European Neuropsychopharmacology (2013) 23, 1577–1586

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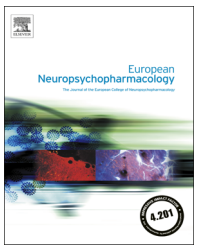

Dopaminergic modulation of the reward system in schizophrenia: A placebo-controlled dopamine depletion fMRI study

Fabiana da Silva Alves[a], Geor Bakker[g], Nicole Schmitz[a], Nico Abeling[b], Gregor Hasler[c], Johan van der Meer[d], Aart Nederveen[e], Lieuwe de Haan[a], Don Linszen[a], Therese van Amelsvoort[f,*]

[a]*Department of Psychiatry, Academic Medical Centre Amsterdam, The Netherlands*
[b]*Department of Genetic and Metabolic Disorders, Academic Medical Centre Amsterdam, The Netherlands*
[c]*Department of Psychiatry, University Hospital, University of Berne, Switzerland*
[d]*Department of Clinical Neurophysiology, Academic Medical Centre Amsterdam, The Netherlands*
[e]*Department of Radiology, Academic Medical Centre Amsterdam, The Netherlands*
[f]*Department of Psychiatry & Psychology, University of Maastricht, The Netherlands*
[g]*Department of Psychiatry & Psychology, University of Maastricht, The Netherlands*

Received 2 January 2013; received in revised form 19 April 2013; accepted 24 June 2013

KEYWORDS
Dopamine;
Brain;
Schizophrenia;
Reward;
Striatum;
AMPT

Abstract
Background: The brain reward circuitry innervated by dopamine is critically disturbed in schizophrenia. This study aims to investigate the role of dopamine-related brain activity during prediction of monetary reward and loss in first episode schizophrenia patients.
Methods: We measured blood-oxygen-level dependent (BOLD) activity in 10 patients with schizophrenia (SCH) and 12 healthy controls during dopamine depletion with α-methylparatyrosine (AMPT) and during a placebo condition (PLA).
Results: AMPT reduced the activation of striatal and cortical brain regions in SCH. In SCH vs. controls reduced activation was found in the AMPT condition in several regions during anticipation of reward and loss, including areas of the striatum and frontal cortex. In SCH vs. controls reduced activation of the superior temporal gyrus and posterior cingulate was observed in PLA during anticipation of rewarding stimuli. PLA patients had reduced activation in the ventral striatum, frontal and cingulate cortex in anticipation of loss. The findings of reduced dopamine-related brain activity during AMPT

*Correspondence to: Department of Psychiatry and Psychology, School for Mental Health and Neuroscience, Maastricht University, Postbox 616, 6200 MD Maastricht, The Netherlands. Tel.: +31 433883584.
E-mail addresses: F.daSilvaAlves@amc.nl (F. da Silva Alves), t.vanamelsvoort@maastrichtuniversity.nl (T. van Amelsvoort).

0924-977X/$ - see front matter
http://dx.doi.org/10.1016/j.euroneuro.2013.06.008

were verified by reduced levels of dopamine in urine, homovanillic-acid in plasma and increased prolactin levels.
Conclusions: Our results indicate that dopamine depletion affects functioning of the cortico-striatal reward circuitry in SCH. The findings also suggest that neuronal functions associated with dopamine neurotransmission and attribution of salience to reward predicting stimuli are altered in schizophrenia.

1. Introduction

Schizophrenia is a serious mental disorder with onset clinical symptoms typically emerging during adolescence. The estimated lifetime prevalence is approximately 0.3–0.7% and genetic and environmental factors play a role in the aetiology of schizophrenia (van Os and Kapur, 2009). Positive (delusions, hallucinations, thought disorganization) and negative (affective flattening, social withdrawal, anhedonia, lack of motivation) symptoms of schizophrenia have been attributed to disrupted dopaminergic neurotransmission and dysfunction of the brain reward circuitry.

Reward processing is mediated by cortical regions, including orbitofrontal, medial dorsolateral prefrontal and cingulate cortices and subcortical brain areas such as ventral striatum, amygdala, thalamus and hippocampus in people exempt from illness (Bjorklund and Dunnett, 2007; Mogenson et al., 1980). This circuitry is innervated by dopaminergic neurotransmission via mesolimbic and mesocortical pathways and is disrupted in patients diagnosed with schizophrenia (Davis et al., 1991; Heinz and Schlagenhauf, 2010). People with schizophrenia have problems with incentive motivation, prediction of reward, difficulty with anticipation of future positive and negative reward outcomes and difficulties learning from them (Gold et al., 2008; Ziauddeen and Murray, 2010). In particular, enhanced release of dopamine in the mesolimbic pathway in schizophrenia may lead to inappropriate motivational significance or aberrant salience to external and internal stimuli (Kapur, 2003). Hence, investigation of reward-dopamine related impairment in schizophrenia may provide insight to the neurobiological mechanisms of reward and motivation in this disorder.

