Testing antidepressant compounds in a neuropsychological model of drug action

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Chapter 2

Testing the Antidepressant Properties of the Peptide ARA290 in a Human Neuropsychological Model of Drug Action

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Under review

Abstract

Studies on the cognitive and neural effects of Erythropoietin (EPO) indicate that EPO may have antidepressant effects. Due to its hematopoietic effects, EPO may cause serious side-effects with repeated administration if patients are not monitored extensively. ARA290 is an EPO-analogue peptide without such hematopoietic side-effects but may have neurotrophic and antidepressant effects. The aim of this study was to investigate the possible antidepressant effects of ARA290 in a neuropsychological model of drug action.

Healthy participants (N=36) received ARA290 (2 mg) or placebo in a double-blind, randomized, parallel-group design. Neural and cognitive effects were assessed one week after administration. Primary outcome measures were the neural processing of fearful *vs* happy faces and the behavioural recognition of emotional facial expressions.

ARA290-treated individuals displayed lower neural responses to happy faces in the fusiform gyrus. ARA290 tended to lower the recognition of happy and disgust facial expressions. Although ARA290 was not associated with a better memory for positive words, it was associated with faster categorization of positive ***vs*** negative words. Finally, ARA290 increased attention towards positive emotional pictures. No effects were observed on mood and affective symptoms.

ARA290 may modulate some aspects of emotional processing, however, the direction and the strength of its effects do not unequivocally support an antidepressant-like profile for ARA290. Future studies may investigate the effects of different timing and dose.

Registration Clinical Trial:

Eudract reg. nr: 2010-024364-18; ClinicalTrials.gov Identifier: NCT 02070783
URL: https://clinicaltrials.gov/ct2/show/NCT02070783?term=02070783&rank=1

Introduction

Despite the large number of pharmacological treatment options for depression, many patients show partial or no recovery, and a significant time-lag for the onset of clinical effects. Furthermore, the majority of patients experience side effects such as weight gain and sexual dysfunction (Masand and Gupta, 2002). There is a need for the development of treatments that target different molecular pathways.

Erythropoietin (EPO) is a glycoprotein that regulates erythropoiesis. EPO also crosses the blood brain barrier (BBB) and has neuroprotective and neurotrophic effects when delivered in high doses (Brines *et al.*, 2000; Shingo *et al.*, 2001; Gonzalez *et al.*, 2009). The finding that cells in ischemic/hypoxic brain tissue express EPO and its receptor (EpoR) (Sirén *et al.*, 2001) has led to studies investigating the role of EPO in different types of brain injuries (reviewed by Brines and Cerami, 2005).

In humans, long-term administration of high dose EPO improved cognitive functions in patients with schizophrenia (Ehrenreich *et al.*, 2007a) and multiple sclerosis (Ehrenreich *et al.*, 2007b), whereas lower-dose EPO produced no cognitive benefits (Ehrenreich *et al.*, 2007b). The effects were not correlated with changes in haematological parameters, suggesting that they were mediated by a mechanism other than that of red blood cell increase. In patients with treatment-resistant depression, eight weeks of weekly EPO versus saline infusions as augmentation treatment had no effect on clinician-rated depression severity (primary outcome), but did have small positive effects on self-rated depressive symptoms, quality of life, psychosocial function and cognition (Miskowiak *et al.*, 2014). Earlier research in healthy volunteers had shown that one dose of EPO ***vs*** saline down-regulates neural response to fearful faces in the fusiform gyrus and reduces the recognition of fearful facial expressions (Miskowiak *et al.*, 2007a). EPO increased bilateral hippocampus activation during a picture memory task one week after administration (Miskowiak *et al.*, 2007b), but not three days after administration (Miskowiak *et al.*, 2007c). EPO also caused transient improvements in self-reported mood in healthy volunteers which lasted for three days (Miskowiak *et al.*, 2007a, Miskowiak *et al.*, 2008). These studies use a neurocognitive model of antidepressant drug action (Harmer *et al.*, 2009) which is based on the finding that various established antidepressants have immediate effects (after a single dose or short-term treatment) on emotional information processing in healthy individuals (reviewed by Harmer *et al.*, 2009, 2010). These early neurocognitive changes induced by antidepressants or by other monoaminergic manipulations may be related to subsequent mood changes (Tranter *et al.*, 2009; Booij and Van der Does, 2011).

In summary, there is evidence to suggest that the effects of EPO mimic antidepressant actions both at a behavioural and at a neural level. However, the human proof-of-concept studies were conducted in relatively small samples. Another limitation is the limited clinical potential of EPO to treat depressive symptoms in non-anemic patients, due to the hematopoietic actions of EPO with repeated administration (Wolf *et al.*, 1997; Stohlawets *et al.*, 2000).

The 11-amino acid, linear peptide ARA290 is an EPO-derivative that exerts tissue-protective effects but is not a hematopoietic stimulant (Brines *et al.*, 2008). ARA290 exerts anti-inflammatory actions in animals (Swartjes *et al.*, 2011; Pulman *et al.*, 2013). In humans, ARA290 had beneficial effects on neuropathic symptoms in patients with sarcoidosis (Heij *et al.*, 2012; Dahan *et al.*, 2013; Niesters *et al.*, 2013). The anti-inflammatory actions of ARA290 are mediated by the innate repair receptor (IRR). (Niesters *et al.*, 2013). Activation of the IRR initiates multiple signalling pathways initiating tissue-protective actions, one of which is the inhibition of inflammation-induced apoptosis (Brines and Cerami, 2012). The effects of ARA290 on neurocognitive processing of emotional information relevant to depression have not yet been assessed.

The aim of this study was to investigate whether ARA290 produces antidepressant-like effects. We carried out a double-blind, randomized placebo-controlled clinical trial in healthy volunteers. Primary outcome measures were the accuracy and speed of recognition of facial expressions of emotions and the neural processing of emotions, in particular amygdala, hippocampal and ventromedial prefrontal cortex (vmPFC) response to viewing fearful versus happy facial expressions. These effects were measured one week after administration of ARA290.

Methods

Participants

Participants were recruited via advertisements at various sites within Leiden University. Inclusion criteria were: Dutch-speaking males and females (not pregnant and not breastfeeding); age 18-35 years; right-handedness; BMI of 18-33kg/m^2. Exclusion criteria were major physical illness; current or past psychiatric disorder (Mini International Neuropsychiatric Interview; M.I.N.I.; Sheehan *et al.*, 1997; Van Vliet *et al.*, 2000); current use of medication (including over the counter medication); lifetime use of hard drugs; any use of nicotine products in the past week; use of soft drugs in the past three months; use of more than 14 alcohol units per week and more than 4 units on any day during the past week; general MRI contraindications. Participants received €90 for the whole study. This study was approved by the Medical Ethics Committee (METC) of Leiden University Medical Centre (LUMC).

