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ORIGINAL ARTICLE

Genome-wide association study of positive emotion identifies a genetic variant and a role for microRNAs

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Positive affect denotes a state of pleasurable engagement with the environment eliciting positive emotion such as contentment, enthusiasm or happiness. Positive affect is associated with favorable psychological, physical and economic outcomes in many longitudinal studies. With a heritability of ⩽64%, positive affect is substantially influenced by genetic factors; however, our understanding of genetic pathways underlying individual differences in positive affect is still limited. Here, through a genome-wide association study of positive affect in African-American participants, we identify a single-nucleotide polymorphism, rs322931, significantly associated with positive affect at $P < 5 \times 10^{-8}$, and replicate this association in another cohort. Furthermore, we show that the minor allele of rs322931 predicts expression of microRNAs miR-181a and miR-181b in human brain and blood, greater nucleus accumbens reactivity to positive emotional stimuli and enhanced fear inhibition. Prior studies have suggested that miR-181a is part of the reward neurocircuitry. Taken together, we identify a novel genetic variant for further elucidation of genetic underpinning of positive affect that mediates positive emotionality potentially via the nucleus accumbens and miR-181.

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INTRODUCTION

Positive affect and negative affect constitute two broad general factors consistently identified as the dominant dimensions of emotional experience in interindividual and intraindividual analyses across diverse descriptor sets, time frames, languages and cultures.[1–4] Positive affect, that is, a state of pleasurable engagement with the environment eliciting positive emotions,[5,6] has been shown to be associated with favorable psychological, physical and economic outcomes in many longitudinal studies.[5–9] For instance, positive affect was a source of resilience for women having chronic pain,[7] was a buffer against risk for depression[8] and it protected against psychiatric symptoms in children exposed to natural disaster.[9] Furthermore, a systematic review of longitudinal studies in healthy populations and populations with medical illness found that positive affect was associated with reduced mortality in both populations, independently of negative affect.[5] Moreover, a large US representative longitudinal study found that young adults who reported higher positive affect went on to earn significantly higher income a decade later, even after the adjustment of potential confounders.[6]

Despite the many important impacts of positive affect, we still have very limited knowledge of its underlying biological mechanisms. Positive affect has a heritability of ⩽64%,[10,11] suggesting that genetic factors moderately contribute to this construct; however, the specific molecular loci contributing to this heritability remain to be identified. Hence, to elucidate genetic variants contributing to this phenotype, we performed a genome-wide association study (GWAS) of positive affect and a quasi-replication of our findings in positive affect in the realm of spiritual well-being in an independent cohort. Subsequently, we investigated functional significance of the identified genetic variants using brain and blood gene expression data and behavioral and neuroimaging data.

MATERIALS AND METHODS

The discovery sample

Participants in the discovery sample were recruited by the Grady Trauma Project (GTP) using the inclusion criteria of age ⩾18 years, understanding English and being able to give informed consent. Exclusion criteria included being acutely suicidal, psychotic or having acute medical problems. Participants gave informed consent and the institutional review boards of Emory University and Grady Memorial Hospital approved the study.

We assessed the trait-level positive affect with the PANAS (Positive And Negative Affect Schedule).[12] Positive affect was represented by total score of the 10-item scale for positive emotional state (that is, feeling interested, excited, strong, enthusiastic, proud, inspired, determined, attentive and active). Each item was rated by participants using a 5-point scale ranging from 1 = *very slightly to not at all* to 4 = *quite a bit* and 5 = *extremely* for the extent that they feel a particular positive emotion *in general*, that is, *on average*. The score ranges from 0 to 50, with higher score reflecting more positive affect. The PANAS has been validated in college students, other adults and clinical populations and showed high internal consistency (Cronbach's α of 0.88), good stability over a 2-month period (test–retest correlation of 0.68[12]) and substantial temporal stability over 7 years (test–retest correlation of 0.42).[13]

Childhood maltreatment was assessed with the well-validated Childhood Trauma Questionnaire.[14]

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Genotypes and principal components in the GTP discovery sample. DNA was extracted from saliva or blood and genotyped on Illumina Omni1-Quad BeadChip. We used Illumina (San Diego, CA, USA) GenomeStudio to call genotypes and PLINK[15] to perform quality control (QC) analyses (see Supplementary Figure 1). We removed single-nucleotide polymorphism (SNPs) with call rates $\leqslant$98% or minor allele frequency (MAF) <0.01, and individuals with $>2\%$ missing data or having heterozygosity $\geqslant 2.5$ s.d. away from the mean. We used PLINK to estimate the proportion of identity by descent for each pair of individuals. Among pairs of individuals with an identity by descent >0.12 (indicating cousins or a closer relation), we removed the individual in each pair with the higher rate of missing genotype data. Using autosomal data pruned in PLINK, we performed principal component analysis (PCA) to infer axes of ancestry and remove outlier subjects. Based on PCA, we retained those African-American individuals who fell within 3 s.d. of the medians of the first and second principal components (PCs) in our sample. After completion of QC and PCA, our sample consisted of 3728 African-American individuals genotyped for 883 511 SNPs. Of the individuals included in the genetic sample, 68% had positive affect data and were included in the GWAS ($N=2522$). We compared the $N=2522$ group with the $N=3728$ group and found that they had very similar distributions of the MAFs and demographic and psychological characteristics (Supplementary Table 1), suggesting that the discovery sample was representative of the overall $N=3728$ sample.

GWAS of positive affect in the discovery sample. The positive affect score was transformed using the one-parameter Box–Cox transformation with λ-value of 2 to increase normality.[16,17] Using PLINK, we regressed the positive affect score on allele count assuming an additive model and included gender, childhood maltreatment total score and the top 10 PCs as covariates. We use the commonly accepted genome-wide significance level of $P<5\times10e-8$.[18,19]

cis-eQTL analysis for rs322931 using brain data set

The publicly accessible data set from the UK Human Brain Expression Consortium (BRAINEAC; braineac.org) consists of post-mortem brain samples from 134 neuropathologically normal individuals of European descent.[20,21] We examined the average gene expression across the 10 available regions in this data set: cerebellar cortex, frontal cortex, hippocampus, medulla, occipital cortex, putamen, substantia nigra, temporal cortex, thalamus and intralobular white matter. Affymetrix GeneChip Human Exon 1.0 ST arrays (Santa Clara, CA, USA) were used to obtain whole transcriptome profiling.[20] The QC process has been previously described in detail.[20,21] Expression data were corrected for gender and batch effects of hybridization date and brain bank before expression quantitative trait locus (eQTL) analyses.

