI)	22	-2	-22	0.042
	Drug x Memory x Valence	R Amygdala (ROI)	26	0	-24	0.044

Table 1 displays the coordinates of each of the significant effects found in the statistical analysis. X-, Y- and Z-coordinate refer to coordinates in MNI space for local maxima found in the analyses where MNI space is an approximation to Talairach space. P-value refers to the significance value after correcting for multiple comparisons at the whole brain level or within the region of interest (ROI). R –right hemisphere, L-left hemisphere.

Titles and legends to figures

Figure 1 Overview of the experimental set-up. Mood-induction was interspersed throughout the entire experiment, prior to each of the encoding and recognition session. At study, subjects were required to make a valence decision. At test, participants saw the same amount of previous presented and new photos and were required to make an old/new judgment. For details see methods section.

MI - mood induction; E - encoding block; R - retrieval block

Figure 2 Displayed are the activation maps (significance threshold at $p<0.001$ uncorrected for displaying purposes) showing significant main effects of memory found during memory formation and memory retrieval. Note that all results displayed in this and the subsequent figures were family-wise error rate corrected for multiple comparisons at the cluster level across the entire brain ($p < 0.05$), or the search volume of interests (amygdala, hippocampus) using a small volume correction ($p < 0.05$). A) On the left panel activation maps from the whole brain analysis during encoding are superimposed on a standard T1 image provided with MRIcron showing significantly larger activations related to successful versus unsuccessful memory formation in bilateral amygdala. B) Whole brain analysis during encoding further revealed that successful memory formation was associated with significant activity in bilateral inferior frontal regions displayed superimposed on a rendered brain provided with SPM 5. Panel C) shows results from the region of interest analysis during retrieval. Greater activity for recognized compared to forgotten stimuli is shown in bilateral amygdala/hippocampus superimposed on a standard T1 image provided with MRIcron. Panel D) shows the significant recognition effect in right middle cingulate cortex.

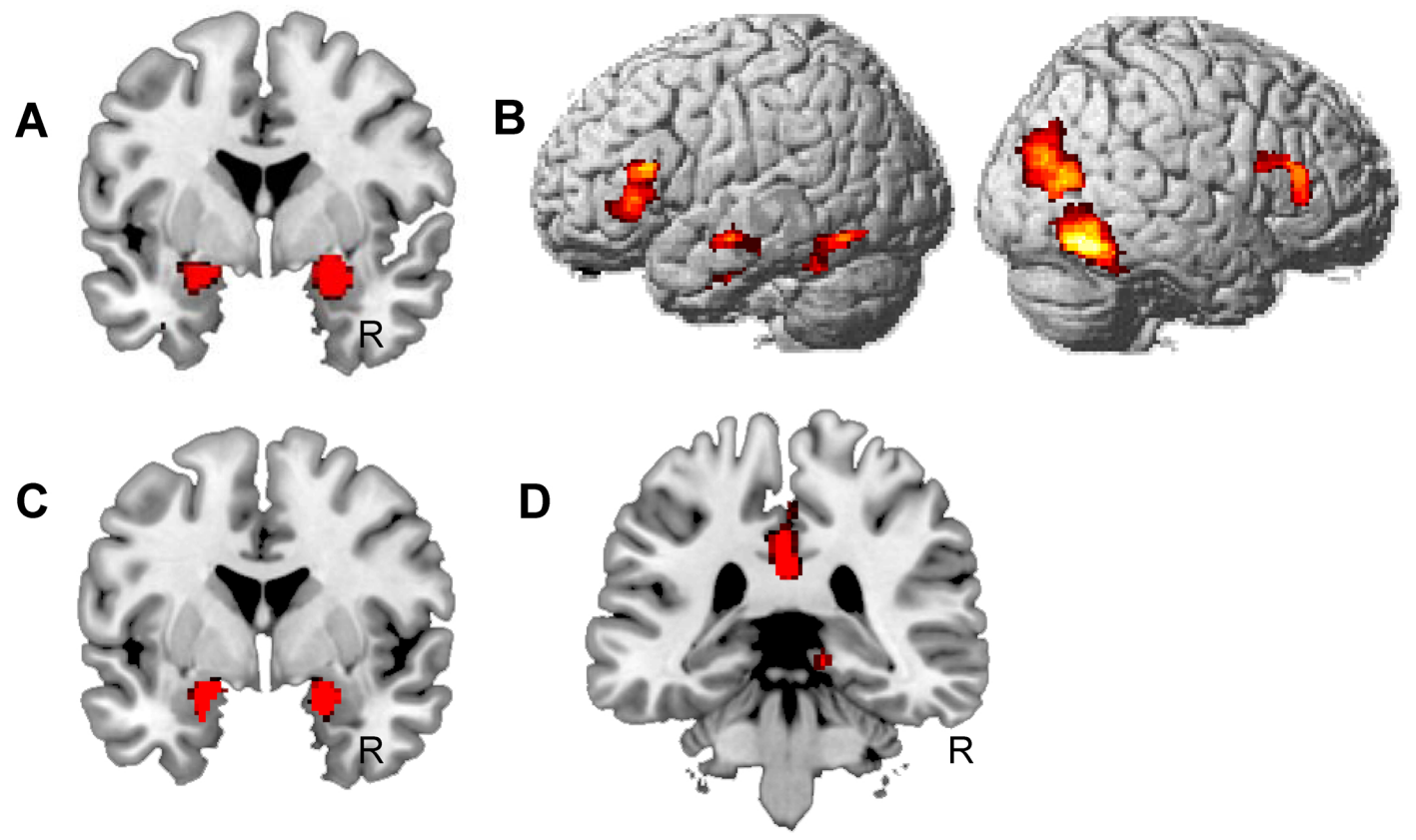
Figure 3 Activation maps (threshold at p<0.001 uncorrected for displaying purposes) from the whole-brain analysis superimposed on sagittal slices of a standard T1 image provided with MRIcron are displaying the significant interaction between the factors of drug and memory formation. As described in the results section, this interaction is based on a larger subsequent memory effect in the placebo than duloxetine condition in A) the right putamen and middle frontal gyrus as well as in B) the right middle and anterior cingulate cortex. Z-coordinates displayed on the figure refer to coordinates in MNI space for local maxima found in the analyses where MNI space is an approximation to Talairach space.