Instruments

Affective symptoms and mood states

Participants filled out the 20-item Positive and Negative Affectivity Schedule (PANAS; state version) (Watson *et al.*, 1988), the 14-item Hospital Anxiety and Depression Scale (HADS; Zigmond and Snaith, 1983; Spinhoven *et al.*, 1997) and single-item Mood States Scales (MSS; 0-10) (Sadness, Annoyance, Tension, Cheerfulness, Energy). The time frame of the HADS was changed into 'the past three days'. IQ was estimated with the National Adult Reading Test (Nelson, 1982; Schmand *et al.*, 1992).

fMRI tasks

E-prime v1.0 was used for presentation of fMRI tasks and recording of responses. Task stimuli were back-projected on a screen located at the end of the scanner bore, which participants could see through a mirror. Description and results of the secondary measure (Picture Recognition Task) and Visual Stimulation Condition are described in the Supplementary Material.

Facial Expression Processing

Pictures of fearful and happy facial expressions were taken from the Radboud Faces Database (Langner *et al.*, 2010) and were presented in blocks. Eight blocks of fearful (4 blocks) and happy (4 blocks) were presented (48 sec/block) in fixed order interspersed with 30 seconds of fixation cross. Each block consisted of 16 faces (8 male and 8 female, randomly presented within a block) presented for 100ms. After presentation of each face a black screen with a white "X" was presented for 2900ms. Responses were only recorded within the first 2000ms

after onset of a trial. The starting block (fearful or happy) was randomized, stratified for sex. Participants performed a simple gender discrimination task. The task took 11 minutes.

fMRI data acquisition

Imaging data were acquired on a Philips 3.0-T Achieva MRI scanner using a 32-channel SENSE head coil for radiofrequency reception (Philips Healthcare, Best, Netherlands).

Whole-brain fMRI data sets were acquired using T2*-weighted gradient echo planar imaging with the following scan parameters: 301 (Facial Expression Processing Task)/170 (Picture Recognition Task)/ 137 (Visual Stimulation Condition) volumes; 38 axial slices scanned in ascending order; repetition time (TR)= 2200ms; echo time (TE)= 30ms; flip angle= 80˚; FOV= 220 x 220 mm; 2.75 mm isotropic voxels with a 0.275 mm slice gap.

A high-resolution anatomical image (T1- weighted ultra-fast gradient-echo acquisition; TR= 9.76ms; TE= 4.59ms; flip angle= 8˚;140 axial slices; FOV= 224 x 177.33 mm; in-plane resolution= 0.875 mm x 0.875 mm; slice thickness= 1.2 mm), and a high-resolution T2*-weighted gradient echo EPI scan (TR= 2.2 s; TE = 30ms; flip angle= 80˚; 84 axial slices; FOV= 220 x 220 mm; in-plane resolution= 1.96 x 1.96 mm, slice thickness= 2 mm) were acquired for registration and normalization to standard space.

fMRI data pre-processing

Prior to analyses, all fMRI data sets were submitted to a visual quality control check to ensure that no gross artefacts were present in the data. Next, data were analysed using FSL Version 4.1.6 (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl).

The following pre-processing steps were applied to the EPI data sets: motion correction (Jenkinson *et al.*, 2002), non-brain removal (Smith, 2002), spatial smoothing using a Gaussian kernel of 6 mm full width at half maximum (FWHM) for the Facial Expression Recognition and Picture Recognition tasks and 8mm FWHM for the Visual Stimulation Condition, grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor, a high-pass temporal filter of 165 sec. (i.e., 0.006 Hz) for the Facial Expression Processing Task, 60 sec. (i.e., 0.017 Hz) for the Picture Recognition Task and 40 sec. (i.e., 0.025 Hz) for the Visual Stimulation Condition. Time-series statistical analysis was carried out with local autocorrelation correction (Woolrich *et al.*, 2001). fMRI EPI datasets were registered to the high resolution EPI image, the high resolution EPI image to the T1-weighted image, and the T1-weighted image to the 2 mm isotropic MNI-152 standard space image (T1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, Canada) (Jenkinson and Smith, 2001; Jenkinson *et al.*, 2002).

fMRI data analysis

For each participant, two explanatory variables (EVs) were included in a general linear model, representing the Fearful and Happy facial expression blocks. Besides the main effects of both expression separately, two other contrasts of interest were defined: Fearful > Happy, Happy > Fearful.

The first-level images of the contrasts of parameter estimates and their corresponding variances were registered to standard space and fed into a second level mixed effects group analysis (Woolrich *et al.*, 2004). We tested for group main effects (one-sample *t*-tests) and between group effects (independent *t*-tests).

The resulting statistic images were cluster corrected for multiple comparisons using an initial cluster forming threshold of $z>2.0$, and a corrected cluster significance of $p<.05$. For clusters or regions where significant effects were observed, mean *z*-scores were extracted from those clusters for each individual participant to create bar graphs for illustration of the effects. The Harvard-Oxford cortical and subcortical atlases, available in FSL, were used for anatomical reference.

Region of interest (ROI) analysis:

The bilateral amygdala was selected for the Facial Expression Processing, using the Harvard-Oxford Subcortical Probability atlas. All regions were thresholded at a 50% probability, binarized, and used as pre-threshold masks in the respective group level mixed effects analyses. Cluster correction was also used within the ROI ($z>2.0$, $p< .05$).

Emotional Test Battery

Facial Expression Recognition

The facial expression recognition task (FERT) used a different set of facial stimuli than the one used in the scanner. Pictures of faces from Ekman and Friesen (1976) were presented sequentially on a computer screen in randomized order for 500ms. Faces expressed one of five emotions: happiness, sadness, fear, anger, or disgust. Participants were instructed to identify the emotion by pressing the corresponding key on the response box as quickly and accurately as possible. Emotional expressions had been morphed between two standard images, 0% (neutral) and 100% (full emotion) in 10% steps. Four examples of every emotion at each intensity level (40/Emotion) were presented together with neutral expressions (4 trials) making a total of 204 stimuli presentations. Accuracy, reaction time for correct choices, and misclassifications were recorded.

Emotion categorization and memory

– ***Categorization.*** Sixty personality characteristics generally considered to be disagreeable (e.g., untidy, hostile) or agreeable (e.g., honest, optimistic) were presented on the computer screen for 500ms. Positive and negative words were matched in terms of word length, ratings of usage frequency. Volunteers were asked to categorize these words as quickly as possible. Specifically, they were asked to indicate whether they would be pleased or upset if they overheard someone else referring to them as possessing this characteristic. Reaction times to positive and negative traits were computed.

– ***Free Recall.*** Fifteen minutes after completion of the categorization task, participants were asked to recall as many of the personality traits as possible within two minutes. Hits (correct responses) and intrusions (false responses) were analysed.

– ***Recognition.*** The 30 disagreeable and 30 agreeable characteristics were intermixed with an equal number of distracters that were not presented previously. The number of hits (correct recognitions) and reaction times were computed. Sensitivity (*d'*) and response bias (β) were computed as in Tranter *et al.*,2009.

