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Investigating the Impact of a Genome-Wide Supported Bipolar Risk Variant of MAD1L1 on the Human Reward System

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Recent genome-wide association studies have identified MAD1L1 (mitotic arrest deficient-like 1) as a susceptibility gene for bipolar disorder and schizophrenia. The minor allele of the single-nucleotide polymorphism (SNP) rs11764590 in MAD1L1 was associated with bipolar disorder. Both diseases, bipolar disorder and schizophrenia, are linked to functional alterations in the reward system. We aimed at investigating possible effects of the MAD1L1 rs11764590 risk allele on reward systems functioning in healthy adults. A large homogenous sample of 224 young (aged 18–31 years) participants was genotyped and underwent functional magnetic resonance imaging (fMRI). All participants performed the 'Desire-Reason Dilemma' paradigm investigating the neural correlates that underlie reward processing and active reward dismissal in favor of a long-term goal. We found significant hypoactivations of the ventral tegmental area (VTA), the bilateral striatum and bilateral frontal and parietal cortices in response to conditioned reward stimuli in the risk allele carriers compared with major allele carriers. In the dilemma situation, functional connectivity between prefrontal brain regions and the ventral striatum was significantly diminished in the risk allele carriers. Healthy risk allele carriers showed a significant deficit of their bottom-up response to conditioned reward stimuli in the bilateral VTA and striatum. Furthermore, functional connectivity between the ventral striatum and prefrontal areas exerting top-down control on the mesolimbic reward system was reduced in this group. Similar alterations in reward processing and disturbances of prefrontal control mechanisms on mesolimbic brain circuits have also been reported in bipolar disorder and schizophrenia. Together, these findings suggest the existence of an intermediate phenotype associated with MAD1L1.
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INTRODUCTION

Bipolar disorder and schizophrenia are severe chronic and highly heritable psychiatric disorders. Family, twin and adoption studies have provided strong evidence for a substantial familial overlap with both bipolar disorder and schizophrenia, suggesting partially shared genetic underpinnings of these diseases (Cardno and Owen, 2014).

Through the use of large genome-wide association studies (GWAS), the search for genetic risk loci for bipolar disorder and schizophrenia has further moved forward (Zhang *et al*, 2012). Recently, Cichon *et al* (2011) identified MAD1L1 (mitotic arrest deficient-like 1) as a potential susceptibility factor for bipolar disorder in a GWAS. The minor allele of the single-nucleotide polymorphism (SNP) rs11764590 was significantly associated with bipolar disorder. Subsequent GWAS also found significant associations of MAD1L1 with bipolar disorder (Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011) as well as associations with both bipolar disorder and schizophrenia (Bergen *et al*, 2012; Ruderfer *et al*, 2014).

MAD1L1 [OMIM 602686] with the intronic SNP rs11764590 is located on chromosome 7p22.3 and is expressed in numerous human tissues (Sun *et al*, 2013; Tsukasaki *et al*, 2001). In the brain, MAD1L1 expression is measurable in cortical and subcortical areas including the basal ganglia (including the dorsal and ventral striatum), the ventral tegmental area (VTA) (Hawrylycz *et al*, 2012), and the hippocampus (Cichon *et al*, 2011; 'GTEx Portal', n.d.; http://www.gtexportal.org/home/gene/MAD1L1), ('Microarray Data :: Allen Brain Atlas: Human Brain', n.d.; http://human.brain-map.org/microarray/search/show?exact_

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match = false&search_term = MAD1L1&search_type = gene). MAD1L1 is a checkpoint gene; it is involved in regulating the spindle assembly checkpoint during mitosis. Variable gene expression levels and mutations of the MAD1L1 gene are associated with chromosomal instability and have a role in carcinogenesis and aging (Cichon *et al*, 2011; Sun *et al*, 2013; Tsukasaki *et al*, 2001). Recently, it was shown that experimental manipulation of the transcription of MIR137, another leading candidate schizophrenia susceptibility gene (Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011), was followed by altered expression levels of MAD1L1 in a neural cell line (Hill *et al*, 2014). These findings suggest common molecular pathways through which genetic variation in these susceptibility genes could confer the risk for schizophrenia or bipolar disorder (Hill *et al*, 2014). Another protein that has been shown to influence MAD1L1 gene expression is the Cockayne syndrome complementation B (CSB) protein (Lake *et al*, 2014). Mutations in CSB are accountable for the majority of Cockayne syndrome cases, an inherited premature aging disease linked to numerous developmental and neurological deficits such as microcephaly, hypomyelination, calcification and neuronal loss, mental retardation, ataxia, and intellectual decline (Lake *et al*, 2014; Vessoni *et al*, 2016). This potentially regulatory effect of CSB on MAD1L1 expression possibly indicates a role of MAD1L1 in the central nervous system.