Genomic DNA was extracted from brain tissues and genotyped on Illumina Infinium Omni1-Quad BeadChip and ImmunoChip. After standard QC, both data sets were combined and only SNPs with good post-imputation quality ($r^2>0.50$) and MAF $\geqslant 0.05$ were included for analyses.[20,21] For rs322931, only one subject had imputed genotype in this data set.

We obtained exon-level and transcript-level probes for the genes located within 1 Mb of rs322931 for *cis*-eQTL analysis,[21] in which the expression level was the outcome and rs322931 genotype the independent variable (using additive model). Bonferroni adjustment was used for multiple testing correction.

rs322931 vs miR-181 in blood

RNA was extracted from whole blood of 68 GTP participants. Small RNA was size fractionated from total RNA and then used as the starting material to generate small RNA libraries following the Illumina TruSeq Small RNA protocol. Quality of the sequencing data was inspected with FastQC. Adapters were trimmed with Trimmomatic.[22] After trimming, sequences <14 nucleotides were excluded. Trimmed reads were aligned to the miRBase v.21,[23] with SHRiMP aligner,[24] and only reads with a PHRED score of $\geqslant 10$ were considered for alignment. Percent reads mapped to mature microRNAs (miRNAs) ranged from 81 to 98%, indicating high-quality libraries and sequencing data. Among the 68 samples, one was an outlier with raw read counts 2.4 s.d. below the mean and was excluded from the analysis. Counts of aligned miRNAs were normalized with regularized log-transformation using DESeq2.[25] The following miR-181a/b was extracted from this data set: miR-181a-2-3p, miR-181a-3p, miR-181a-5p, miR-181b-2-3p, miR-181b-3p and miR-181b-5p. Above 90% of the 67 samples had 0 abundance count for miR-181b-2-3p and miR-181b-3p, and 90% of the samples had counts $\leqslant 7$ for miR-181a-3p; thus, these three miRNAs were excluded from the eQTL analysis based on the threshold criteria for calling expressed miRNAs with high confidence.[23] eQTL analysis for the remaining three miR-181a and miR-181b was performed using linear regression, where the normalized expression of the miRNA was the outcome, genotype was the independent variable (additive model) and gender, the first genotypic PC and batch were the covariates. Multiple testing was addressed with Bonferroni correction.

Functional MRI study

Procedure. A total of 55 women from the GTP cohort (2 TT, 16 TC and 37 CC for rs322931) completed magnetic resonance imaging (MRI scan), as illustrated in Figure 1. Two participants were excluded for falx calcification and eight because of excessive head motion (see below). The final sample for analysis included $N=45$: 2 TT, 12 TC and 31 CC (demographics in Supplementary Table 4). Participants viewed scene stimuli from the International Affective Picture Series.[26] Trials began with a white fixation cross, centered on a black background for a jittered intertrial interval of 1.5–2.5 s. The scene was then presented for 1.5 s, followed by a rating period of 1.5 s. Scene stimuli were displayed in color full-screen at a resolution of 1024×768. The rating screen included a black background with 'like/neutral/dislike' centered in white, 48 pt Helvetica font. In all, 36 positive, 36 negative and 36 neutral scenes were presented in a semi-random order such that no more than two pictures of the same valence preceded one another. Following scanning, participants reported subjective emotional arousal responses to each scene on a 1–5 scale (1: very little or no arousal; 5: high arousal). Item order in the viewing and rating tasks was counterbalanced across participants.

MRI acquisition. Scanning took place on a 3.0T Siemens Trio with echo-planar imaging (Siemens, Malvern, PA, USA). T1-weighted scans were collected using three-dimensional MP-RAGE with 176 contiguous 1 mm axial slices (TR/TE/TI = 2600/3.02/900 ms, 1 mm^3 voxel size). Functional images were gathered using 37 3-mm axial slices in a descending interleaved sequence (TR/TE = 2000/3.00 ms, 3 mm^3 voxel size).

MRI preprocessing and analysis. Initial data quality checks were performed using ArtRepair.[27] Slices containing spike artifacts were identified and replaced using linear interpolation, with no more than 4% of slices repaired per participant. Volumes affected by motion artifact were repaired using linear interpolation, with no more than 5% of volumes repaired per participant. Participants with head motion >1.5 mm/TR in more than 5% of volumes were excluded from further analyses. Additional image preprocessing steps and statistical analyses were implemented in SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK). Volumes were slice-timing corrected to the middle slice in time, and spatially realigned to the first image of the run. A 128 Hz high-pass filter removed low-frequency noise.[28] Segmented T1s and co-registered functional images were normalized to the Montreal Neurological Institute (MNI) template. Functional images were visually examined to verify that no participant had dropout in any substantial portion of the nucleus accumbens (NAc) or amygdala. Images were smoothed with a 6 mm Gaussian kernel.

Task-related activity was modeled by convolving the onset times of scene stimuli in the positive, negative and neutral emotion conditions with a canonical hemodynamic response function. First-level models included the three emotion conditions, and covariates for six rigid-body motion parameters. Contrasts of the Negative > Neutral conditions, and Positive > Neutral conditions from each subject were used in group-level random effects analyses. Genotype effects on emotion-related activation were investigated for rs322931. A dominance model was used to compare emotion-related activation in the CC group ($N=31$) with activation in T-carriers (TC, TT; $N=14$). Regions of interest were defined anatomically, using the masks from the IBASPM MaxPro MNI atlas[29] (bilateral NAc), and probabilistic maps following Amunts *et al.*[30] (bilateral amygdala). Statistical significance levels for regions of interest and exploratory whole-brain analyses were height extent corrected to a threshold of $P<0.05$, using Alphasim Monte Carlo simulation with 1000 iterations for voxels within the NAc and amygdala regions of interest, and a gray-matter mask created from the segmented MNI 152-subject average T1 for whole-brain analysis. With a cluster-forming threshold of $P<0.05$, extents of $k=5$ for the NAc (voxel-wise $P<0.008$) and $k=18$ for the amygdala (voxel-wise $P<0.003$) were required for a corrected threshold of $P<0.05$. Exploratory whole-brain analyses with a cluster-forming threshold of $P<0.01$ required an extent threshold of $k=25$ to reach a corrected threshold of $P<0.05$