The ventral striatum, an area of the mesolimbic pathway, receives much attention in studies investigating the brain reward circuitry. One of the first studies that found evidence for disrupted striatal dopaminergic neurotransmission in schizophrenia used single photon emission computed tomography and dopamine depletion with alphamethyl-paratyrosine (AMPT) (Abi-Dargham et al., 2000). This study showed a greater increase in striatal dopamine D_2 receptor radioligand binding after dopamine depletion in unmedicated schizophrenia patients compared with healthy controls. Also, functional magnetic resonance imaging (fMRI) studies on reward processing have shown decreased activation of the ventral striatum in response to monetary reward-predicting stimuli in unmedicated schizophrenia patients. Probably, increased phasic dopaminergic activity in striatum in patients with schizophrenia interferes with salience attribution and functional activation to reward-predicting stimuli (Heinz and Schlagenhauf, 2010; Juckel et al., 2006a, 2006b).

Our previous study in healthy individuals (da Silva Alves et al., 2011) showed that acute dopaminergic depletion attenuated activation of the striatum and cingulate cortices during anticipation of reward and loss – advocating dopamine's key role. In the present study, we were interested in brain activation following dopamine depletion in patients with schizophrenia compared to healthy controls. We employed fMRI during AMPT and placebo conditions to investigate how dopamine depletion affects functional response during reward processing in schizophrenia patients.

Empirical evidence shows high striatal dopamine turnover, as seen in unmedicated schizophrenia patients, to be associated with changes in phasic dopaminergic signalling of reward stimuli (Juckel et al., 2006b). Top-down modulation of this signalling by frontal regions, insula, and cingulate cortex has also been found compromised for both reward and loss processing in schizophrenia (Cohen et al., 2012). In medicated patients, second generation antipsychotics, which block dopaminergic neurotransmission restored striatal brain activation (Juckel et al., 2006a). Therefore, we expect no differences in functional response in our group of schizophrenia patients during anticipation of reward and loss in the placebo condition in comparison healthy control subjects. Furthermore, we hypothesize that acute dopamine depletion will reduce activation in frontal regions and striatum, as these areas have shown to be highly sensitive to dopaminergic depletion and have been implicated in schizophrenia. Finally, we expect overall brain activation in schizophrenia to be reduced compared to that of healthy controls.

2. Experimental procedures

2.1. Subjects

A total of 10 male patients with schizophrenia (mean age $\pm$SD; 22.70$\pm$3.2 years) and 12 male healthy controls (34.55$\pm$11.21 years) were included in the study. Individuals with schizophrenia (first episode patients, duration of illness 18$\pm$0.8 months) were recruited from the Adolescent Clinic of the Department of Psychiatry, Academic Medical Centre, University of Amsterdam (AMC). Healthy volunteers were recruited by local advertisement. The study was conducted at the Department of Psychiatry, AMC and was approved by the local Medical Ethics Committee. All participants were capable of giving written informed consent and did so, after receiving full information on the study.

All healthy individuals were interviewed by a physician using semi-structured psychiatric interview. None of the healthy participants had a history of psychiatric disorders, medical conditions affecting brain function, substance or alcohol abuse and they were not using any medication at the time of testing. Clinical diagnoses

of individuals with idiopathic schizophrenia were made according to the DSM-IV criteria by two psychiatrists independent of the study. All patients included met the criteria of the paranoid subtype of schizophrenia. Patients with schizoaffective psychosis or with history of drug use were excluded. All patients were receiving care at the psychiatric open-ward inpatient and day care units of AMC, and were all medicated at the time of testing. Urine drug screening (cocaine, tetrahydrocannabinol, opiates, amphetamines, benzodiazepines) was performed at the beginning of the first and second study day and was negative in all participating subjects.

The Positive and Negative Symptom Scale (PANSS) (Kay et al., 1987) was used to assess positive, negative and general psychopathology in the patient group. In addition, for assessment of intelligence quotient (IQ) we used the shortened Dutch version of the Wechsler Adult Intelligence Scale (WAIS-III-NL) consisting of five subtests: vocabulary, comprehension, similarities (verbal IQ), block design, and object assembly (performance IQ) (Canavan et al., 1986; Wechsler, 1997). For demographics and clinical variables see Table 1.

2.2. Study design and dopamine challenge

All subjects underwent two fMRI measurements (Day I and Day II) with an inter-scan interval of approximately 8 days. The fMRI study was conducted as a randomized double blind controlled study, with two conditions: (1) administration of AMPT and (2) administration of placebo (cellulose, corn starch) tablets. On day 1, baseline samples of blood and urine were collected and AMPT or placebo was administered at 8.00 h (T0). Subsequently, AMPT or placebo was administered again 2 h later at 10.00 h (T2) followed by collection of blood samples at 11.00 h (T3). At 12.00 h (T4) the last dose of AMPT or placebo was administrated. The fMRI scanning started 1 h after the last dose of AMPT at 13.00 h (T5). At the end of the fMRI session at 14.00 h (T6) the last blood and urine samples were collected. On day 2 the same procedures were employed, however this time subjects were assigned to the other treatment condition (AMPT/placebo) according to the crossover design. To assess the effects of AMPT on subjective well being we used the 'subjective well-being under neuroleptic' questionnaire (SWN) (de Haan et al., 2002; Naber, 1995) on both study days.

Three doses of 500 mg AMPT were orally administered (total dose 1.5 g) over two intervals of 2 h. These doses were similar to those used by Boot et al. (2008) and da Silva Alves et al. (2011).

Table 1 Demographics and clinical variables.