Bipolar disorder and schizophrenia do not only partially share a genetic basis, but also show phenotypic similarities, eg, with regard to symptomatology (Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011; Tamminga *et al*, 2013). Moreover, both disorders are characterized by altered functional brain activation patterns in prefrontal and subcortical networks (Calhoun *et al*, 2011; Chai *et al*, 2011), including the reward system (Deserno *et al*, 2013; Gradin *et al*, 2011; Whalley *et al*, 2012).

The reward system in the brain is based on a neural circuitry including regions of the mesolimbic dopamine system, in particular the ventral striatum and VTA (Diekhof and Gruber, 2010). In a recent functional magnetic resonance imaging (fMRI) study, we found that euthymic to mildly depressed bipolar I patients showed a reduced bottom-up responsiveness of the ventral striatum and a disturbed top-down control of the mesolimbic reward system by prefrontal brain regions while performing a specific reward paradigm, the 'Desire-Reason Dilemma' paradigm (DRD paradigm) (Trost *et al*, 2014). The DRD paradigm assesses the neural mechanisms underlying reward processing and active reward dismissal in favor of a long-term goal (Diekhof and Gruber, 2010). With regard to schizophrenia, recent fMRI studies showed that schizophrenic patients also exhibited abnormal subcortical reward processing (Juckel *et al*, 2006; Subramaniam *et al*, 2015) and associated alterations in functional connectivity within the salience network and reward regions (Gradin *et al*, 2013). Similar effects with regard to the ventral striatum were shown in healthy first-degree relatives of schizophrenic patients (Grimm *et al*, 2014). As already similarly reported for prodromal, drug-naive and chronic schizophrenia patients (Deserno *et al*, 2013), these first-degree relatives exhibited an attenuated striatal response during reward anticipation (Grimm *et al*, 2014).

Imaging genetics is a promising approach to shed light on the neurophysiological impact of susceptibility genes on the human brain. It offers the possibility to investigate the genetic effects on the neuronal level that may mediate the vulnerability to psychiatric disorders in the sense of an intermediate phenotype (Gottesman and Gould, 2003).

In the present study, we aimed to investigate the effects of the MAD1L1 risk allele on the neurofunctional level. To this end, a homogenous group of healthy adults underwent fMRI. All participants performed the DRD paradigm to investigate possible gene effects of the MAD1L1 risk allele on the mesolimbic reward system and other brain regions. We hypothesized that risk allele carriers would show alterations in subcortical reward processing similar to those found in bipolar and schizophrenic patients as well as in their first-degree relatives according to the concept of an intermediate phenotype.

MATERIALS AND METHODS

Subjects

Participants in the Genomic Imaging Goettingen (GIG) study ($n = 299$) were recruited by advertisements in intern online student networks and local newsletters in the Georg-August-University Goettingen and the University Medical Center Goettingen. Healthy young adults aged 18–31 years were included. Exclusion criteria were past or present psychiatric disorders according to ICD-10, a positive family history of psychiatric disorders, substance abuse during the last month, cannabis abuse during the last 2 weeks, mental retardation, dementia, neurological or metabolic diseases, and pregnancy in women. All participants were Caucasians with European ancestry.

Imaging and performance data for at least 70% of all trials, and MAD1L1 rs11764590 genotype were available for 224 participants (exclusion criteria were unavailability of genotype in 12 cases, failure of fMRI data acquisition in 7 cases, and fMRI motion artifacts and/or less than 70% correct answers across all task conditions in 56 cases).

The mean age of the participants was 24.01 years (± 2.45 years; range 18–31 years), 212 subjects were right handed, 12 were left handed or both (1 case ambidextrous), and 87 subjects were male. All participants had passed a higher general school qualification.

The Temperament and Character Inventory (TCI) (Cloninger *et al*, 1998) and the Barratt Impulsiveness Scale 11 (BIS) (Patton *et al*, 1995) were performed by all participants to assess personality traits associated with dopaminergic neurotransmission and reward-related brain activation. Both scales investigate aspects of personality traits characteristic for bipolar disorder, eg, enhanced impulsivity (Strakowski *et al*, 2010). TCI and BIS data were available for 220 participants.