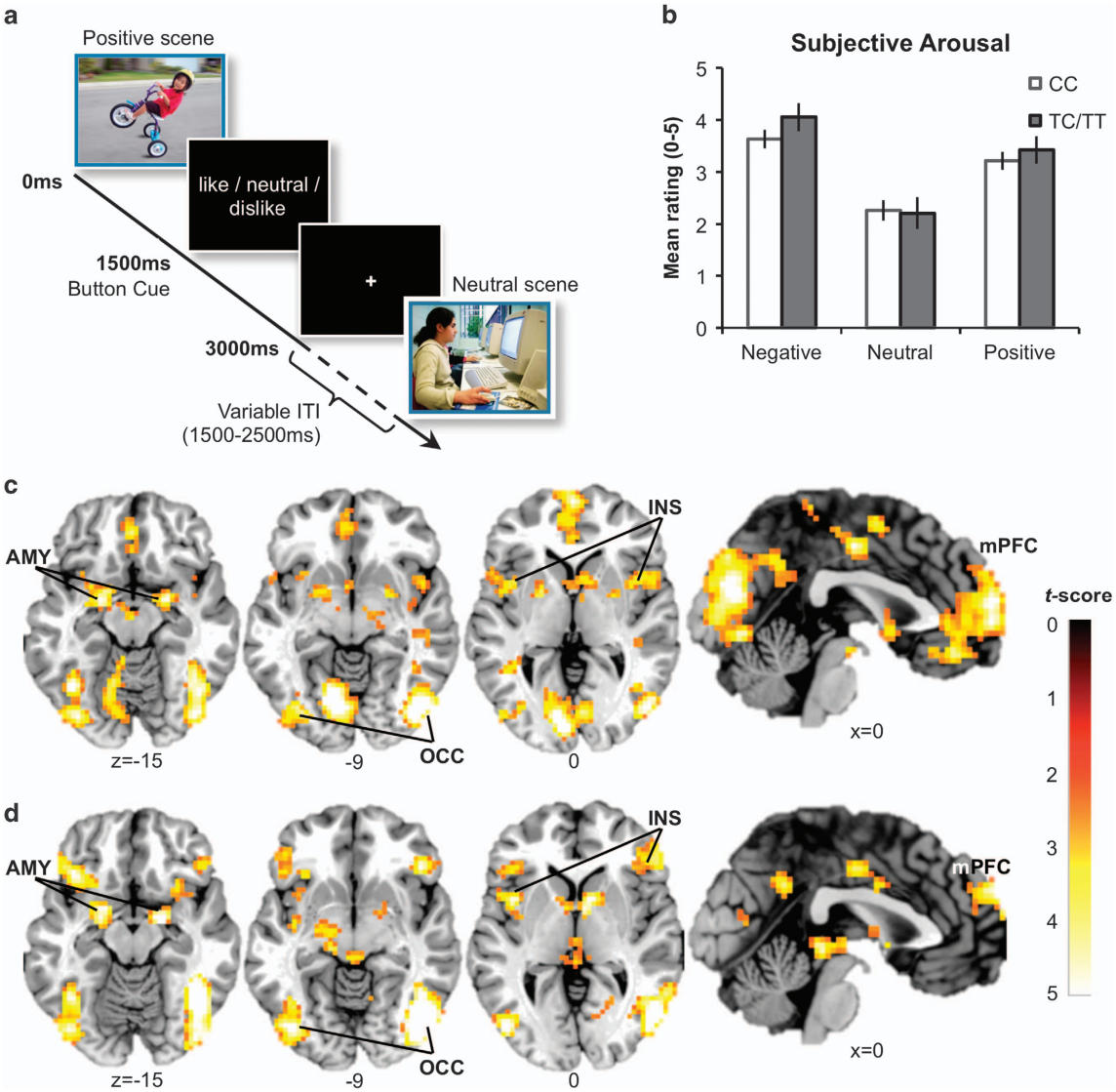

Figure 1. Functional magnetic resonance imaging (fMRI) task measuring response to emotional scene stimuli. (**a**) Example trial structure during the scene-viewing task. (**b**) Subjective arousal ratings, shown as the mean across participants for each emotion condition, by rs322931 genotype group. Emotion condition significantly influenced arousal ratings, $F[2, 60] = 40.9$, $P < 0.001$), and there was no effect of genotype or genotype × emotion interaction (P-values > 0.10). Error bars represent 1 s.e. (**c**) Significant clusters of fMRI activation for positive relative to neutral scenes across the whole sample, $P < 0.05$, corrected at the whole-brain level. (**d**) Significant clusters of fMRI activation for negative relative to neutral scenes across the whole sample, $P < 0.05$, corrected at the whole-brain level. The fMRI activation clusters are overlaid on a representative single subject in Montreal Neurological Institute (MNI) space, and axial slices are shown in neurological orientation. Positive and negative stimuli elicited greater fMRI activation than neutral stimuli in brain regions consistent with emotional arousal and regulation, including the bilateral amygdala, insula, mPFC and occipital cortex. AMY, amygdala; INS, insula; mPFC, medial prefrontal cortex; OCC, occipital cortex.

(voxel-wise $P < 0.0004$). Subjective emotional arousal ratings were tested using a mixed-effects 3 × 2 analysis of variance with emotion as a within-subjects predictor (positive, negative, neutral), and rs322931 group as between-subjects predictor (TT/TC vs CC).

Fear-potentiated startle

Demographic data on the 248 GTP participants (14 TT, 87 TC and 147 CC for rs322931) who completed the fear-potentiated startle (FPS) task are shown in Supplementary Table 7. The FPS protocol was based on previous work[31] shown to produce robust conditioning and consisted of a habitual phase in which the conditioned stimuli (CSs) were presented without any reinforcement and a conditioning phase that consisted of three blocks, each containing four presentations of each trial type, a reinforced CS (CS+), nonreinforced CS (CS−) and noise probe alone. A response keypad was used during fear conditioning to record expectancies of the unconditioned stimulus on each CS presentation.

As previously described,[32] we measured the eyeblink component of the acoustic startle response by electromyography recordings of the right orbicularis oculi muscle. Data were acquired using Biopac MP150 (Goleta, CA, USA) for Windows and were filtered, rectified, and smoothed using MindWare software (MindWare Technologies, Gahanna, OH, USA). Startle magnitude was measured as the maximum amplitude of the eyeblink muscle contraction 20–200 ms after presentation of the startle probe. FPS was calculated by subtracting the mean startle magnitude for noise probe alone trials from the CS+ or CS− trials. A dominance model was used to compare the FPS with the CS+ (threat condition) and FPS with CS− (safety condition) in the CC group ($N = 147$) to T-carriers (TC, TT; $N = 101$).