Visual-Probe

Biased attention was assessed using the visual-probe task. Stimulus pictures were selected from the International Affective Picture Set (Lang *et al.*,2005). Sixteen of these had a negative valence, 16 a positive valence and 32 were neutral. The categories negative and positive valence were matched on arousal ratings (*M*= 5.7) and differed in valence (*M*=7.3 for positive, *M*=2.4 for negative). Mean arousal and valence ratings for the neutral pictures were 3.2 and 5.1, respectively. All stimulus pictures were presented in grayscale. The probes consisted of images of either one or two black dots, four pixels wide and high.

The task started with a number of practice trials, in which a separate set of neutral pictures was used. Practice trials continued until six consecutive trials were correctly answered with a minimum of eight trials. The actual assessment was based on 192 trials, divided in six cycles of 32 trials. Within each cycle, each positive and negative image was randomly selected once and randomly paired with one of the 32 neutral images. Within trials of each valence (positive-neutral, negative-neutral), the location of the emotional stimulus, the probe identity and the congruency were counterbalanced and randomized.

The stimulus display showed two pictures in a horizontal arrangement. Timing of a trial was as follows: cross (500ms)- stimuli (500ms)- probe (until response)- inter trial interval (750ms). A short self-paced break was offered every 30 trials. Participants were instructed to respond as accurately and as fast as possible.

Design

Randomized, double-blind placebo-controlled, parallel-group study. Randomization was carried out in blocks of six, and was stratified for sex. The study was conducted at the LUMC and randomization was carried out by the LUMC pharmacy, Leiden. The study included two lab visits separated by 6 or 7 days.

Procedure

First Lab Visit

Participants who showed interest were provided with information by email and underwent a brief telephone screening. Upon arrival at the laboratory, participants provided written informed consent after the study had been fully explained. Participants underwent a screening procedure including the M.I.N.I. interview and a physical examination. Screening for alcohol use was done by means of a breath test, drugs screening (QD1 220 Drug card-Quantum diagnostics,UK) and a pregnancy test (QuickVue-Quidel Corporation San Diego,USA) were done by urine tests. Next, participants completed questionnaires and the IQ test. After ARA290 or placebo (i.v. 2 mg) administration, the participant was monitored for 10 minutes. At the end of the session participants were given written and oral instructions for the coming week: no nutritional supplements, no drugs or tobacco and limitation of alcohol use to 4 units/day with a maximum of 14 units/week. Caffeine intake was forbidden within one hour before the second lab visit. Participants received a diary in which they were asked to record any violations of these instructions.

Second Lab visit

Upon arrival the participant handed in the diary and was interviewed to check for compliance. Following screening for alcohol use, drug use and pregnancy, participants completed questionnaires and the Picture Encoding Task (Supplementary Material). Participants underwent the MRI scanning session (60 minutes) and completed the Emotional Test Battery afterwards.

Results

Sample characteristics

Of the 45 individuals who were invited to an intake session, two did not show up (illness (*N*=1); did not provide a reason (*N*=1)). Five participants did not meet in- and exclusion criteria (use of soft drugs (*N*=1); excess alcohol use (*N*=1); history of MDD (*N*=2); impaired vision (*N*=1). One eligible participant could not be included due to miscommunication among staff (no certified person available to administer treatment). One participant dropped out after the first lab visit for personal reasons unrelated to the project. Thirty-six participants completed the whole study (Figure 1). The analyses were conducted on 36 participants, except for the fMRI data for which 34 participants were included. fMRI data of two participants were excluded due to head motion (>3mm or 3° in any direction). Groups did not differ in age, IQ, sex distribution and clinical characteristics (Table 1).

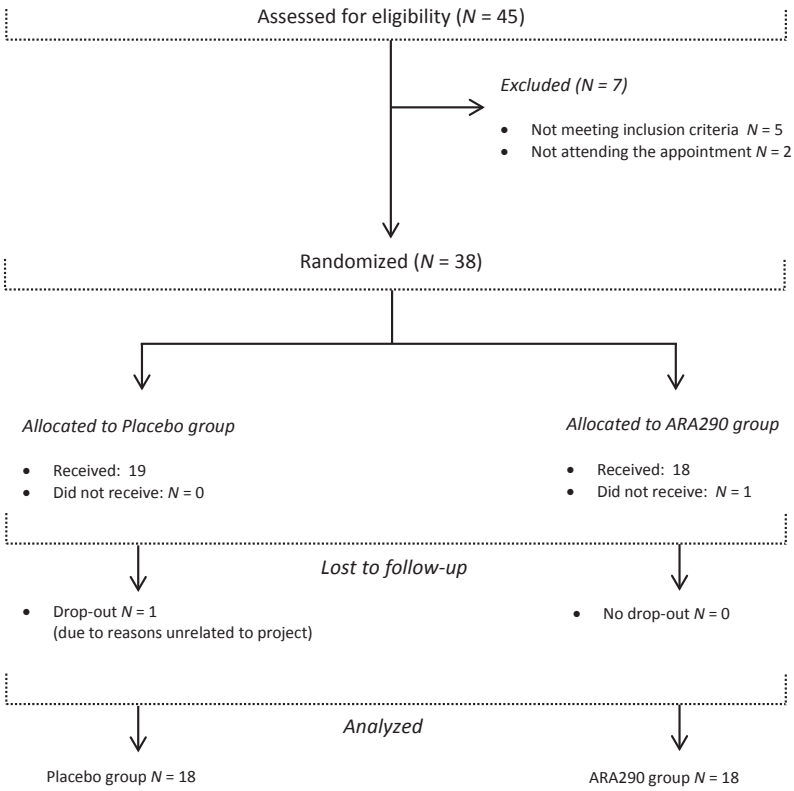

Figure 1. Numbers of participants assessed and included in the data analysis of the double-blind, randomized placebo-controlled clinical trial.

Table 1. Demographics and Characteristics

	ARA290 (*M±SD)*	PLC (*M±SD)*
Sex (f/m)	9/9	9/9
Age	21.3 ± 3.5	20.5 ± 2.0
IQ	104.4 ± 7.9	104.8 ± 7.8
PANAS-S *Positive*		
Pre-treatment	31.5 ± 5.0	33.8 ± 6.0
Post-treatment	31.4 ± 5.6	31.7 ± 6.3
PANAS-S *Negative*		
Pre-treatment	12.6 ± 2.3	13.2 ± 2.6
Post-treatment	12.7 ± 1.6	12.1 ± 1.8
HADS *Anxiety*		
Pre-treatment	2.4 ± 1.8	4.0 ± 2.7
Post-treatment	2.9 ± 1.3	4.0 ± 2.8
HADS *Depression*		
Pre-treatment	1.4 ± 1.2	2.0 ± 2.0
Post-treatment	2.2 ± 1.9	2.1 ± 2.2

PLC = Placebo; f = female; *M* = Mean; *SD* = Standard Deviation

Effects on affective symptoms and mood states

RM-ANOVA with Time (pre-post) as within-subject factor and Treatment (ARA290-Placebo) as between-subject factor was conducted separately on the Anxiety and Depression scale scores of the HADS. No main effects of Time were found in either analysis. A main effect of Treatment was found at trend level on the Anxiety scale ($F(1, 34)=4.10, p=0.051$), with lower anxiety levels in the ARA290 group (Table 1). No interactions effects were found. RM-ANOVAs on the Positive and Negative Affect scores revealed no main effect of Time, Treatment, or interaction effect (Table 1).