All participants provided written informed consent after the study procedure had been fully explained. The study was carried out in accordance with the latest version of the Declaration of Helsinki and was approved by the local ethics committee.

Experimental Procedure/DRD Paradigm

Initially, participants underwent an operant conditioning task. Eight differently colored squares were presented as

Table 1 Demographic Variables and Personality Traits

	All participants	C/C homozygotes	C/T and T/T carriers	*P*-values
Number	224	131	93	
Age	24.01 ± 2.45	24.20 ± 2.51	23.74 ± 2.35	0.165
Gender	87 M/137 F	57 M/74 F	30 M/63 F	0.086
Handedness	212 R/12 L	124 R/7 L	88 R/5 L	0.991
Nicotine[a]	17 yes/207 no	11 yes/120 no	6 yes/87 no	0.582
Caffeine[a]	54 yes/170 no	34 yes/97 no	20 yes/73 no	0.440
Alcohol[a]	0 yes/224 no	0 yes/131 no	0 yes/93 no	
BIS total	62.20 ± 8.44	62.43 ± 7.98	61.88 ± 9.08	0.645
BIS non-planning	23.64 ± 4.29	23.64 ± 3.94	23.65 ± 4.75	0.990
BIS congitive	22.31 ± 3.59	22.57 ± 3.60	21.96 ± 3.58	0.208
BIS motor	16.24 ± 2.97	16.21 ± 2.75	16.28 ± 3.26	0.873
TCI persistance	4.70 ± 2.14	4.79 ± 2.12	4.57 ± 2.17	0.446
TCI reward dependence	16.55 ± 3.42	16.70 ± 3.37	16.34 ± 3.51	0.439
TCI harm avoidance	13.64 ± 6.16	12.98 ± 5.97	14.55 ± 6.33	0.065
TCI novelty	21.32 ± 5.76	21.62 ± 5.68	20.90 ± 5.89	0.368

Abbreviations: BIS, Barratt Impulsiveness Scale; F, female; L, left handed; M, male; R, right handed; TCI, Temperament and Character Inventory.
Data are presented as mean ± standard deviation.
[a]Nicotine, caffeine, or alcohol use during the last 2 h before fMRI.

stimuli on a monitor in a shuffled mode. Subjects were instructed to respond to each of the stimuli by button press with their right hand. Button choice was free and subjects were encouraged to explore the stimulus-response-reward contingencies. By doing so, subjects were conditioned to associate two colors (red and green) with an immediate reward (bonus of +10 points), while the other six colors were associated with a neutral outcome. The goal of this operant conditioning task was to establish stimulus-response-reward contingencies for the next phase of the experiment.

Subsequently, subjects were familiarized with the actual experimental task, the DRD paradigm, a delayed matching to sample task. Subjects had to perform blocks of four or eight trials. At the beginning of every block, subjects were shown two targets (two different neutral colors, not the previously conditioned colors red and green). In the following, four or eight colored squares were presented one after another. To achieve the superordinate goal (50 points at the end of each block), subjects had to accept the two target colors shown at the beginning and to reject non-target colors by button press. Two different types of blocks had to be performed. In the first type of blocks, the 'Desire Context' (DC), subjects were allowed to accept the previously conditioned reward stimuli in addition to the two target colors and win bonus points (+10 points). In the second type of blocks, the 'Reason Context' (RC), the conditioned reward stimuli had to be rejected in order to successfully pursue the long-term goal (50 points at the end of the block). So, during the RC, subjects were forced to overcome the tendency to acquire immediate reward (reject the previously conditioned reward stimuli) in order to reach the superordinate long-term goal. This situation therefore constituted a 'desire-reason dilemma' (Diekhof *et al*, 2012; Diekhof and Gruber, 2010; Trost *et al*, 2014). For more information, see also Supplementary Figure S1.

Genotyping and Sample Structure

DNA of all participants was isolated from saliva. Saliva was collected into Oragene saliva DNA kits (DNA Genotek, Kanata, Ontario, Canada) using the Gentra Puregene Blood Kit (Qiagen) with standardized protocols. Genome-wide SNP genotyping was performed using Illumina OmniExpress Genotyping BeadChips according to the manufacturer's standard protocols and using 400 ng of DNA.

EIGENSOFT (Price *et al*, 2006) was used to identify population outliers based on a principal components analysis. Taken together, these analyses showed that a majority of the subjects included in this study cluster together with HapMap3 European-descent populations. This fact makes unlikely that the results of the study are due to population stratification.