CHDWB replication sample

The Center for Health Discovery and Well Being (CHDWB) was established to evaluate the effectiveness and utility of a health and prevention-focused rather than disease-focused care setting. Inclusion criteria and exclusion

criteria have been detailed previously.[33] Spiritual well-being was assessed with the FACIT Spiritual Well-Being scale, a well-validated, 12-item scale measuring sense of inner peace, strength from spiritual beliefs and sense of purpose in life in the past 7 days.[34] Its score ranges from 0 to 48, with the higher score reflecting more spiritual well-being. The scale has been translated and validated in 15 different languages and used in many studies.[34] We included the two main ethnic groups, African American ($N=103$) and Caucasian ($N=323$) in the analysis, and excluded one American-Indian and 24 Asian-American individuals. Per dbSNP, rs322931 has similar MAF in African Americans (MAF = 0.184) and Caucasians (MAF = 0.183).

Genotypes and PCs in the CHDWB. DNA was extracted from blood and typed either on Illumina OmniQuad or Core+Exome arrays to obtain genome-wide genotypes. SNPs with MAF <0.01, with missing data in $>5\%$ of the samples, and with Hardy–Weinberg equilibrium *P*-value $<10^{-4}$ were removed. Using PLINK,[15] we pruned the genome-wide autosome SNPs in 50 SNP windows to remove all SNPs with linkage disequilibrium >0.2, and then we computed pairwise identity-by-state metrics, from which the multiple dimensional scaling was computed.

Association between rs322931 and spiritual well-being was examined with linear regression in which genotype for rs322931 was the independent variable (using additive model), total score of spiritual well-being was the outcome and gender and first four PCs were covariates.

RESULTS

A total of 2522 African-American participants, whose socio-demographic characteristics are presented in Supplementary Table 2, were included in the GWAS. We found no evidence to suggest inflation of the association test statistics on the quantile–quantile plot after covarying for 10 PCs, gender and childhood maltreatment (genomic inflation factor $\lambda=1.00$; Supplementary Figure 2). We found two SNPs significantly associated with positive affect after gender, childhood maltreatment and 10 PCs were adjusted for: rs322931 (β (s.e.) = 60.3 (10.8), $P=2.59\times10^{-8}$) and rs7550394 (β (s.e.) = 62.7 (11.4), $P=3.84\times10^{-8}$; Figure 2a). The SNPs reside on chromosome 1 in a locus for LINC01221 and are in high linkage disequilibrium with each other ($D'=0.988$ and $r^2=0.844$). Rs322931 and rs7550394 had a MAF of 0.19 and 0.17, and Hardy–Weinberg equilibrium *P*-value of 0.8 and 1, respectively. The SNPs are closest to miR-181a and miR-181b (Figure 2b). The minor alleles of both SNPs were associated with having more positive affect. These two SNPs also met genome-wide significance level when we only adjusted for gender and 10 PCs (rs322931: $\beta=61.6$; $P=3.86\times10^{-8}$; rs7550394: $\beta=64.4$; $P=4.73\times10^{-8}$). Given their high degree of linkage disequilibrium with each other, in follow-up studies, we focused on rs322931 for further analyses.

rs322931 is a brain *cis*-eQTL for miR-181a and miR-181b

We next investigated whether rs322931 is a *cis*-eQTL in the brain. There are six genes within 1 Mb of rs322931: *MIR181A*, *MIR181B*, *PTPRC*, *ATP6V1G3*, *NEK7* and *NR5A2* (Figure 2b). The six genes were represented by eight transcripts in the BRAINEAC data set (Table 1). Among these transcripts, rs322931 was significantly associated with all four of the transcripts for miR-181a1 and miR-181b1, even after multiple testing correction (uncorrected *P*-values = (1.30×10^{-5} to 6.60×10^{-4}); Bonferroni adjusted *P*-values = ($1.04-10^{-4}$ to 5.28×10^{-3}); Table 1).

At the exon level, there were eight probes for miR-181a1/b1 (Supplementary Table 3), of which six were significantly associated with rs322931 at uncorrected *P*-values ranging from 1.30×10^{-5} to 1.90×10^{-3}. The minor allele of rs322931 was significantly associated with lower expression of miR-181a1 and miR-181b1 for all of these six probes. The top three of these six probes were exprID 2450057 ($\beta=0.29\pm0.06$; $P=1.30\times10^{-5}$; Figure 3a), exprID 2450024 ($\beta=0.22\pm0.06$, $P=1.40\times10^{-4}$; Figure 3b) and exprID 2450059 ($\beta=0.20\pm0.05$, $P=1.40\times10^{-4}$; Figure 3c).

In our secondary analysis we found that rs7550394 was also significantly associated with all four of the transcripts for miR-181a1/b1, with *P*-values ranging from 9.10×10^{-5} to 3.30×10^{-3}. Similarly, its minor allele was associated with decreased expression of miR-181a1 and miR-181b1.

rs322931 is a blood eQTL for miR-181b

As we found that rs322931 is a brain *cis*-eQTL for miR-181a/b, we examined the relationship between this SNP and miR-181a/b in peripheral whole blood. We found that rs322931 was significantly associated with miR-181b-5p after gender, age, population substructure and batch effects were simultaneously adjusted for ($\beta=0.19\pm0.07$, $P=0.0121$, Bonferroni adjusted $P=0.0363$; Figure 3d). The minor allele of rs322931 was associated with higher expression of miR-181b-5p (Figure 3d).

Effects of rs322931 on neuroimaging and psychophysiological measures of emotion

To probe potential effects on systems-level neurobiology, we examined the rs322931 polymorphism in relation to brain and psychophysiological markers of emotional reactivity. In the functional magnetic resonance imaging (fMRI) task comparing positive with neutral stimuli, T-allele carriers showed greater responses than those with CC genotype in the NAc (Figure 4a) and amygdala (Figure 4b) regions of interest. No group differences were observed in the NAc and amygdala for negative versus neutral stimuli. Whole-brain analyses of T-carriers relative to the CC showed greater activation in the left putamen and caudate, left superior and middle frontal gyri and left occipital cortex in response to both positive and negative stimuli, relative to neutral (Figures 4c and d, Supplementary Tables 5 and 6). T-carriers showed less activation than CC for negative versus neutral stimuli in the left thalamus and in midbrain areas including the periaqueductal gray and substantia nigra. In sum, the minor allele of rs322931 was associated with greater fMRI activation of brain regions involved in emotion and reward.

In fear conditioning paradigm we found that T-carriers had less fear to the safety cue than CC group ($P=0.025$; Figure 4e). The groups did not differ in their responses to the fear cue. This finding suggests that the minor allele of rs322931 is associated with better fear inhibition in response to safety cue.