	Patients (10)	Controls (12)
Age	22.70 ± ± 3.2	34.55 ± ± 11.21
Years of education	10.8 (1.5)	13.7 (2.8)
Handedness	8R/2L	10R/2L
WAIS-III IQ	90.30 (16.24)	96.64 (11.72)
PANSS total	51.58 (9.61)	
PANSS positive	12.16 (3.18)	
PANSS negative	14.42 (5.57)	
Antipsychotic medication	Clozapine (1)	
	Risperidone (2)	
	Olanzapine (1)	
	Quetiapine (2)	
	Aripiprazole (3)	
	Haloperidol (1)	

Urine samples were collected at T0 and T6 for determining DA levels. Blood samples were drawn at T0, T3, and T6 for assessment of plasma levels of prolactin (PRL) and HVA, a catecholaminergic metabolite of dopamine. Detailed description of AMPT administration and assessment of catecholamines, their metabolites and prolactin was as previously explained (da Silva Alves et al., 2011).

2.3. fMRI task: monetary incentive delay

We used event-related fMRI to assess BOLD brain activation during the monetary incentive delay (MID) task (Knutson et al., 2001a). In short, the MID task was used to evoke anticipation of potential monetary reward, loss, or no consequential outcome. It consists of two sessions of 72 trials of 6 s, yielding a total of 144 trials and total duration of 14 min. During each trial, subjects were shown one of the seven cues. Cues signalling reward were denoted by circles (n=54), loss by squares (n=54), and no monetary outcome by triangles (n=36). The amount of money that subjects were able to win was indicated by one horizontal line (0.20 Euro), two lines (1.00 Euro) and three lines (5.00 Euros). Similarly, loss cues signalled the possibility of losing the same amounts of money. Subjects had to respond to the white target square that appeared for a variable length of time. To succeed in a trial, volunteers had to press the button during the time that the white square target was visible (target, 160–260 ms). Unlike the MID described by Knutson et al. (2001a, 2001b) we were unable to pay the amount of money earned during the task, reward and loss was based on point scoring.

2.4. fMRI data acquisition

fMRI data were collected using a 3T MRI Philips system equipped with a sense head coil as previously explained(da Silva Alves et al., 2011). The task stimuli were generated using the e-prime software (SCOPE V2.5.4/Pentium). For the MID task 360 event related, transversal multislice T2*-weighted gradient-echo planar images (EPI) were acquired with: echo time (TE) 30 ms, repetition time (TR) 2000 ms, 96 × 96 matrix, 35 slices, 3 × 3 mm in-plane resolution, slice thickness 3 mm with a 1 mm interslice gap, covering the entire brain. For anatomical localization transversal high-resolution structural T1-weighted volumetric images were acquired in the same session, with full head coverage, using 150 contiguous slices (1 mm thick, with 0.89 × 0.89 mm^2 in-plane resolution), a 256 × 256 × 124 matrix and a TR/TE of 24/5 ms (flip angle 45°, FOV 24 cm).

2.5. fMRI data pre-processing

All functional and structural brain images were pre-processed blind for design condition as previously explained (da Silva Alves et al., 2011). Slice time correction was used to adjust for time differences due to multi-slice image acquisition. After the realignment of the scans, visual inspection of motion-correction estimates showed that some subjects had head movements greater than 5 mm. Hence, the movement artefacts were included as regressors in the further pre-processing. The pre-processed fMRI data were then analyzed in the context of the general linear model (GLM) approach (Friston et al., 1995) using a two-level procedure.

3. Statistical analysis

3.1. Catecholamines, their metabolites and prolactin

Compiled data are expressed as mean ± SD. To check whether dependent variables were normally distributed visual

inspection of histograms and Kolmogorov-Smirnov (Lillifors) test were applied. In the event of significantly skewed distributions, natural base logarithmic transformations were applied to allow for parametric statistical procedures. Between-group comparisons were performed using independent-sample *t*-tests. Challenge, time and group main effects and all interaction effects for the dopaminergic markers were tested with GLM repeated. A probability value of 0.05 two-tailed was selected as level of significance. Statistical analyses were performed with SPSS, release 16.0.2 for Windows (SPSS Inc., Chicago, IL, USA, 2008).

3.2. fMRI data analysis

The analyses focused on changes in BOLD contrast that occurred during anticipatory delay periods and were conducted using the SPM8 (http://www.fil.ion.ucl.ac.uk/spm/). The first level data analysis was performed by modelling the different conditions (reward, loss, no monetary outcome). Changes in the BOLD response were assessed using the estimated GLM parameters for the anticipation of potential monetary gain vs. anticipation of no monetary outcome (reward vs. no outcome) and the anticipation of potential monetary loss vs. anticipation of no monetary outcome (loss vs. no outcome). In the second level analysis, individual contrast images of the first level analysis were included in a two-sample *t*-test to detect relevant brain activation in patients with schizophrenia and in healthy controls during AMPT and placebo conditions. To account for differences in age between the groups, age was included as a covariate in the between group analysis. For the whole brain analysis, comparisons were corrected for multiple comparisons using family wise error correction (FWE_{cor}) $P<0.05$ at cluster level (extent threshold of 10 voxels). Voxels and clusters were localized using the MNI coordinates and transformed into Talairach and Tournoux coordinates (Brett et al., 2002). Next to the whole brain analyses, we conducted region of interest (ROI) analyses focusing on activation within the left and right striatum since these brain regions are implicated in reward processing (Knutson et al., 2001b) and show pathology in schizophrenia (Gold et al., 2008; Juckel et al., 2006b). ROIs were structurally defined *a priori* which included the whole striatum. We used the Marsbar toolbox (http://marsbar.sourceforge.net) to extract mean parameter estimates averaged across all voxels in each ROI (FWE_{cor}) $P<0.05$.