Genotype Group Classification

Participants were divided into two groups: homozygous major allele carriers (C/C; $n = 131$) were compared with heterozygous minor allele carriers (C/T; $n = 85$) and homozygous minor allele carriers (T/T; $n = 8$). Gender, age, handedness as well as nicotine, caffeine, and alcohol use (before fMRI) did not differ significantly between groups, see Table 1.

The frequency of the major allele (C) of the MAD1L1 SNP rs11764590 was 0.77 in the present sample. The observed genotype distribution did not deviate from Hardy–Weinberg equilibrium ($X^2 = 1.68$, $p = 0.19$, 1 degree of freedom) (Rodriguez *et al*, 2009).

fMRI Data Acquisition

fMRI was performed on a 3-Tesla Magnetom TIM Trio Siemens scanner (Siemens Healthcare, Erlangen, Germany) equipped with a standard eight-channel phased-array head coil. First, a T1-weighted anatomical data set with 1 mm isotropic resolution was acquired. Parallel to the anterior commissure–posterior commissure line, thirty-one axial slices were acquired in ascending direction for fMRI (slice thickness = 3 mm; interslice gap = 0.6 mm) using a gradient-echo echo-planar imaging sequence (echo time 33 ms, flip angle 70°; field-of-view 192 mm, interscan repetition time 1900 ms).

In 2 functional runs, 185 volumes each were acquired. Subjects responded via button presses on a fiber optic computer response device (Current Designs, Philadelphia, Pennsylvania, USA), and stimuli were viewed through goggles (Resonance Technology, Northridge, California, USA). Presentation Software (Neurobehavioral Systems, Albany, California, USA) was used to present the stimuli in the scanner.

Functional images were preprocessed and analyzed with SPM5 (Statistical Parametric Mapping; www.fil.ion.ucl.ac.uk/spm/software/spm5/) using a general linear model. The study design was event-related and only correctly answered trials were included in the analysis.

Linear *t*-contrasts were defined to assess brain activation effects in the two contexts. We analyzed activation effects elicited by the conditioned reward stimuli in the DC and activation effects elicited by the same conditioned stimuli when being presented in the RC. We contrasted the events of conditioned reward stimuli in the DC with those in the RC to assess the extent of downregulation (suppression) of reward-related activation during a competition between the superordinate goal and the proximal reward option in the RC.

Two sample *t*-tests (C/C > T carriers) were performed using single subject contrast images to assess group effects. Age and gender were entered as covariates of no interest. FWE (family-wise error) correction was performed with respect to our *a priori* regions of interest (small volume analyses of the dopaminergic reward system) and on the whole brain level. Small volume analyses were applied to the bilateral ventral striatum (±12 12 −3) and the VTA (±9 −21 −12) (regions of interest, 10 mm spheres centering *a priori* coordinates from previous studies) (Diekhof *et al*, 2012; Diekhof and Gruber, 2010). Further whole-brain genotype group effects were searched for using $p<0.001$, uncorrected.

Psychophysiological Interaction Analysis

Psychophysiological interaction (PPI) (Diekhof and Gruber, 2010; Friston *et al*, 1997; Trost *et al*, 2014) was used to assess functional interactions of the ventral striatum with prefrontal brain regions, especially the anteroventral prefrontal cortex (avPFC), in the 'DRD' situation. Previous studies using the DRD paradigm in healthy subjects had shown a significant negative functional interaction between the ventral striatum and the avPFC in the dilemma situation (Diekhof and Gruber, 2010), while this negative functional interaction had been significantly impaired in bipolar patients in our latter study comparing bipolar patients with healthy controls (Trost *et al*, 2014). For the PPI analysis, the bilateral ventral striatum (local maximum one-sample *t*-test of all participants in the dilemma situation L ventral striatum −9 12 3 and R ventral striatum 12 12 6) was selected as seed area (5 mm sphere). Small volume analyses using *a priori* coordinates of the avPFC from our previous studies (±30 51 3, 10 mm sphere; FWE-corrected for small volume; Diekhof and Gruber, 2010; Trost *et al*, 2014) were carried out to investigate the negative functional connectivity between the bilateral ventral striatum and the avPFC in the 'DRD'.

Beta Value Extraction

Mean beta estimates were extracted from the bilateral ventral striatum and the VTA using MARSBAR (Brett *et al*, 2002). Beta extraction for each participant was performed using the coordinates of the local maxima of the whole sample (one sample *t*-test, DC, $p<0.05$, FWE-corrected).