Quasi-replication of rs322931 in positive affect in an independent cohort

As spiritual well-being is a dimension of positive affect, we performed a quasi-replication of our GWAS finding in spiritual well-being in the CHDWB cohort. Among the 426 participants (295 women and 131 men), we found a significant association between rs322931 and spiritual well-being after gender and PCs were adjusted for (β (s.e.) = 1.28 (0.63); $P=0.044$). The minor allele of rs322931 was associated with having more spiritual well-being, consistent with our finding in the discovery sample.

Percent variance of positive affect explained by genome-wide common SNPs

Lastly, using GCTA software[35] we estimated the proportion of variance of positive affect explained by genome-wide common SNPs (MAF $\geqslant0.01$) in the GTP discovery sample. We found that the common autosomal SNPs together explained $21.4\pm14.6\%$ of the variance of positive affect when 10 PCs were included as covariates at *P*-value of 0.07, and $21.5\pm14.6\%$ of the variance of positive affect when 10 PCs and gender were included as covariates at *P*-value of 0.07. Our large s.d. values and nearly significant *P*-values for the estimates are likely because of our relatively small sample size.

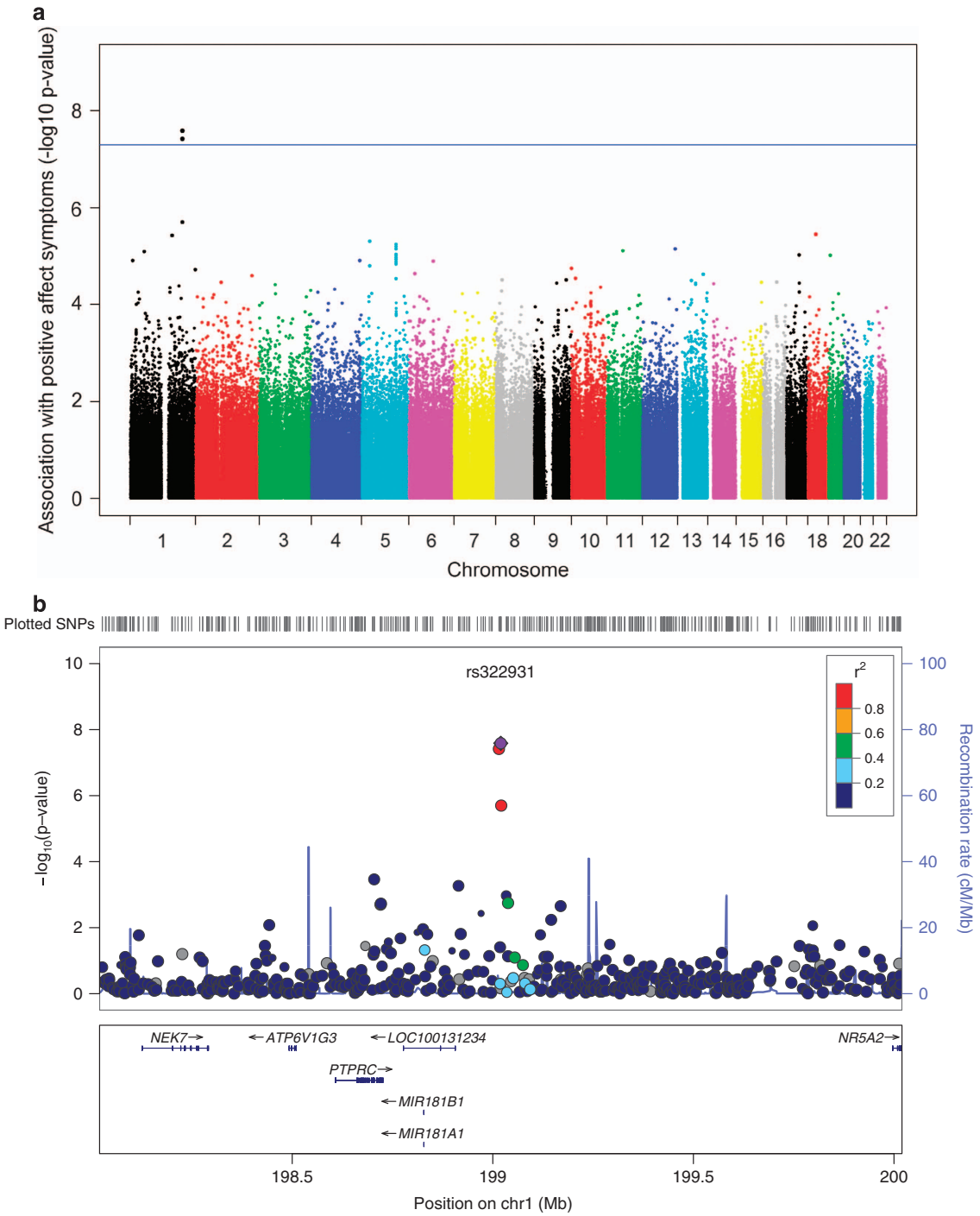

Figure 2. Genome-wide association study of positive affect identifies single-nucleotide polymorphism (SNP) rs322931. (**a**) Manhattan plot of the genome-wide association study (GWAS) for positive affect (rs322931, $P = 2.59 \times 10^{-8}$); the blue line denotes standard genome-wide corrected significance[18] at the negative $\log_{10}(5 \times 10^{-8}) = 7.3$. (**b**) Regional plot of SNP associations within 1 Mb of rs322931 (purple triangle) on chromosome 1; plot generated with LocusZoom.