4. Results

Schizophrenia patients were younger than healthy controls (HC 34.55±11.21, SCZ 22.70±3.2; $P=0.006$) Table 1. There were no differences between schizophrenia patients and healthy controls in total IQ (HC 96.64±11.72, SCZ 90.30±16.24; $P=0.31$). Scores on the subscale of the PANSS were positive 12.16±3.18, negative 14.42±5.57 and general psychopathology 25.00±5.45. Total of PANSS score was 51.58±9.61. The total SWN score did not differ between the two conditions (AMPT was 66.45±9.8, PLA 66.70±9.55; $P=0.95$). However, SWN subscale scores indicated that participants experienced more loss of self control in the AMPT condition than after placebo condition (AMPT 17.18±1.84, PLA 15.50±1.78; $P=0.05$). Four of the healthy controls and four patients were smokers (last cigarette approximately 90 min before scanning).

4.1. Behavioural effects of dopamine depletion

No serious adverse events like acute dystonia or crystalluria were observed. Plasma levels of AMPT showed significant increase from T3 to T6 in both, healthy controls (T3 12.12 mg/l±3.87; T6 16.75 mg/l±4.75, $t(10)=-2.58$, $P=0.03$) and schizophrenia patients (T3 14.19 mg/l±3.05; T6 18.69 mg/l±2.21, $t(9)=-3.71$, $P=0.005$). Plasma levels of AMPT were not significantly different between the groups ($F(1, 19)=3.09$, $P=0.09$).

4.2. Task performance

Healthy control reaction times in reward (PLA 226.9 ms±71.72, AMPT 268.40 ms±84.53; $t(22)=-1.30$, $P=0.21$) and loss (PLA 240.82 ms±72.05, AMPT 280.27 ms±86.15; $t(22)=-1.22$, $P=0.24$) did not differ across challenge conditions. Schizophrenia patients were slower in AMPT than in placebo in both reward (PLA 261.94 ms±96.60, AMPT 346.83 ms±46.31; $t(18)=-2.50$, $P=0.02$) and loss (PLA 276.81 ms±76.82, AMPT 359.59 ms±19.19; $t(18)=-3.31$, $P=0.004$).

Repeated measures ANOVA showed a significant main effect of incentive value ($F(1, 20)=10.96$, $P<0.001$) and a significant main effect of challenge ($F(1, 20)=9.31$, $P<0.001$) and group ($F(1, 20)=5.60$, $P<0.03$). Schizophrenia and patients did not differ for anticipation of reward or loss in the placebo condition. However, in the AMPT condition patients were slower than healthy controls in both, the reward condition ($t(20)=-2.62$, $P=0.02$) and the loss condition ($t(20)=-2.84$, $P=0.01$).

4.3. Neuro-endocrine response and peripheral markers for dopamine

Means and standard deviations for neuro-endocrine (PRL) and peripheral markers for dopamine (DA, HVA) are displayed in Table 2. Effects of AMPT on the peripheral markers of dopamine in schizophrenia patients are shown in Figure 1.

Repeated measures ANOVA for PRL showed a significant main effect of condition ($F(1, 14)=20.84$, $P<0.001$) on plasma PRL and a significant interaction of condition × group ($F(1, 14)=9.34$, $P=0.009$). In both schizophrenia patients and healthy controls levels of plasma PRL decreased between T0 and T6 in the placebo condition, whereas in the AMPT condition the prolactin levels showed an increase between T0 and T6. Schizophrenia patients had marked higher levels of PRL compared to healthy controls in the placebo condition. The difference of PRL levels between placebo and AMPT was larger in healthy controls than in schizophrenia.

Repeated measures ANOVA for urine DA showed a significant effect of condition ($F(1, 16)=16.79$, $P<0.001$) and a significant interaction of condition × time ($F(1, 16)=47.61$, $P<0.001$). DA levels in urine in both groups decreased from T0 to T6 in the AMPT condition. Schizophrenia patients had higher levels of DA in urine compared to healthy controls in both placebo and AMPT condition. The

Table 2 Neuro-endocrine response and peripheral markers.