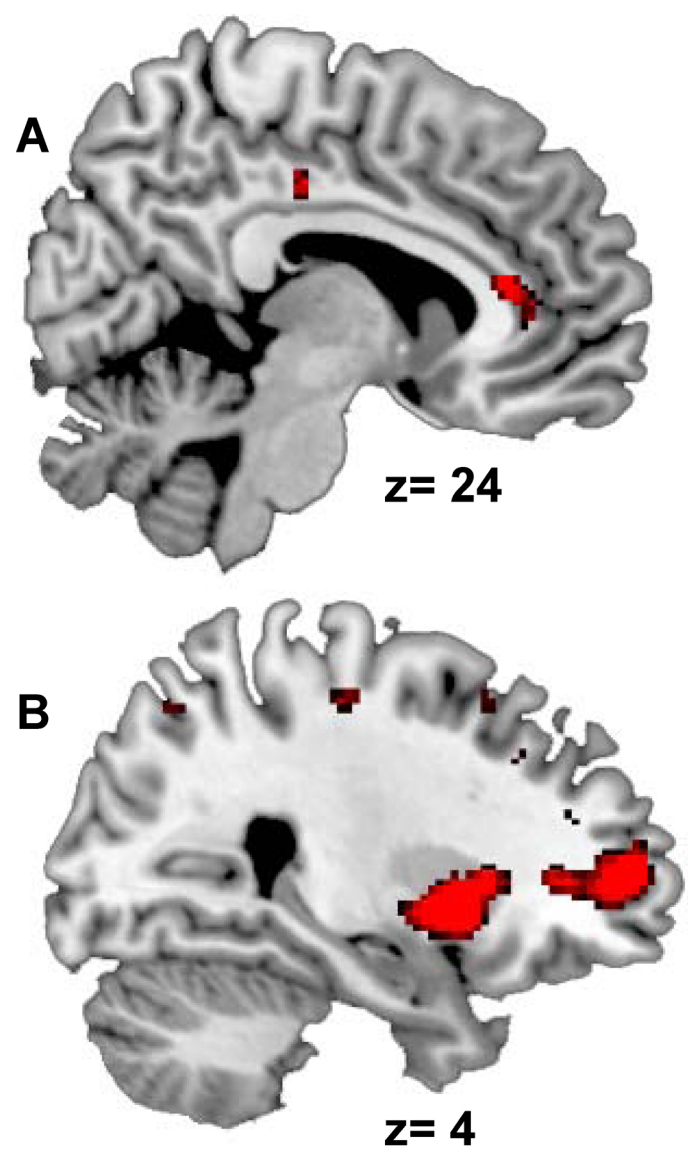
Figure 4 Displayed are the significant results from the three-way-interaction between the factors of drug, valence and memory during retrieval within the amygdala. A) Activation maps (threshold at p<0.001 uncorrected for displaying purposes), superimposed on a coronal slice of a standard T1 image, show a significant three-way-interaction in the right amygdala. B) To further visualize this interaction, parameter estimates are shown as provided by SPM. The bar graphs show beta-estimates of the specific contrast estimates of the recognition memory effect (i.e., hits minus misses ± 90% confidence interval). This demonstrates that the interaction arises due to an elevated recognition effect for happy scenes in the duloxetine condition, which was also statistically confirmed by post-hoc analyses.