A 6x5x2 RM-ANOVA with Time (pre-treatment, day 1-5 post-treatment) and mood state (5 scales) as within-subject factors and Treatment (ARA290-Placebo) as between-subject factor was conducted on 30 participants (ARA290, $N=13$; Placebo, $N=17$) because

eight participants had missing MSS scores on one or more days. A significant main effect of Scale ($F(1.74,48.67)=142.19;p<0.01$) and a trend-level interaction effect of Time x Scale ($F(8.37,234.24)=1.85,p=0.066$) were found. No main or interaction effects involving Treatment were found (Table S1).

BOLD Response-Facial Expression Processing

Whole brain- main effects of emotion. In both the ARA290 and Placebo group, the processing of fearful (Table S2a) and happy (Table S2b) facial expressions activated multiple regions within the occipital cortex, the precentral gyrus and motor cortex. No differences were found between the two groups for each of the emotions separately.

Whole brain- Intervention x Emotion interaction. A between groups difference on the contrast Fear > Happy was found in the lateral occipital cortex, supramarginal gyrus and temporal occipital fusiform cortex (Table 2; Figure 2). The mean *z*-scores of the clusters for each condition (happy and fear) are presented in Figure S1. The interaction of Intervention x Emotion in the fusiform gyrus is mainly driven by the difference in response of the ARA290 and Placebo groups to happy faces. Specifically, processing of happy faces resulted in reduced activation in the ARA290 group compared to Placebo in the bilateral fusiform gyrus, whereas processing of fearful faces elicited increased activation in the right fusiform gyrus in the ARA290 group compared to placebo. A small volume correction applied within the bilateral amygdala mask did not reveal any differences between the two groups.

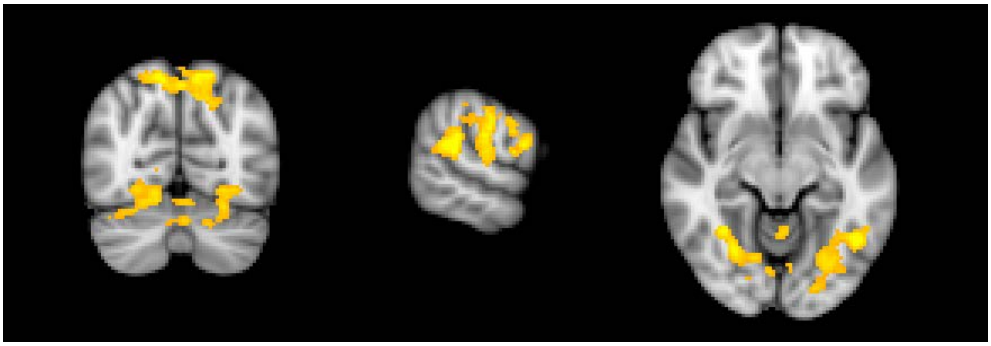

Figure 2. BOLD response during Facial Expression Processing task. Contrast Fear vs. Happy blocks, in clusters where BOLD response by ARA290 is greater than by placebo (x = 62 y = -68 z = -10) *(z>2.0, corrected p<.05).*

Table 2. Contrast Fear > Happy for ARA290 > Placebo

Region	Hemisphere	Cluster size 2 mm	Peak voxel coordinates			Z-value
ARA290 > Placebo			X	Y	Z	
Lateral occipital cortex	R	1461	12	-70	56	3.20
	L		-12	-70	56	3.04
Precuneus	R		4	-80	54	3.05
Cuneus	L		-6	-82	42	2.92
Fusiform gyrus	R	1174	34	-52	-12	3.25
Lingual gyrus	R		24	-62	-4	3.13
Cerebellum	L		-2	-62	-28	2.86
Supramarginal gyrus	R	1026	62	-36	16	3.26
Postcentral gyrus	R		64	-14	28	3.21
Precentral gyrus	R		62	6	18	2.92
Inferior temporal	L	947	-44	-56	-8	3.20
Cerebellum	L		-22	-60	-30	2.88
Fusiform gyrus	L		-30	-72	-10	2.62

z>2.0 and a corrected cluster significance threshold of p=.05

Behavioural response-Facial Expression Processing

Groups did not differ in accuracy of gender discrimination during emotional face processing. Overall accuracy scores were high (95% correct) on both happy and fearful faces. A 2x2 RM-ANOVA on reaction times with Emotion as within-subjects factor and Treatment as between-subjects factor revealed no significant main or interaction effects. This allows us to asses any neural differences unconfounded by differences in performance of the task.

Effects on Emotional Test Battery

- Facial Expression Recognition

RM-ANOVA was conducted with Emotion (six facial expressions) as within-subjects factor and Treatment (ARA290-Placebo) as between-subjects factor on total accuracy scores of each emotion. The main effect of Emotion was significant ($F(3.27,111.41)=240.08, p<0.001$).

A main effect of Treatment on total accuracy was found (F(1,34)=5.15,p=0.030) with reduced performance in the ARA290 group, but no significant interaction effect was found (F(3.27,111.41)=0.99,p=0.402) (Figure S2;Table S3).

Separate RM-ANOVAs were conducted for each emotion of the FERT, with Intensity (10 levels) as within-subjects factor and Treatment (ARA290-Placebo) as between-subjects factor on accuracy scores. The main effect of Intensity was significant for each emotion. Differences between groups were only found on the emotions "Happy" and "Disgust". A main effect of Treatment on the emotion "Happy" was found at trend level (F(1,34)=4.07,p=0.052), but no interaction effects were found. This was driven by worse relative performance in the ARA290 compared to the placebo group. A main effect of Treatment (F(1,34)=5.10,p=0.031) and a trend-level Intensity x Treatment interaction (F(5,169.99)=2.13,p=0.065) were also found on the emotion "Disgust". Overall, the emotion of "Disgust" was recognized less accurately by the ARA290 (M=24.4, SD=4.4) than the Placebo-treated group (M= 27.1, SD= 2.3).

Separate RM-ANOVAs were conducted with Emotion (6 levels) as within-subjects factor and Treatment (ARA290-Placebo) as between-subjects factor on reaction times, target sensitivity (d') and response bias (β) (Table S3). There were no effects of Treatment or interaction effects on the outcomes reaction times or target sensitivity. Only a main effect of Treatment on response bias (β) was found (F(1,34)=4.59,p=0.039), but no significant interaction effect (F(1.82,61.9)=0.90,p=0.40). Independent-samples t-tests revealed that the ARA290-treated group had a higher β value i.e. fewer false alarms (M=0.94, SD=0.070) than the Placebo-treated group (M=0.89, SD=0.08) for the sad faces (t(34)=2.11,p=0.043) (Table S3).