Statistical Analyses

Behavioral data, personality traits (TCI and BIS) and beta estimates were analyzed using SPSS for Windows (version 21.0, SPSS, IBM, Armonk, New York, USA). Behavioral data: percentage of correctly accepted (DC)/rejected (RC) boni and reaction times were compared between groups. Normal distribution of performance data, TCI, BIS, and beta estimates was tested using the Kolmogorov–Smirnov test and Q-Q plots. Mann–Whitney *U*-tests (in case of non-normal distribution of scores) and independent sample *t*-tests were used to test for differences between genotype groups (two-tailed significance). The calculation of correlation coefficients was done according to Pearson (Pearson *r*, $p<0.05$, two-tailed significance).

Chi-Square was used to check Hardy–Weinberg equilibrium ($p>0.05$, 1 degree of freedom) (Rodriguez *et al*, 2009).

RESULTS

Behavioral Data

Homozygous major allele carriers and risk allele carriers did not differ significantly with respect to behavioral performance data (Supplementary Table S1). Reaction times were normally distributed and did not differ significantly between groups. Because of significant deviation from normality ($p<0.001$), group comparisons of percentage of correctly answered bonus trials were done using the non-parametric Mann–Whitney *U*-test. There was no genotype effect on percentage of correctly answered bonus trials (DC and RC).

TCI and BIS Results

TCI and BIS scores were available for 220 participants (C/C $n=128$; C/T $n=84$; T/T $n=8$). Differences between genotype groups in TCI and BIS scores and subscales did not reach statistical significance (see Table 1). However, for the TCI subscale harm avoidance there was a trend toward statistical significance between group means of risk allele carriers and non-carriers ($p=0.065$). Risk carriers showed higher TCI harm avoidance subscale scores than non-carriers (C/C 12.98 ± 5.97; C/T 14.31 ± 6.23; T/T 17.13 ± 7.26). TCI harm avoidance subscale scores were significantly correlated with the number of risk alleles (positive correlation, $r=0.146$, $n=220$, $p=0.030$, two-tailed significance).

fMRI Data

In line with our hypothesis, the risk allele carriers exhibited significantly reduced reward-related bottom-up activation in response to the conditioned stimuli in the bilateral VTA, the ventral (and the dorsal) striatum in the DC (Table 2, Figure 1). Small volume analyses using *a priori* coordinates from our previous studies (Diekhof *et al*, 2012; Diekhof and Gruber, 2010) confirmed significantly reduced activations in the bilateral ventral striatum and the VTA in the risk allele carriers (FWE-corrected for small volume).

In addition to these activations in mesolimbic dopaminergic brain regions, participants also showed activations in a bilateral fronto-parietal cortical network (associated with salience processing) replicating findings of previous studies (Diekhof *et al*, 2012; Diekhof and Gruber, 2010; Trost *et al*, 2014) (Supplementary Table S2).

Within this bilateral fronto-parietal network, activations in the orbitofrontal/insular cortex, the inferior parietal lobule (both regions FWE-corrected across the whole brain), the pre-SMA, and further frontal and parietal cortices were also

significantly diminished in the risk allele carrier group as compared with homozygous major allele carriers (Supplementary Table S2).

In the RC, both groups showed an (attenuated) BOLD signal in the bilateral VTA and the ventral striatum. In the left VTA and the bilateral ventral striatum, the BOLD signal was significantly diminished (FWE-corrected for small volume) in the risk allele carriers compared with the homozygous major allele carriers (Table 3).

In both groups, activations in the reward task-related fronto-parietal network were found (Diekhof *et al*, 2012; Diekhof and Gruber, 2010; Trost *et al*, 2014). The risk allele carriers showed significantly reduced brain activation in the bilateral frontoopercular cortex/insular cortex and the right intraparietal cortex in comparison with the homozygous major allele carriers (Supplementary Table S3).

By contrasting brain activations in the DC with activations in the RC, we assessed the extent of top-down suppression of reward-related cortical and subcortical network activity due to the dilemma. The extent of suppression in the reward regions did not differ significantly between groups. There was a significantly reduced cortical network suppression in the left frontoopercular/anterior insular cortex and the left middle frontal gyrus in the risk allele carriers (Supplementary Table S4).

Additional second-level analyses with TCI harm avoidance subscores as covariates of no interest showed no changes in the main imaging findings (*a priori* regions of interest) in neither the DC nor the RC (Supplementary Table S5).