DISCUSSION

In a GWAS of positive affect we identified a novel SNP on chromosome 1, rs322931, whose minor allele was significantly associated with having more positive affect in the discovery sample and more spiritual well-being in an independent replication sample. Consistently, we found the minor allele of rs322931 significantly associated with increased fMRI activation of the NAc, a key brain region for pleasure, reward and motivation to positive emotional stimuli, and with better inhibition of fear during presentation of a safety cue in a human fear conditioning paradigm. Furthermore, rs322931 influences expression of miR-181a and miR-181b in the brain and miR-181b in blood that is notable for several reasons. First, prior studies suggest that miR-181a is part of the reward neurocircuitry as its expression in hippocampal neurons is induced by cocaine and amphetamine and dopamine signaling,[36] and its expression in the NAc

Table 1. Association between rs322931 and average gene expression across the 10 brain regions for the 6 genes located within 1 Mb of rs322931 (*cis*-eQTLs) at the transcript-level ID

Gene symbol	*Marker*	*rsid*	*exprID*	*Chr*	*Start*	*Stop*	P-*value*
MIR-181A1	Chr1:199019855	rs322931	t2450056	Chr1	198868041	198868274	1.30E − 05
MIR-181A1	Chr1:199019855	rs322931	t2450058	Chr1	198868449	198868844	1.40E − 04
MIR-181A1	Chr1:199019855	rs322931	t2450054	Chr1	198867434	198868019	2.30E − 04
MIR181A1, MIR181B1	Chr1:199019855	rs322931	t2450000	Chr1	198776723	198906548	6.60E − 04
NR5A2	Chr1:199019855	rs322931	t2374126	Chr1	199845845	200146534	6.30E − 03
NEK7	Chr1:199019855	rs322931	t2373736	Chr1	197958385	198292204	1.20E − 01
ATP6V1G3	Chr1:199019855	rs322931	t2449922	Chr1	198492257	198510075	2.00E − 01
PTPRC	Chr1:199019855	rs322931	t2373842	Chr1	198519065	198726605	2.10E − 01

Abbreviations: Chr, chromosome; eQTL, expression quantitative trait locus. LOC100131234 is identified as miR-181A in pubmed.gov.

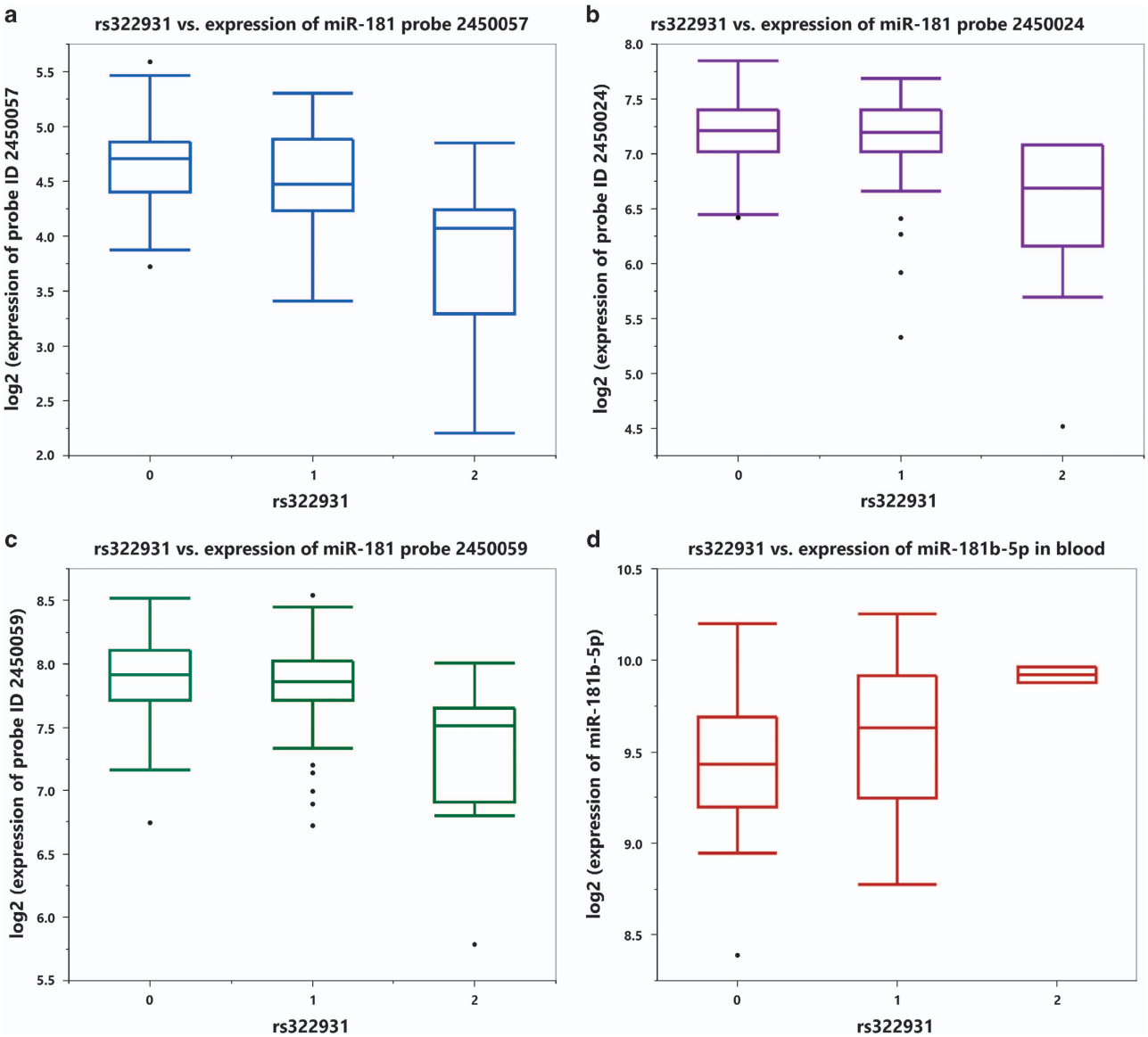

Figure 3. Single-nucleotide polymorphism (SNP) rs322931 associates with levels of miR-181 in brain and blood. The minor allele of rs322931 is significantly associated with lower expression of miR-181a1 and miR-181b1 in the brain for all six of their exon-level probes. Shown on the x axis are the number of minor allele of rs322931 and on the y axis $\log_2$ of level of expression for each of the top three exon-level probes of miR-181a1 and miR-181b1: (**a**) exprID 2450057 ($P = 1.30 \times 10^{-5}$); (**b**) exprID 2450024 ($P = 1.40 \times 10^{-4}$); and (**c**) exprID 2450059 ($P = 1.40 \times 10^{-4}$). (**d**) The minor allele of rs322931 is significantly associated with higher expression of miR-181-5p in blood. Shown on the x axis is the number of minor alleles of rs322931 and on the y axis level of RNA expression of miR-181b-5p. Outliers are depicted as black dots and the median is the middle line in the box.