- Emotion categorization

A 2x2 RM-ANOVA on the reaction times, with Valence (Positive-Negative) as within-subjects factor and Treatment (ARA290-Placebo) as between-subjects factor revealed a trend effect of Valence (F(1,34)=3.06,p=0.09) and a Valence x Treatment interaction effect (F(1,34)=5.61,p=0.024). This was driven by increased speed to positive (M=770, SD=134.9) *vs* negative (M=831, SD=156.8) words following ARA290 (t(17)= 3.50,p=0.003) (Table 3). There was no main effect of Treatment on reaction times (F(1,34)=0.261),p=0.613). The same analyses on the accuracy scores revealed no main or interaction effects (Table 3).

- Emotional Memory -Free Recall

A 2x2 RM-ANOVA on the recall scores with Valence (Positive-Negative) as within-subjects factor and Treatment (ARA290-Placebo) as between-subjects factor revealed no main or interaction effects (Table 3). The same analysis on Intrusive memory scores revealed only a main effect of Valence (F(1,34)=30.3,p<0.001). This was driven by increased false recalls (intrusions) in positive *vs* negative words in both groups (Table 3).

- Emotional Memory -Recognition

A 2x2 RM-ANOVAs on Hits with Valence (Positive-Negative) as within-subjects factor and Treatment (ARA290-Placebo) as between-subjects factor revealed only a main effect of Valence ($F(1,34)=36.50, p<0.001$). This was driven by increased Hits in positive vs negative words in both groups (Table 3).

The same analysis on reaction time (for hits) revealed only a main effect of Valence ($F(1,34)=8.94, p=0.005$). This effect was driven by longer reaction times to negative ($M=1038.9$, $SD=254.6$) *vs* positive ($M=975.5$, $SD=252.1$) words in the Placebo group ($t(17)=2.75, p=0.014$.

Separate RM-ANOVAs were conducted with Valence (Positive-Negative) as within-subjects factor and Treatment (ARA290-Placebo) as between-subjects factor on target sensitivity (*d'*) and response bias (β). These analyses revealed only a main effect of Valence on response bias. In both groups conservative response (i.e., a higher β value) was given to negative compared to positive words.

Visual-Probe

RM-ANOVA with Valence (Negative-Positive Bias Index) as within-subject factor and Treatment (ARA290-Placebo) as between-subject factors revealed no main effect of Valence ($F(1,34)=2.89, p=0.098$) or interaction effect. A main effect of Treatment ($F(1,34)=6.82, p=0.013$) was found. The ARA290 group had higher bias indexes for both valences (Figure 3).

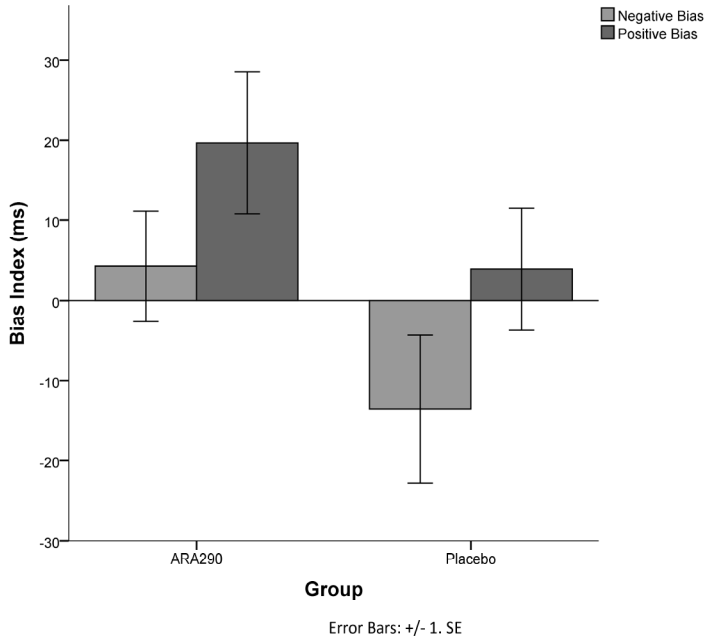

Figure 3. Attentional Bias for positive and negative pictures.

Table 3. Emotion Categorization and Memory (Free Recall and Recognition)

			ARA290 (*M* ± *SD*)	Placebo (*M* ± *SD*)
Categorization	Hits (# correct out of 30)	*Positive*	28.0 ± 2.1	28.1 ± 1.9
		Negative	28.3 ± 1.2	26.7 ± 6.2
	Reaction Time (ms)	*Positive*	770 ± 134.9*	834 ± 232.2
		Negative	831 ± 156.8*	825 ± 166.5
Free Recall	Hits (# correct recall)	*Positive*	3.8 ± 2.6	4.3 ± 1.5
		Negative	3.8 ± 1.9	3.4 ± 1.6
	Intrusions (# false recall)	*Positive*	2.0 ± 1.7	1.9 ± 1.8
		Negative	0.3 ± 0.6	0.6 ± 0.7
Recognition	Hits (# correct out of 60)	*Positive*	24.2 ± 4.1	25.7 ± 2.5
		Negative	20.4 ± 4.4	19.9 ± 5.9
	Reaction Time (ms)	*Positive*	947.6 ± 232.4	975.5 ± 252.1
		Negative	998.1 ± 254.3	1038.9 ± 254.6

Target sensitivity (*d'*)	*Positive*	0.851 ± 0.102	0.841 ± 0.162
	Negative	0.866 ± 0.072	0.859 ± 0.101
Response bias (β)	*Positive*	-0.148 ± 0.437	-0.173 ± 0.384
	Negative	0.393 ± 0.354	0.370 ± 0.318

* Valence x Treatment interaction effect (p=0.024). ACC = Accuracy; RT = Reaction Times; ms = milliseconds; *M* = Mean; *SD* = Standard Deviation

Discussion

We used a cognitive neuropsychological model of antidepressant drug action to investigate antidepressant-like effects of ARA290, a derivative of EPO. Based on the literature on EPO, a single dose of ARA290 or placebo was administered to healthy volunteers and the effects were measured one week after administration.

On the primary outcome measures, we observed two small trend-level differences between ARA290- and placebo-treated individuals. The recognition of both happy and disgusted facial expressions in the behavioural task tended to be lower after ARA290 than after placebo. ARA290 had no effect on the neural processing of facial stimuli (positive ***vs*** negative expressions) in the amygdala, hippocampus or vmPFC in healthy individuals. However, ARA290-treated individuals did show reduced neural responses to happy ***vs*** fearful faces in the fusiform gyrus, a region involved in face- specific processing (McCarthy *et al.*, 1997).