Marker	Condition	Schizophrenia			Healthy controls		
		T0	*T3*	*T6*	*T0*	*T3*	*T6*
PRL (μg/l)	PLA	35.00±31.58	29.53±30.95	25.86±29.36	11.39±5.85	8.37±4.08	7.95±3.32
	AMPT	36.41±30.53	45.12±35.34	37.21±33.50	14.10±5.70	42.65±20.94	29.30±11.32
		P=0.87	*P=0.42*	*P=0.66*	*P=0.14*	*P<0.001*	*P<0.001*
DA urine (nmol/mmol creat)	PLA	156.90±51.87	-	162.25±46.24	121.10± ±34.80	-	132.90± ±46.98
	AMPT	159.56±50.69	-	72.89±32.03	135.70±37.99	-	65.50±18.34
		P=0.85		*P=0.002*	*P=0.37*		*P<0.001*
HVA plasma (nmol/l)	PLA	82.46±32.38	62.32±28.77	59.14±25.05	70.73±30.84	57.35±22.06	47.29±13.49
	AMPT	91.04±48.99	44.32±17.04	32.23±15.44	72.10±41.20	39.37±20.07	26.50±13.49
		P=0.34	*P=0.04*	*P=0.004*	*P<0.11*	*P=0.03*	*P=0.001*

T0=8.00 h; T3=11.00 h; T6=14.00 h.
PRL=prolactin; DA=dopamine; HVA=homovanillic acid; NE=norepinephrine.
AMPT=α-methylparatyrosine; PLA=placebo.

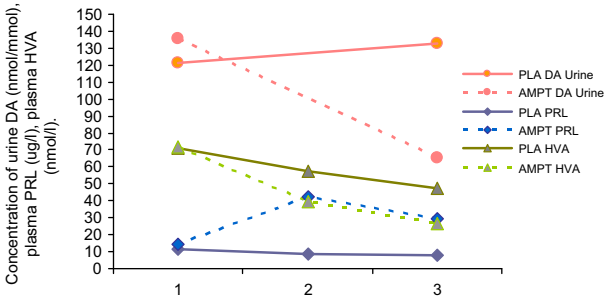

Fig. 1 Effect of AMPT on peripheral markers of dopamine in schizophrenia patients.

difference of DA in urine between T0 and T6 was larger in AMPT than in placebo condition.

Repeated measures ANOVA for plasma HVA showed a significant effect of condition ($F(1, 15)=22.37$, $P<0.001$) and a significant interaction of condition × time ($F(2, 14)=12.54$, $P<0.001$). Levels of plasma HVA decreased over time and were lower in AMPT than in placebo condition. Although not significant, in both the placebo and AMPT condition the schizophrenia had higher levels of plasma HVA at all three time intervals compared to healthy controls.

5. fMRI findings

5.1. Schizophrenia patients

5.1.1. Placebo vs. AMPT condition

During *anticipation of reward,* schizophrenia patients significantly activated the right inferior frontal gyrus, insula and middle frontal gyrus ($P_c<0.001$, P_c: corrected for multiple comparisons at cluster level) (Figure 2). In addition, they activated the medial frontal gyrus bilaterally and the left anterior cingulate ($P_c<0.05$) (Table 3). During the *anticipation of loss*, patients activated the right inferior frontal gyrus ($P_c<0.001$).

ROI analysis showed significant activation of the left ventral striatum $P_c<0.001$.

5.1.2. AMPT vs. placebo condition

Schizophrenia patients showed no significant brain activation during anticipation of reward or anticipation of loss, anticipation of reward vs. loss or loss vs. reward.

5.1.3. Schizophrenia vs. healthy controls

5.1.3.1. Placebo condition. During *anticipation of reward*, schizophrenia patients compared to controls showed reduced brain activation ($P_c<0.001$) in the right superior temporal gyrus and left posterior cingulate (Table 4, Figure 3). During *anticipation of loss* schizophrenia patients vs. controls showed reduced activation in several areas of the brain including the left cingulate gyrus and left ventral striatum ($P_c<0.05$).

ROI analysis showed reduced activation of the left ventral striatum ($P_c<0.001$) during anticipation of loss.

5.1.3.2. AMPT condition. During *anticipation of reward*, schizophrenia patients compared to controls showed reduced brain activation in the right inferior and middle frontal gyrus ($P_c<0.001$). Schizophrenia patients vs. controls showed no significant brain activation during *anticipation of loss*.

ROI analysis showed reduced activation of the left (ventral) striatum (caudate head and body) ($P_c<0.001$) during anticipation of reward and anticipation of loss.

6. Discussion

The current study is the first to assess the modulatory role of dopamine on activation of in the brain reward system in patients with schizophrenia through an AMPT depletion paradigm. Our main findings show that schizophrenia patients had overall reduced brain activation during anticipation of monetary reward and loss following dopamine depletion. Therefore, in schizophrenia activation patterns in reward related processing are directly affected by concentration of dopamine.

Previous studies show aberrant reward and loss stimuli cueing in schizophrenia and have attributed this to changes in dopaminergic neurotransmission (Abi-Dargham, 2004) but none have discerned the modulatory