PPI Results

As shown in previous studies (Diekhof and Gruber, 2010), both genotype groups exhibited functional interactions of the ventral striatum with the avPFC in the dilemma situation. The homozygous major allele carriers showed a significant negative functional interaction of the left ventral striatum (seed) with the left avPFC (−24 54 9; $t=3.62$; $p<0.05$, FWE-corrected for small volume, 10 mm sphere around *a priori* coordinates). The minor allele carriers did not show this negative functional connectivity, but showed subthreshold positive connectivity between the ventral striatum (seed) and the left avPFC (−24 51 0; $t=2.43$; $p<0.05$, uncorrected). In the group comparison, minor allele carriers showed a significantly reduced negative functional connectivity of the left ventral striatum (seed L ventral striatum: −9 12 3, 5 mm sphere) with the left avPFC (−27 51 3; $t=3.57$; $p<0.05$, FWE-corrected for small volume, 10 mm sphere around *a priori* coordinates).

Table 2 Brain Activations *A Priori* Regions of Interest 'Desire Context' (DC): C/C > T Carriers

***A priori* regions of interest**	**C/C > T carriers MNI coordinates (*t*-values)**
L VTA	−3 −27 −24 (4.27)
R VTA	12 −27 −15 (3.56)
L ventral striatum	−21 15 −3 (3.74)
R ventral striatum	9 15 6 (3.55)

Abbreviations: L, left; R, right; VTA, ventral tegmental area.
Two sample *t*-test C/C > T carriers, $p<0.05$, FWE-corrected for small volume (10 mm spheres around *a priori* coordinates bilateral striatum ±12 12 −3 and bilateral VTA ±9 −21 −12).

Table 3 Brain Activations *A Priori* Regions of Interest 'Reason Context' (RC): C/C > T Carriers

***A priori* regions of interest**	**C/C > T carriers MNI coordinates (*t*-values)**
L VTA	0 −30 −21 (3.47)
R VTA	n.s.
L ventral striatum	−15 18 0 (3.35)
R ventral striatum	6 15 3 (3.99)

Abbreviations: L, left; R, right; n.s., not significant; VTA, ventral tegmental area.
Two sample *t*-test C/C > T carriers, $p<0.05$, FWE-corrected for small volume (10 mm spheres around *a priori* coordinates bilateral striatum ±12 12 −3 and bilateral VTA ±9 −21 −12).

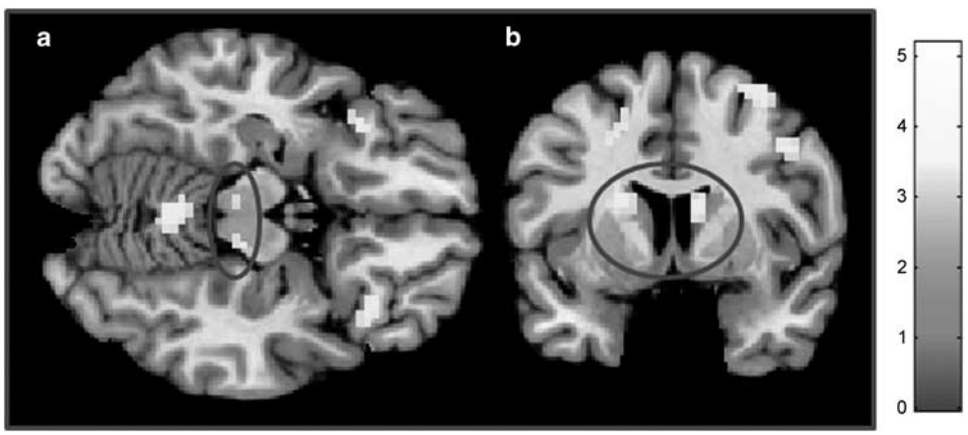

Figure 1 C/C > T carriers in the DC. (a) Bilateral VTA (blue circle) ($z=-15$). (b) Bilateral dorsal striatum (blue circle) ($y=6$). For presentation purposes at $p<0.001$, uncorrected. A full color version of this figure is available at the *Neuropsychopharmacology* journal online.

Beta Value Extraction and Exploratory Correlational Analyses

Mean beta estimates extracted from the bilateral ventral striatum and the VTA in the DC differed significantly between groups. Risk allele carriers showed significantly lower mean beta estimates than homozygous major allele carriers (Supplementary Table S6).

There was no significant correlation of the beta estimates extracted from the bilateral ventral striatum with nicotine or caffeine use.

Post hoc analyses revealed no significant correlation of the TCI harm avoidance subscores with beta estimates extracted from the bilateral ventral striatum or the VTA.