On secondary outcomes, we found faster categorization of positive (self-referential) words compared to negative words in the ARA290-treated group, but not a better memory for positive words. Furthermore, ARA290-treated individuals had a higher positive attentional bias score than placebo-treated individuals.

Unlike EPO (Miskowiak *et al.*, 2007b), ARA290 did not increase memory-relevant neural response in the hippocampus during a picture recognition task. Finally, ARA290 did not have an effect on self-reported mood states or affective symptoms.

We hypothesized that ARA290 would be associated with reduced recognition of fearful and/or increased recognition of happy facial expressions. The observed trend-level effects on happy and disgust expressions were both in the same direction, which cannot be interpreted as an antidepressant-like effect. The reduced neural response to happy faces in the fusiform gyrus found in the ARA290 group, is the same region as reported by Miskowiak *et al.* (2007a) for EPO. However the direction of this effect was opposite: in Miskowiak *et al.* (2007a), EPO reduced the response to fearful ***vs*** neutral faces. In our study the difference was mainly driven by the effect of ARA290 on the happy faces which resulted in lower activation in the ARA290 group in the bilateral fusiform gyrus. Processing of fearful faces elicited increased activation in the right fusiform gyrus in the ARA290 group compared to placebo.

In contrast with the previous demonstration of enhanced bilateral hippocampus response in healthy participants after EPO (Miskowiak *et al.*, 2007b), ARA290 did not reliably increase the neural response in the hippocampi during the same picture recognition task. A methodological difference between the current study and the EPO study is that our participants completed the encoding task outside the scanner, meaning that the encoding task was completed one hour before recognition. The longer time frame between the two related tasks may have weakened the hippocampus response.

Consistent with a possible anti-depressant effect, ARA290 was associated with faster categorization of positive ***vs*** negative self-referential words. This is in line with the effects of single dosages of conventional antidepressants in healthy individuals (Harmer *et al.*, 2003; 2004) and could suggest that ARA290 may lead to a shift from negative towards more positive information processing, particularly regarding self- image. However, ARA290 had no effect on memory (i.e., recall and recognition) of positive ***vs*** negative self-referential words. Although there are no EPO studies with this specific outcome, we would expect improvement in memory for positive ***vs*** negative words, based on studies conducted with conventional antidepressant drugs (Harmer *et al.*, 2004; Arnone *et al.*, 2009). ARA290 did increase the attention for positive stimuli similar to the effect of citalopram on attention in healthy volunteers (Browning *et al.*, 2007; Murphy *et al.*, 2009).

Taken together, on our primary outcomes we found that ARA290 tended to lower the recognition of both positive (happy) and negative (disgust) faces. ARA290 also elicited a differential neural response compared to placebo during processing of facial expressions, though in an unexpected direction. However, on the secondary outcomes of emotional categorization and attention ARA290 did show antidepressant-like effects. Our findings show that while ARA290 modulates some aspects of emotional processing the direction and the strength of its effects is overall not congruent with the biomarker model of early antidepressant effects (Harmer *et al.*, 2009, 2010), in which antidepressants produce a marked shift from a negative towards a positive emotional processing bias.

A strength of our study is that we had a relatively large sample size. Therefore, there is little risk that the present negative findings are due to type II error. However, the study has some limitations which need to be addressed. Except for measures related to mood, affective symptoms, we did not include baseline measurements prior to ARA290 administration. The reason for this is that all other measures include emotional stimuli which are subjective to learning and habituation effects. Therefore, all measurements related to emotional processing were completed one week after administration of ARA290 or placebo. Since we had to limit the baseline measurements we cannot be certain whether the several small effects found in this study are due to a pre-existing difference between the groups (i.e., randomization failure) or due to the ARA290 treatment. However, we did control for changes in mood and subjective states and participants were matched for age, sex and IQ.

We based our current study design on findings with EPO (Miskowiak *et al.*, 2007a; 2007b), as data published on ARA290 in healthy populations is still sparse. Therefore, we might not have been able to examine the effect of ARA290 with the highest effective dose and the time point on which ARA290 is the most effective. Administration of this dose (2 mg i.v.) to somatic patients (i.e., neuropathic pain patients) raised no safety concerns (Heij *et al.*, 2012; Niesters *et al.*, 2013). Since this is the first study assessing the antidepressant properties of ARA290 in humans, a single dose of 2 mg might have been too low to exert an antidepressant-like effect in healthy participants and/or the effect of ARA290 may have lasted shorter than one week.

ARA290 acts on the receptor that initiates tissue-protective and anti-inflammatory actions. ARA290 might exert its beneficial effects on mood and cognition by decreasing inflammation in the central nervous system, and therefore it may be interesting to look at the effect of ARA290 in a subtype of depression, namely depressive patients with high inflammation biomarkers in their blood.

As a first step towards a clinical trial in patients, we tested the effects of a single dose in healthy volunteers on the cognitive and neural processing of emotions one week after ARA290 administration. The model we have used has been validated with various registered antidepressants (reviewed by Harmer *et al.*, 2009). Although ARA290 does not seem to cause a marked shift from negative to positive emotional processing, it does have an effect on emotional processing in general. Our study needs replication and future studies may benefit from: a) higher doses and/or repeated administration of ARA290; and b) earlier measurement of the effects of ARA290 after its administration.

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Supplemental Material

Methods

fMRI tasks

Picture Encoding and Recognition Task: The picture encoding task was completed before scanning in a quiet room. One hour later participants completed the picture recognition task inside the scanner. The encoding and recognition tasks were selected because of hippocampal engagement in encoding and recognition of complex visual scenes (Stern *et al.*, 1996; Hariri *et al.*, 2003), and were the same as the ones used by Miskowiak *et al.* (2007b). Pictures were matched for emotional valence, arousal, and visual complexity, and were presented in a blocked paradigm to maximize sensitivity for hippocampal blood oxygenation level dependent (BOLD) signal change (Birn *et al.*, 2002; Miskowiak *et al.*, 2007b). In both tasks, each of the eight picture blocks (24 sec.) were preceded by brief instructions (2 sec.) and presented interleaved with 20 sec. of fixation cross, resulting in a total task duration of 6 min. Blocks consisted of 6 pictures presented serially for 3 sec. interleaved with a 1 sec. fixation cross. The encoding and recognition tasks contained an equal number of pictures representing indoor and outdoor scenes. In addition, the recognition task contained an equal number of old (i.e., previously encoded) and new pictures. During encoding, volunteers determined whether the picture represented an "indoor" or "outdoor" scene, while in the subsequent recognition task they needed to determine whether the picture was "old" or "new".

Visual Stimulation Condition: To explore whether drug-related effects observed during the Facial Expression Processing and Picture Recognition tasks were attributable to global effects of ARA290 on the BOLD signal, a control visual stimulation condition was used. A flashing checkerboard (frequency, 8 Hz) was presented in blocks of 15 sec. alternating with 15 sec. of a fixation cross for a total of 10 cycles (total duration of 5 min.). Participants were instructed to passively view the screen.

fMRI data analysis

Picture Recognition Task: One explanatory variable (EV) was included in a general linear model, representing the recognition blocks during which participants had to respond whether they had seen the picture before or not. Contrasts were made for task-related activation and deactivation.