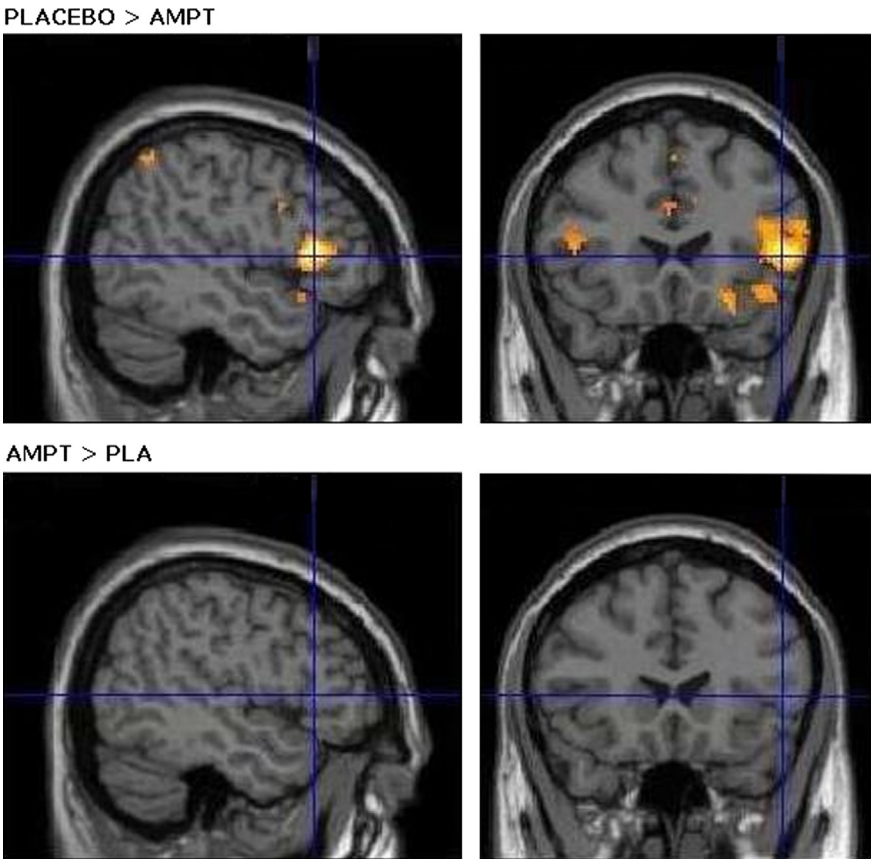

Fig. 2 Brain activation in schizophrenia patients during anticipation of reward ($x=48 y=22 z=6$). AMPT impaired recruitment of striatal and cortical regions in schizophrenia.

Table 3 Brain regions showing significant BOLD activation associated with anticipation of reward and loss in schizophrenia.

Conditions	Brain regions	BA		Talairach coordinates			*t*-Value
				X	*y*	*z*	
PLA > AMPT	**1a. Anticipation of reward > no outcome**						
	Inferior frontal gyrus	BA 45	R	48	22	6	7.2
	Medial frontal gyrus	BA 10	R	32	42	14	6.8
	Insula	BA 13	R	36	12	0	4
	Middle frontal gyrus	BA 46	R	44	20	18	4
	Anterior cingulate	BA 32	L	−6	30	20	4.12
	1b. Anticipation of loss > no outcome						
	Inferior frontal gyrus	BA 45	R	48	24	8	7.05
	Insula	BA 47	R	34	16	−2	5.59
	Lentiform nucleus putamen*		L	−22	10	−10	5.17
AMPT > PLA			-	-	-	-	

AMPT=α-methylparatyrosine; PLA=placebo; BA=Brodmann area; L=left; R=right.
$p<0.05$ corrected for multiple comparisons at cluster level.
*ROI, $p<0.001$ corrected.

role of dopamine on activation of critical nodes in the reward brain circuitry. In the present study reduced overall brain activation was found during salience processing of monetary reward and loss cues in the AMPT condition in schizophrenia. An earlier study conducted by our research group found that dopamine depletion attenuates activation in the cingulate cortex and ventral striatum (da Silva Alves et al., 2011) in health subjects. A similar effect was found in the patients with schizophrenia in the present study. These findings are consistent

Table 4 Brain regions showing significant BOLD activation associated with anticipation of reward and loss in schizophrenia compared to healthy controls.

Conditions	Brain regions	BA		Talairach coordinates			*t*-Value
				X	*y*	*z*	
HC > SCZ PLACEBO	**1a. Anticipation of reward > no outcome**						
	Superior temporal gyrus	41	R	34	−30	6	4.65
	Posterior cingulate	29	L	−2	−46	8	3.93
	2a. Anticipation of LOSS > no outcome						
	Inferior frontal gyrus	47	R	38	16	−14	5.84
	Lentiform nucleus		L	−16	16	−4	4.65
	Superior frontal gyrus	6	L	−4	16	62	4.3
	Cingulate gyrus	32	L	−6	26	32	4.64
	Medial frontal gyrus	9	L	−16	24	26	4.19
	Anterior cingulate	24	L	−4	24	22	4.01
	Lentiform nucleus*		L	−20	14	−6	4.7
HC > SCZAMPT	**1b. Anticipation of reward > no outcome**						
	Inferior frontal gyrus	13	R	38	24	12	5.63
	Middle frontal gyrus	10	R	32	42	14	4.75
	Lentiform nucleus*		L	−20	12	−6	3.83
			L	−22	10	−12	
	2b. Anticipation of loss > no outcome						
	Caudate head*		L	−14	18	−8	4.81
	Caudate body*		L	−18	16	−10	4.63

AMPT = α-methylparatyrosine; PLA = placebo; BA = Brodmann area; L = left; R = right.
$p < 0.05$ corrected for multiple comparisons at cluster level.
*ROI, $p < 0.001$ corrected.