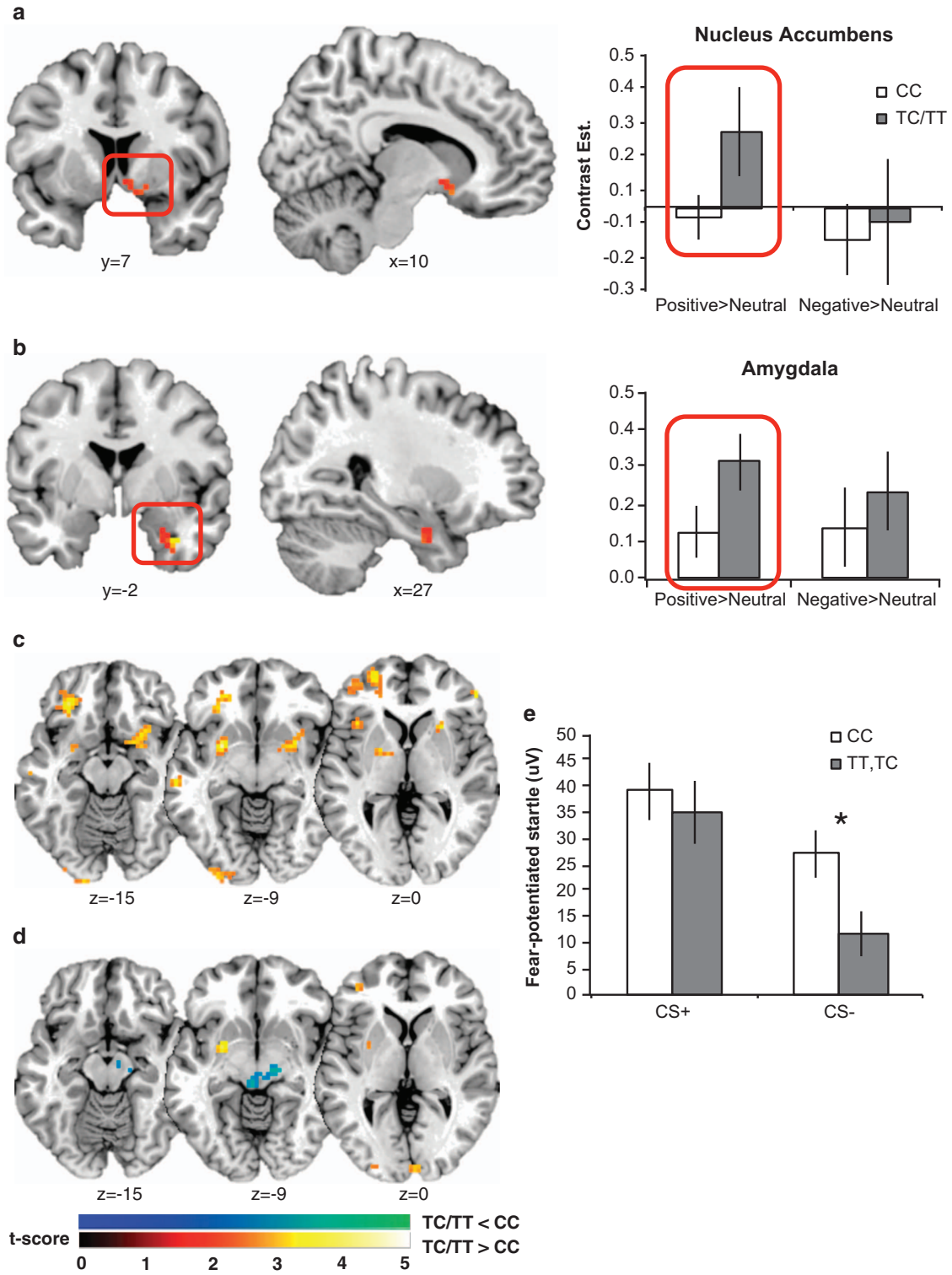

Figure 4. Effect of single-nucleotide polymorphism (SNP) rs322931 genotype on functional magnetic resonance imaging (fMRI) responses to emotional scene stimuli. In both the nucleus accumbens (NAc) (**a**) and amygdala (**b**) regions of interest (ROIs), rs322931 T-carriers showed a greater response than the CC group to positive stimuli (positive > neutral; NAc: xyz = 15,5, − 14, $Z = 2.6$, $k = 12$; amygdala: xyz = 36, − 1, − 32, $Z = 3.3$, $k = 21$). Bar graphs show mean contrast estimates across voxels within the bilateral anatomical ROI masks; error bars show one s.e. Genotype did not influence the response to negative stimuli (negative > neutral) in either ROI. Additional regions that showed group differences in the whole-brain analysis are shown in (**c**) for the positive > neutral contrast and in (**d**) for the negative > neutral contrast. For all fMRI results, significant clusters are overlaid on slices from a representative template brain in Montreal Neurological Institute (MNI) space, in neurological orientation. (**e**) Effect of rs322931 genotype (TT/TC vs CC) on fear-potentiated startle ($P < 0.05$ marked with *).

influences cocaine-induced additive behavior.[37] We postulate that rs322931 mediates positive affect via the reward neurocircuitry by influencing miR-181a expression. Second, miR-181a is strongly enriched in the synaptodendritic compartment of the NAc and influences synaptic plasticity through regulating glutamate receptor 2 subunit of AMPA-type glutamate receptors.[36] Hence, rs322931 can be inferred to mediate synaptic plasticity, a dynamic process that is important for learning and memory,[38] and its decline is associated with cognitive aging and age-related neurological disorders.[39] Such effects on plasticity may explain the finding that the minor allele of rs322931 was associated with better learning of the safety signal in the fear conditioning experiment. Third, miR-181a/b are intrinsic regulators of T-cell receptor sensitivity and signaling strength;[40,41] thus, by influencing expression of miR-181a/b, the SNP rs322931 may have some influence on the immune system. Taken together, we postulate that rs322931 influences positive emotion via miR-181 and the nucleus accumbens and may also influence synaptic plasticity and immune functioning, consistent with the psychosomatic interplay among affect, neuroplasticity and physical health observed in prospective epidemiological studies.