Visual Stimulation Condition: One explanatory variable (EV) was included in a general linear model, representing the visual stimulation block during which participants had to view the flickering checkerboard. One contrast of interest was made for task-related activation.

The first-level images of the contrasts of parameter estimates and their corresponding variances were registered to standard space and fed into a second level mixed effects group analysis (Woolrich *et al.*, 2004). We tested for group main effects (one-sample *t*-tests) and between group effects (independent *t*-tests). The resulting statistic images were cluster corrected for multiple comparisons using an initial cluster forming threshold of $z>2.0$, and a corrected cluster significance of $p<.05$.

Region of interest (ROI) analysis

The bilateral hippocampus was selected for the Picture Recognition Task, and area V1 for the Visual Stimulation Condition. To define the hippocampus ROI's the Harvard-Oxford Subcortical Probability atlas was used, for area V1 the Juelich Histological atlas. All regions were thresholded at a 50% probability, binarized, and used as pre-threshold masks in the respective group level mixed effects analyses. Cluster correction was also used within the ROI ($z>2.0$, $p<0.05$).

Supplemental Material

Results

BOLD Response- Picture Recognition Task

Whole brain - main effect of task. In the ARA290 group, recognition of in- or outdoor scenes activated the visual cortex and a cluster in the paracingulate gyrus and supplementary motor cortex (Table S4). A similar pattern of activation was observed in the Placebo group (Table S4). The ARA290 group did not differ from the Placebo group in neural response during recognition of pictures, neither when the analysis was reduced to the ROI comprising voxels within the bilateral hippocampus.

Behavioural Response- Picture Recognition Task

Picture Encoding:

Independent-samples *t*- tests revealed no significant differences between the ARA290-treated group (M = 44, SD = 10.8) and the Placebo-treated group (M = 47, SD = 1.9) on accuracy (t (33) = -1.084, p = 0.29). The same analysis revealed no differences between the ARA290-treated group (M = 762, SD = 157.5) and the Placebo-treated group (M = 784, SD = 157.9) on reaction times (t (33) = -0.418, p = 0.68).

Picture Recognition:

Independent-samples *t*- tests on the four categories (misses, false alarms, correct rejections and hits) with Treatment as grouping variable revealed no differences between the two groups. Independent-samples *t*- tests on Target sensitivity (d') and Response bias (β) with Treatment as grouping variable revealed a trend for response bias (p = 0.058). There was a trend toward a higher β value i.e. fewer false alarms in ARA290-treated (M = 0.13, SD = 0.15) vs Placebo-treated group (M = 0.04, SD, = 0.13) (t (34) = 1.963, p = 0.058).

BOLD Response- Visual Stimulation Condition

Both the whole brain and ROI analysis (within area V1) revealed no differences between the two groups in response to the visual stimulation condition.

Table S1 Mood State Scale

	Group	Pre (*M* ± *SD*)	D1 (*M* ± *SD*)	D2 (*M* ± *SD*)	D3 (*M* ± *SD*)	D4 (*M* ± *SD*)	D5 (*M* ± *SD*)
Sadness	ARA290	0.5 ± 0.8	1.5 ± 1.3	1.5 ± 1.9	1.6 ± 1.7	1.7 ± 1.5	1.2 ± 1.7
	Placebo	0.6 ± 0.9	1.6 ± 2.1	1.7 ± 2.1	1.6 ± 1.8	0.6 ± 0.9	0.9 ± 1.3
Annoyance	ARA290	0.2 ± 0.4	1.5 ± 1.5	1.8 ± 2.2	1.7 ± 1.7	1.2 ± 1.3	1.5 ± 1.6
	Placebo	0.8 ± 1.3	1.4 ± 1.6	2.1 ± 2.3	1.8 ± 2.3	1.2 ± 1.3	1.5 ± 2.3
Tension	ARA290	2.0 ± 1.4	2.1 ± 2.0	2.1 ± 2.2	2.5 ± 2.3	1.9 ± 1.7	2.0 ± 1.5
	Placebo	2.5 ± 1.5	2.2 ± 2.3	2.5 ± 1.7	2.5 ± 1.7	2.4 ± 2.0	2.4 ± 2.3
Cheerfulness	ARA290	5.6 ± 1.7	5.8 ± 1.5	4.9 ± 2.3	5.1 ± 1.8	5.4 ± 1.5	5.8 ± 2.0
	Placebo	6.5 ± 1.2	5.5 ± 1.8	5.5 ± 1.8	5.3 ± 1.8	6.1 ± 1.4	5.3 ± 2.1
Energetic	ARA290	5.4 ± 1.3	5.8 ± 1.9	5.3 ± 2.1	5.3 ± 1.9	5.3 ± 1.7	5.7 ± 1.8
	Placebo	6.3 ± 1.5	5.3 ± 2.1	5.5 ± 1.5	5.1 ± 2.2	6.1 ± 1.3	5.4 ± 2.2

M = Mean; *SD* = Standard Deviation

Table S2A BOLD response during fear blocks - ARA290 and placebo groups

Region	Hemisphere	Cluster size 2 mm	Peak voxel coordinates			Z-value
ARA290			X	Y	Z	
Occipital pole	L	31647	-14	-100	-4	6.27
Lateral occipital cortex	R		42	-78	-14	6.07
Fusiform gyrus	R		38	-54	-26	5.88
Lingual gyrus	R		8	-76	-16	5.62
Postcentral gyrus	L	6314	-40	-26	56	6.35
Precentral gyrus	L		-38	-8	64	5.19
Supplementary motor	L	1527	-4	2	52	5.04
	R		10	4	46	4.48
Anterior cingulate	L		-10	18	30	2.33
Superior frontal gyrus	R		8	14	54	2.25
Placebo						
Supplementary motor	L	41478	-8	8	50	5.96
Cerebellum	R		24	-52	-24	5.64
Occipital pole	R		22	-100	-4	5.28
Fusiform gyrus	R		38	-58	-18	5.22
Inferior frontal gyrus	R		50	12	6	5.03
Precentral gyrus	L		-38	-20	68	4.80

z>2.0 and a corrected cluster significance threshold of p=0.05

Table S2B BOLD response during happy blocks - ARA290 and Placebo groups

Region	Hemisphere	Cluster size 2 mm	Peak voxel coordinates			Z-value
ARA290			X	Y	Z	
Fusiform gyrus	L	33197	-34	-78	-16	6.25
	R		36	-60	-22	5.91
Lateral occipital cortex	R		42	-76	-14	6.20
Occipital pole	L		-20	-92	0	5.80
Cerebellum	R		36	-52	-28	5.68
Precentral gyrus	R	1999	46	-2	46	4.68
Placebo						
Cerebellum	R	53450	26	-52	-24	5.89
Lateral occipital cortex	R		38	-76	-20	5.36
Occipital pole	R		22	-100	-4	5.30
	L		-14	-100	-4	5.26
Fusiform gyrus	R		38	-58	-20	5.26
Supplementary motor	R		10	2	50	5.18