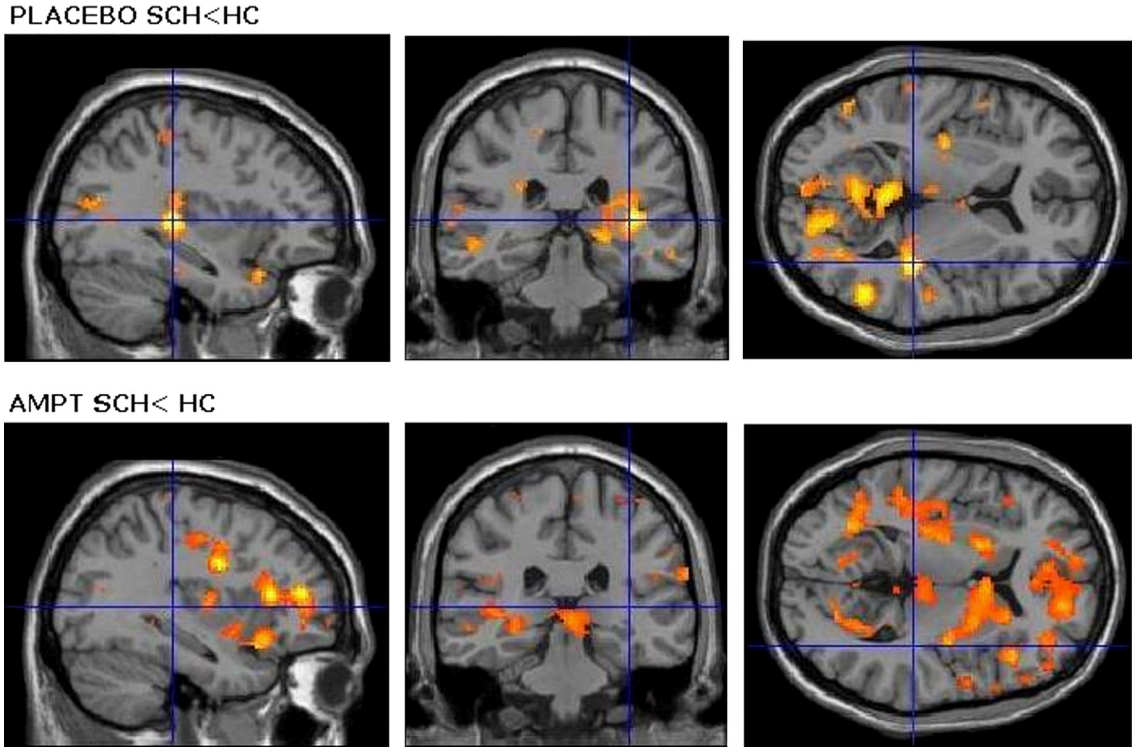

Fig. 3 Areas of reduced brain activation in schizophrenia patients vs. healthy controls during anticipation of reward ($x = 34 y = -30 z = 6$).

with neuroleptic-induced blunting of the brain reward system (Abler et al., 2007; Menon et al., 2007).

A depletion paradigm in schizophrenia patients allows for investigation of changes in systemic functional response inherent to dopamine that are absent in healthy controls, and thus attributable to illness pathology. Comparisons in the current study between healthy control subjects and patients with schizophrenia in AMPT showed reduced functional response of the inferior and middle frontal gyrus, and ventral striatum during anticipation of reward. During anticipation of loss of incentive reduced activation was found in the striatum, including the caudate head and body. These findings may suggest higher sensitivity of striatocortical reward circuitry to dopamine depletion in schizophrenia. In addition empirical evidence shows functional domain specificity of the inferior frontal gyrus and middle frontal gyrus in mediating signalling of reward and loss. The inferior frontal gyrus exerts response suppression and plays a role in modulation of attention (Aron et al., 2003; Carter et al., 1995). Middle frontal regions mediate integration of internal and external cues to adjusting response strategies and attentional bias – such as to rewarding event (Ridderinkhof et al., 2004). Therefore results show these processes to be suscept to dopamine modulation and pathologically affected in schizophrenia.

In unmedicated patients with depression AMPT reduced function of brain reward system and proposed to represent a trait-like biological marker of major depression (Hasler et al., 2009). Because our group of schizophrenia patients was medicated we cannot conclude on the state-trait effects. Given that dopamine imbalance is a consistent finding in schizophrenia we believe that the sensitivity to dopamine depletion observed in our study may also represent an inherent aspect of schizophrenia.

In the placebo condition, within group results show activity concentrated in frontal areas and insular cortex in the brains of schizophrenia patients during anticipation of reward. Contrary to expectation patients did not show activation of the striatum. This is in contrast to other studies which show normal activation patterns in medicated schizophrenia patients to monetary reward in the ventral striatum (Juckel et al., 2006a; Schlagenhauf et al., 2008; Walter et al., 2009). However, Cohen et al. (2012) established that during reward anticipation there is frontal top-down directed EEG synchrony to the ventral striatum. Activation of these regions in placebo condition was found in schizophrenia in the current study, yet this activation was hypoactive in comparison to healthy control subjects. Therefore failure to find significant activation of the ventral striatum may be the result of changes in top-down innervations of frontal regions.