DISCUSSION

Consistent with our primary hypothesis, young healthy MAD1L1 rs11764590 risk allele carriers showed a reduced bottom-up responsiveness and an altered top-down regulation of the mesolimbic reward system similar to a recently investigated sample of euthymic to mildly depressed bipolar patients, both study samples performing the DRD paradigm (Trost *et al*, 2014).

The MAD1L1 risk allele carriers showed significantly reduced brain activation in response to conditioned reward stimuli in the VTA and the ventral striatum. This effect was seen bilaterally in the DC when participants were allowed to accept the conditioned reward stimuli and win additional bonus points, but was also present in the RC in the left VTA and the bilateral ventral striatum when subjects had to refrain from accepting the conditioned reward stimuli. In the dilemma situation, a reduced functional connectivity between the avPFC and the ventral striatum in terms of an altered top-down regulation was found in the MAD1L1 risk carriers.

Moreover, risk allele carriers also showed reduced activations in the reward task-related bilateral fronto-parietal network similar to the findings in bipolar patients (Trost *et al*, 2014). Especially in the anterior insula/frontoopercular cortex, the risk allele carriers exhibited diminished brain activation in response to the conditioned reward stimuli compared with homozygous major allele carriers. This reduced activation occurred in both contexts (in the RC at $p<0.001$, uncorrected). Therefore, we interpret these reduced activations in mesolimbic and cortical regions as a reduced bottom-up response to highly salient, conditioned reward stimuli. Risk allele carriers showed a neurofunctional deficit of their reward responsiveness, in mesolimbic reward areas, but also in task-related cortical regions associated with salience processing.

In schizophrenia research, support for the aberrant salience processing hypothesis is growing (Whitton *et al*, 2015). Mediated by a dysregulated dopamine transmission in schizophrenia, salience attribution mechanisms are impaired resulting in either inappropriate responses to irrelevant cues or failure to respond adequately to significant events (Kapur, 2003; Whitton *et al*, 2015).

The finding of an attenuated brain activation in response to reward cues in mesolimbic dopaminergic regions has been multiply replicated in schizophrenia, but is also found in affective disorders (Bogdan *et al*, 2013; Deserno *et al*, 2013; Gradin *et al*, 2011; Hall *et al*, 2014; Trost *et al*, 2014). Drug-naive, first-episode, but also chronic schizophrenia patients show reduced ventral striatal activations during reward anticipation (Deserno *et al*, 2013); and attenuated positive prediction error signal in the midbrain is another replicated finding in schizophrenia (Deserno *et al*, 2013; Waltz *et al*, 2009). Recently, attenuation of striatal activation during reward anticipation was shown in a sample of unaffected first-degree relatives of schizophrenic patients and interpreted as a potential intermediate phenotype for schizophrenia (Grimm *et al*, 2014).

In bipolar disorder, reward processing is also altered in mesolimbic dopaminergic regions. Recent fMRI studies showed alterations in reward-related striatal and cortical brain activations, state-dependent, but also during euthymia (Abler *et al*, 2008; Caseras *et al*, 2013; Nusslock *et al*, 2012; Trost *et al*, 2014; Whitton *et al*, 2015). A number of studies have reported elevated striatal activations in response to rewarding stimuli in hypomanic individuals (O'Sullivan *et al*, 2011), euthymic (Mason *et al*, 2014; Nusslock *et al*, 2012) and manic (Abler *et al*, 2008) bipolar patients. However, other studies in bipolar patients have shown a reduced reward responsiveness challenging the hypothesis of a general reward hypersensitivity model of bipolar disorder. Decreased striatal activations in response to reward stimuli were found in euthymic to mildly depressed (Trost *et al*, 2014), depressed bipolar patients (Redlich *et al*, 2015; Satterthwaite *et al*, 2015) and euthymic bipolar II/bipolar not otherwise specified patients (Yip *et al*, 2015); with the latter study reporting differential hypoactivations of both the dorsal and the ventral striatum during reward processing in bipolar disorder (Yip *et al*, 2015). These results are in line with the present findings of a reduced bottom-up responsivity of striatal regions (dorsal and ventral) in healthy MAD1L1 risk allele carriers.

Furthermore, in our previous study investigating bipolar patients, reduced suppression of brain activation in the dilemma situation was seen in frontal cortical regions (ie, middle frontal gyrus) (Trost *et al*, 2014), which is also found in the middle frontal gyrus and frontoopercular/anterior insular cortex in risk allele carriers in the present study (Supplementary Table S2, $p<0.001$, uncorrected). In addition to the finding of reduced functional connectivity in the dilemma situation, this further suggests that not only bottom-up, but also cortically driven top-down mechanisms involved in reward processing are altered in MAD1L1 risk allele carriers.