z>2.0 and a corrected cluster significance threshold of p=0.05

Table S3 Facial Expression Recognition Task

	ARA290 (*M* ± *SD*)	PLC (*M* ± *SD*)
Accuracy (Hits)		
Anger	18.1 ± 5.1	20.6 ± 4.2
Disgusted	24.4 ± 4.4	27.1 ± 2.3
Fearful	24.9 ± 2.6	25.7 ± 2.8
Happy	26.9 ± 3.8	29.2 ± 2.7
Sad	17.0 ± 3.8	19.1 ± 7.6
Neutral	3.9 ± 0.3	3.7 ± 0.7
Reaction time (ms)		
Anger	1275.2 ± 299.5	1236.5 ± 297.8
Disgusted	1028.9 ± 433.5	991.9 ± 219.2
Fearful	1016.1 ± 309.4	1133.1 ± 317.2
Happy	866.1 ± 250.4	810.6 ± 198.0
Sad	1154.0 ± 239.6	1148.5 ± 391.3
Neutral	788.0 ± 387.3	882.3 ± 393.0
Target sensitivity (*d'*)		
Anger	0.84 ± 0.04	0.87 ± 0.03
Disgusted	0.88 ± 0.05	0.91 ± 0.02
Fearful	0.89 ± 0.02	0.90 ± 0.02
Happy	0.92 ± 0.02	0.93 ± 0.02
Sad	0.85 ± 0.02	0.86 ± 0.05
Neutral	0.90 ± 0.04	0.88 ± 0.07
Response Bias (*β*)		
Anger	0.83 ± 0.15	0.90 ± 0.10
Disgusted	0.71 ± 0.21	0.78 ± 0.15
Fearful	0.75 ± 0.13	0.83 ± 0.15
Happy	0.96 ± 0.06	0.98 ± 0.04
Sad	0.94 ± 0.07*	0.89 ± 0.08
Neutral	-0.89 ± 0.31	-0.76 ± 0.46

PLC = Placebo, *M* = Mean; *SD* = Standard Deviation

Table S4 Main Effects Picture Recognition task (activation)

Region	Hemisphere	Cluster size 2 mm	Peak voxel coordinates			Z-value
ARA290			X	Y	Z	
Lingual gyrus	L	60522	-4	-88	-8	7.37
Temporal occipital fusiform cortex	R		28	-54	-10	7.32
Occipital pole	L		-26	-90	6	7.18
Lateral occipital cortex	R		26	-86	16	6.96
Lateral occipital cortex, superior	R		30	-84	14	6.94
Paracingulate gyrus	L	2975	-6	12	46	6.50
	R		2	22	44	5.79
Supplementary motor	L		-6	4	52	5.74
Placebo						
Parahippocampal	L	74570	-22	-40	-16	6.87
Temporal occipital fusiform cortex	R		30	-48	-12	6.85
Occipital pole	L		-8	-94	-8	6.75
	R		20	-98	6	6.53
Lingual gyrus	L		-20	-48	-12	6.64

z>2.0 and a corrected cluster significance threshold of p=0.05

Supplemental Figures

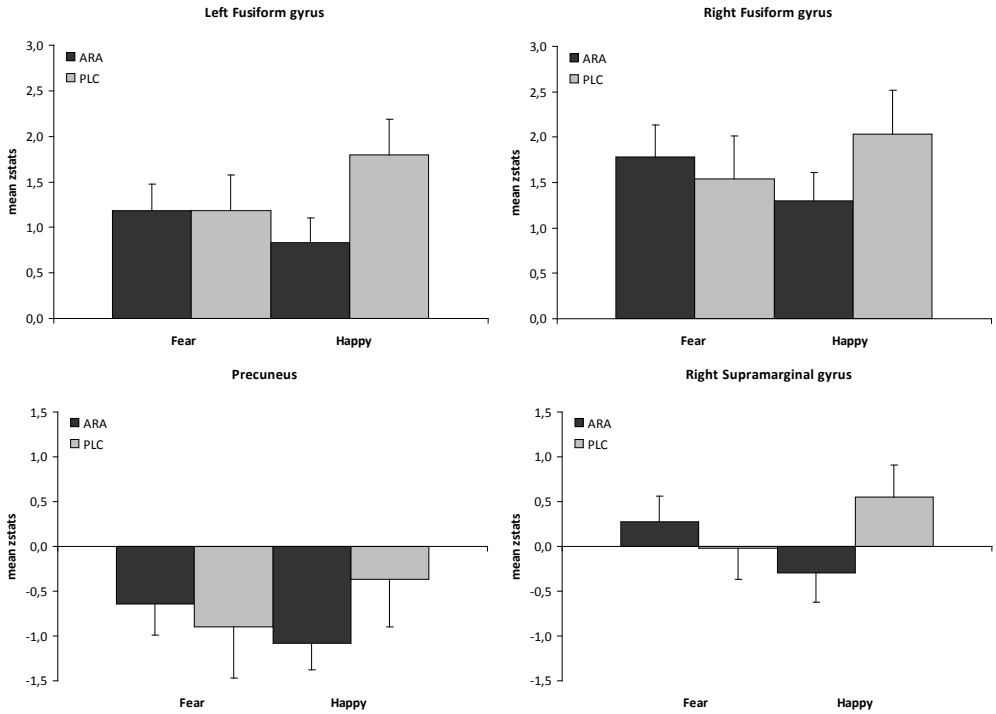

Figure S1. Mean z-stats (SE) for Fear and Happy conditions separately of the clusters resulting from contrast Fear > Happy (ARA = ARA290; PLC = Placebo).

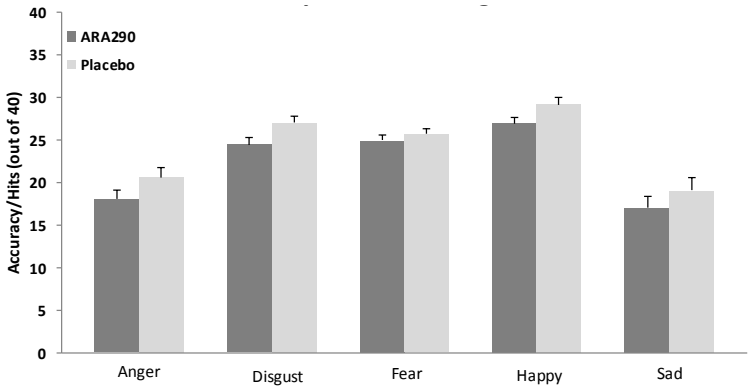

Figure S2. Accuracy scores (SE) on the Facial Expression Recognition task.