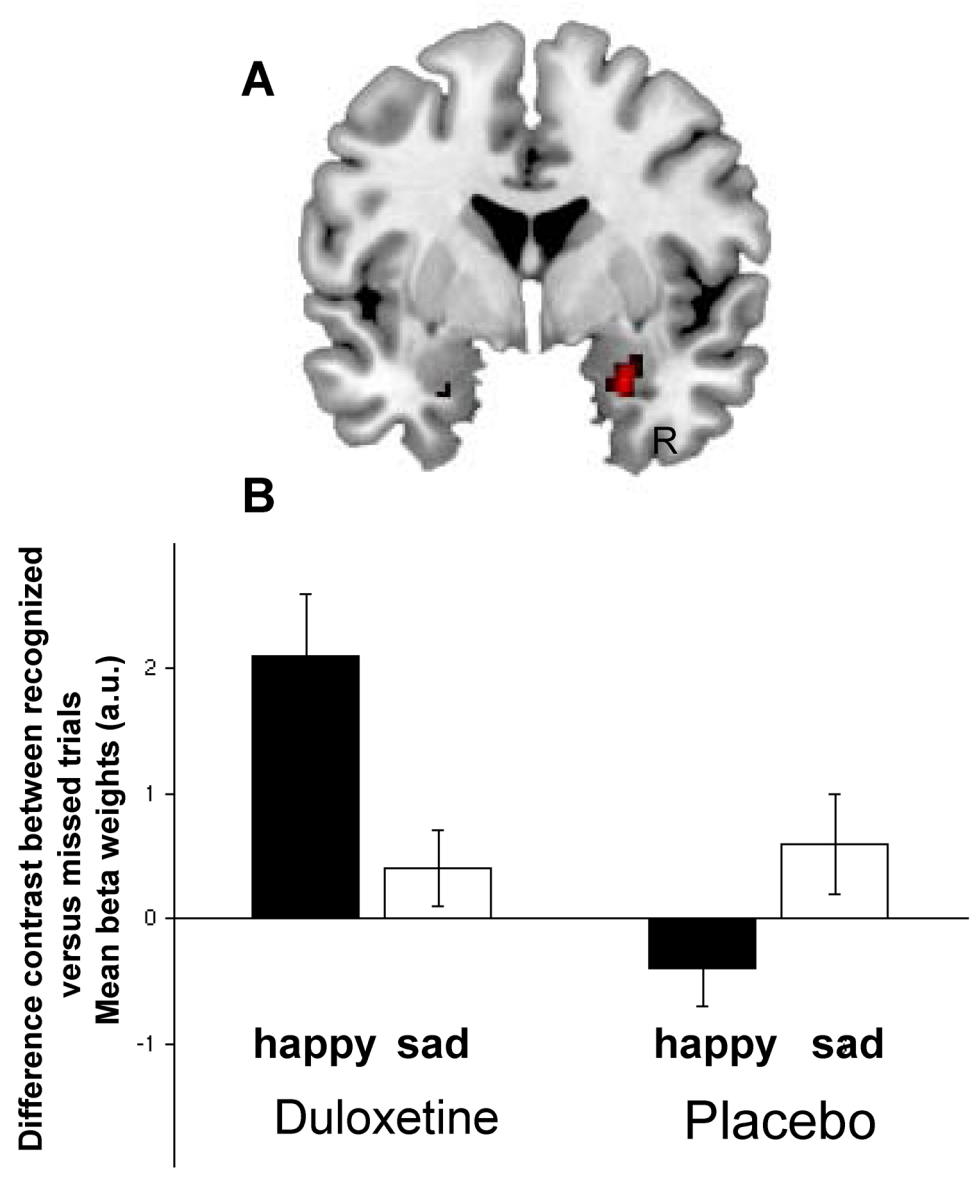
MI E MI E MI R MI R MI R MI R

Sad or happy?

New or old?


A
R
B
C
R
D
R


A
z= 24
B
z= 4


A
R
B
Difference contrast between recognized versus missed trials
Mean beta weights (a.u.)
2
1
0
-1
happy
sad
Duloxetine
happy
sad
Placebo