Risk allele carriers and non-carriers in our study did not differ significantly with respect to demographic or behavioral data. Enhanced trait impulsivity, a characteristic of bipolar disorder (Strakowski *et al*, 2010), was not found in the risk allele carriers. However, with regard to personality traits associated with MAD1L1, we found a significant correlation of the risk allele with the TCI subscale scores harm avoidance. Subjects scoring high in harm avoidance are described as rather worrying, fearful, shy, and fatigable (Cloninger *et al*, 1993). Several studies found elevated harm avoidance scores in schizophrenia patients (Jetha *et al*, 2013; Ohi *et al*, 2012) and subjects at ultra-high risk for psychosis or experiencing psychotic-like symptoms (Fresán *et al*, 2015; Nitzburg *et al*, 2014). With regard to bipolar disorder, harm avoidance was associated with mood episode recurrence in

bipolar offspring in a longitudinal study (Kemner *et al*, 2015) and with the overall burden of depressive episodes during lifetime in a sample of bipolar (I and II) and unipolar patients (Zaninotto *et al*, 2015). Bipolar II patients and patients with major depressive disorder showed higher harm avoidance scores than controls (Zaninotto *et al*, 2015). In a general population sample, high harm avoidance scores predicted elevated dysphoria rates (Rosenström *et al*, 2014).

Referring to our imaging findings in association with MAD1L1, the reduced ventral striatal reward responsiveness on the neurofunctional level in the risk allele carriers was not correlated with harm avoidance subscores, but is complemented by findings of personality traits partly overlapping with negative and depressive symptoms in clinical populations. Reduced ventral striatal activation has been associated with apathy in schizophrenia (Kirschner *et al*, 2015), with anhedonia in depression (Keedwell *et al*, 2005) and was predominantly present in bipolar patients suffering from depressive symptoms (Redlich *et al*, 2015; Trost *et al*, 2014). Therefore, a reduced mesolimbic reward responsivity associated with MAD1L1 may be paralleled by personality traits linked to subthreshold psychopathologic symptoms.

The results of the present study are limited concerning the comparability with other studies investigating the dopaminergic reward system due to the specific fMRI reward paradigm we used. A number of studies cited above used other reward paradigms rather focusing on the neurofunctional underpinnings of reward anticipation and outcome (Caseras *et al*, 2013; Knutson *et al*, 2001; Yip *et al*, 2015) than the neural responses to conditioned reward stimuli under changing conditions with respect to salience of the stimuli. However, despite these methodological differences growing evidence suggests differentially altered reward processing mechanisms in bipolar disorder (with a mesolimbic hyporesponsiveness rather linked to depressive symptoms) and in schizophrenia (failure to respond adequately to significant events) throughout the literature.

Apart from our *a priori* regions of interest, the findings of reduced cortical activations in the risk allele carriers are limited by the statistical threshold of $p<0.001$ uncorrected. Our objective was to present genotype-associated alterations within the robustly replicated task-related fronto-parietal network linked to salience processing in addition to the subcortical key regions of the mesolimbic reward system.

Another limitation is the fact that only one SNP was investigated in the present study. Our intention was to investigate the possible effects of this one SNP in MAD1L1 which was strongly associated with bipolar disorder (Cichon *et al*, 2011) and to compare the results to the findings in our bipolar sample (Trost *et al*, 2014). Moreover, we aimed to integrate our findings into the growing evidence for a phenomenological and genetic overlap of bipolar disorder and schizophrenia (Tamminga *et al*, 2013). Further studies will be needed to replicate and complement our findings integrating additional genetic data.

Concluding, we suggest that abnormal reward processing can be regarded as a potential endophenotype for both bipolar disorder and schizophrenia. Here, we show that MAD1L1 as a susceptibility gene for both of these genetically overlapping disorders is associated with a decreased bottom-up responsiveness of the mesolimbic reward system and related cortical regions involved in the salience network as well as with reduced top-down control processes. By modulating the functionality of these specific subcortical and cortical networks, the MAD1L1 risk variant may increase individual vulnerability for bipolar, affective, or psychotic disorders and contribute to clinical disease manifestation according to the concept of an intermediate phenotype. Furthermore, in parallel to the neurofunctional findings, specific personality traits in the form of higher harm avoidance subscale scores in the risk allele carriers may represent discrete subthreshold symptoms associated with MAD1L1.

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