During anticipation of loss significant activation in the ventral striatum was found in placebo compared to AMPT condition. Between group comparisons show schizophrenia patients compared to controls had reduced activation in ventral striatum and more anterior areas, including frontal and cingulate cortex, during anticipation of loss. Therefore, results indicate that dopaminergic neurotransmission in subcortical regions of the brain reward system may have not been normalized by medication in our group of schizophrenia patients. Results also indicate that in as early as first episode schizophrenia patients changes in systemic functional response to dopaminergic neurotransmission seem to have manifested. A recent study has not detected these differences in activation in reward and loss cueing in prodromal patients (Juckel et al., 2012). Research of this nature is highly relevant in better understanding effects of antipsychotics vs. illness pathology.

Abnormal activity of the ventral striatum during reward processing in schizophrenia was proposed to be a characteristic of a subset of medicated schizophrenia patients with high negative symptoms (Simon et al., 2010; Waltz et al., 2010). This was probably not the case in our group of patients. The schizophrenia patients had low scores on the three subscales of the PANSS confirming remission of symptoms. It seems that antipsychotic medication stabilized the symptoms but we could not detect this normalizing effect in brain activity. It is important to note that the patients in this study were first episode psychotic patients in the initial phase of antipsychotic treatment, mostly with atypical antipsychotics that reach their therapeutic effect by blocking striatal dopamine D_2 receptors, perhaps disallowing activation to reach statistical significance.

This is the first study to investigate the effects of dopamine depletion in the reward system of schizophrenia patients. The randomized double blind placebo approach and the comparison with healthy individuals is a major strength of this study. Moreover, extent of dopamine depletion was assessed by peripheral dopamine markers confirming the effect of AMPT in both groups. Additional verification of the effect AMPT was found in impaired performance on the reward task in the schizophrenia group.

Several limitations of this study need to be addressed in future research. First, the patients were treated with various types and dosages of atypical antipsychotic medication which may have some differential effect on the dopamine function and metabolism (Bressan et al., 2003; Seeman, 2002). However, inferences of functional differences as a function of dopamine can still be made. The therapeutic window of antipsychotic medication lies at 65–80% blockade at D2 (Uchida et al., 2011), rendering a maximum of 20–35% of D2 receptors free to bind with endogenous dopamine. Therefore, although the patients were on treatment with antipsychotics, we could still access the functional effects of the dopamine depletion. The dopamine depletion design with AMPT allows us to assess overall endogenous dopamine function and reflects pre-synaptic dopaminergic function, whereas antipsychotic medication reflects post-synaptic dopaminergic function through D2 receptor blockade.

Future research must also address the potential effect of age on functional response of the brain circuitry. In the current study the patient group was younger than the healthy controls. Aging is associated with changes in maturation of the mesolimbic circuitry probably altering dopaminergic signals (Backman et al., 2006) and striatal activation was shown to vary with age (Bjork et al., 2004; Samanez-Larkin et al., 2007; Schott et al., 2007). The latter study reported intact striatal activation to reward in old age and a relative reduction during loss anticipation (Samanez-Larkin et al., 2007). Although we have co-varied for age, implications of age for reward brain function further studies are necessary to address this issue.

Another aspect to take into consideration is the explorative nature of the present study with a relatively small sample size. However, the number of subjects was sufficient for a power of 80% and alpha of 0.002 (Desmond and Glover, 2002).

Earlier fMRI studies of the reward system have found results sustaining the proposed expectations with similar or smaller sample sizes (Knutson et al., 2001a, 2004). The subjects in our study did not earn the actual amount of money presented during the MID task. The task was based in point scoring which is commonly used to investigate functional activation of the reward system. However, we cannot discharge the possibility that this could have resulted in lower drive to accomplish the best performance and consequently having a differential effect on striatal activation. Furthermore our study only measures the BOLD response, which rather indirectly reflects brain activation, and does not directly address dopaminergic neurotransmission.

This study provides insight in the impairment of dopamine-related reward system in schizophrenia. We found overall reduced brain activation during anticipation of monetary reward and loss after dopamine depletion in medicated schizophrenia patients. We show that this system in schizophrenia is sensitive to dopamine depletion during reward predicting stimuli. We observed a clear imbalance of dopamine-related brain activity in the early phase of antipsychotic treatment. Neurobiological mechanisms of reward in schizophrenia involve a complex dopaminergic loop including, next to striatal regions, also frontal brain regions. Future studies that combine fMRI with other brain imaging techniques measuring the degree of dopamine depletion may help to further understand the therapeutic effects and resistance to antipsychotic drugs.

Role of funding source

The study was funded by the National Alliance for Research on Schizophrenia and Depression (NARSAD). NARSAD had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

Fabiana da Silva Alves managed the literature searches, performed the experiments, analyses and wrote the manuscript. Geor Bakker wrote the manuscript and contributed with analysis of this manuscript. Nicole Schmitz wrote the protocol and contributed with analysis of this manuscript. Johan van der Meer analyzed the data for this manuscript. Aart Nederveen, Nico Abeling, Gregor Hasler, Lieuwe de Haan, Don Linszen contributed materials, analysis tools and wrote the manuscript. Therese van Amelsvoort designed the study, wrote the protocol and the manuscript. All authors contributed to and have approved the final manuscript.

Conflicts of interest

The authors declare that, except for income received from their primary employer, no other financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest

Acknowledgements

We are grateful to all the participants and mental health workers involved in this project.

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