The minor allele of rs322931 was associated with having more positive affect and decreased expression of miR-181a/b in the brain but increased expression of miR-181b in the blood, consistent with adaptive effects of downregulation of miR-181a/b in the brain and upregulation of miR-181b in the blood. For instance, patients with schizophrenia had upregulated expression of miR-181b in the temporal cortex.[42,43] Similarly, depressed mice had upregulated expression of miR-181a in the prefrontal cortex versus controls.[44] Similarly, overexpression of miR-181 in rat hippocampal neurons inhibited dendritic growth[45] and decreased the size and number of dendritic spines.[36] In peripheral blood, posttraumatic stress disorder patients had reduced expression of miR-181a/b versus controls.[46]

It is notable that rs322931 genotype influenced NAc and amygdala responses to positive stimuli but had no effect on responses to negative stimuli. These findings suggest potential differences in the genetic pathways contributing to positive and negative emotion traits. For disease models, the findings highlight that positive emotional traits do not simply reflect decreased negative emotion but may confer resilience to co-occurring negative mood or stress. However, given the observation that rs322931 minor allele predicted greater reactivity to both positive and negative stimuli in striatal clusters outside the NAc, additional research is needed to specifically probe the effects of positive versus negative stimuli on various aspects of reward-related circuitry. The minor allele of rs322931was also associated with lower activation to negative stimuli in the left thalamus, and in midbrain nuclei that play major roles in regulating arousal responses, whose lesion can block arousal-related potentiation of the acoustic startle responses.[47–49] Downregulation of midbrain activation in minor allele carriers may contribute to the decreased startle response to the CS− observed in the fear conditioning experiment. Moreover, conditioned safety signals such as the one used in this study have been shown to activate the NAc because of their having rewarding properties.[50]

We observed that the amygdala responded similarly to the NAc to positive stimuli but not to negative stimuli in the rs322931 T-carriers vs CC group (Figure 4). Specifically, the amygdala showed increased activation to negative stimuli whereas the NAc did not, perhaps because a 1:1 association between these regions is not expected. Indeed, previous fMRI studies have often shown some disconnect in the responses of the amygdala versus NAc to positive and negative outcomes,[51] and a meta-analysis observes a consistent coactivation of the NAc and amygdala across studies, but much stronger associations between NAc and regions including anterior cingulate, medial frontal gyrus, parahippocampal gyrus and other aspects of the striatum.[52] Both the amygdala and NAc are directly connected with many cortical and subcortical regions, and it is likely that they may receive different regulatory inputs from regions such as the prefrontal cortex and hippocampus depending upon the task or context.

For the GWAS of positive affect, we included childhood maltreatment as a covariate in the regression model for two reasons. First, childhood is a sensitive developmental window period when upbringing experiences can powerfully shape emotional development and psychological functioning in adulthood.[53,54] In our data set, there is a significant inverse correlation between childhood maltreatment total score and positive affect score ($P < 0.0001$). Second, studies have shown that genetic variants can moderate one's susceptibility to environmental insults.[55,56]

We examined rs322931 in relation to depression, posttraumatic stress disorder symptoms, alcohol use, drug use, negative affect and resilience in the GTP sample, adjusting for gender and population substructure. Rs322931 was not significantly associated with depression, posttraumatic stress disorder, alcohol use or drug use. However, its minor allele was significantly associated with having less negative affect ($P = 0.002$) and having more resilience ($P = 0.0018$), consistent with our positive affect finding (Supplementary Table 8).

Okbay *et al.*[57] recently published a very large GWAS of subjective well-being, operationally defined as having life satisfaction or positive affect, in which they identified three SNPs meeting genome-wide significance level for subjective well-being. In addition, the authors listed the top 44 SNPs for subjective well-being and the top 30 SNPs for positive affect in particular from their meta-analyses of original, unimputed data sets, as well as the top 76 SNPs for subjective well-being from a meta-analysis of cohorts with imputed genotypes. Our GTP data set has original, unimputed genotypes. We examined the top SNPs from the meta-analysis of Okbay *et al.*[57] of GWAS of unimputed data sets in our GTP sample. Of their top 30 positive affect SNPs, 15 were found in the GTP sample. Of these 15 SNPs, one SNP, rs6581971 (on chromosome 12, Okbay $P = 4.84 \times 10^{-6}$), was significantly associated with positive affect in our GTP sample at uncorrected *P*-value of 0.007. Of the top 44 SNPs for subjective well-being of Okbay *et al.*,[57] 9 SNPs were found in the GTP sample. Of these nine SNPs, one SNP, rs13102973 (on chromosome 4, Okbay $P = 3.05 \times 10^{-6}$), was significantly associated with positive affect at the uncorrected *P*-value of 0.0487. Next we examined the top 76 SNPs from the meta-analysis of Okbay *et al.*[57] of imputed data sets in our GTP sample. Of the 76 top SNPs for subjective well-being of Okbay *et al.*,[57] 5 SNPs were found in the GTP data set but none was associated with positive affect at uncorrected *P*-value of < 0.05. These observations may be because of three factors. First, our GTP participants were all African Americans compared with the majority of Caucasian participants in the GWAS of Okbay *et al.*[57] Second, the majority of the studies included in the meta-analysis of Okbay *et al.*[57] used one item, usually extracted from various scales, to assess subjective well-being or positive affect, whereas ours used a 10-item, psychometrically validated positive affect scale. Although the GWAS of Okbay *et al.*[57] has great power from their enormous sample size, our study may have more comprehensive and fine-grained assessment of positive affect. Third, our GTP discovery sample consisted of inner-city participants with high levels of stress and trauma exposure. Hence, positive affect in this context reflects positive emotions in the face of environmental adversity that arguably allowed us to observe adaptive effects of polymorphisms that may not be observable in populations with lower trauma or stress exposure.

Our results should be interpreted in light of their limitations. First, the sample sizes for both the discovery and replication samples are relatively small. With relatively few studies on genetic underpinnings of positive emotions, we face the challenges of a nascent research area. Second, our replication is a quasi-replication as trait-level positive affect was assessed in the discovery sample and state-level spiritual

well-being was used in the replication sample. Nevertheless, spiritual well-being represents positive affect specific to the dimensions of inner peace, sense of purpose in life and strength from spiritual beliefs. Similar to positive affect, spiritual well-being has been shown to be associated with better quality of life ($r = 0.48$), better life satisfaction ($r = 0.318$) and better physical well-being ($r = 0.47$), and is inversely associated with hopelessness ($r = -0.55$), anxious preoccupation ($r = -0.49$) and depression ($r = -0.34$).[34] Notably, genetic factors influencing the expression of the different dimensions of psychological well-being have been shown to be largely shared, as reflected by the high estimates of genetic correlations among the different dimensions (ranging from 0.77 to 0.99).[11]

Future studies are needed to replicate our findings and to deeply sequence the region around rs322931 to elucidate the causal genetic variants for positive emotions. Understanding the neurobiology of positive affect and emotional well-being may provide a complementary and powerful approach to the current predominant focus on the biology of psychopathology.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors edited and commented on the manuscript. KJR obtained funding for the GWAS of positive affect, oversaw the GTP study and revised the manuscript. APW, LMA and JSS wrote the first draft of the manuscript. APW and LMA performed the GWAS of positive affect. APW performed the eQTL analyses in brain and blood. JSS designed the fMRI study and analyzed the fMRI data. GG provided the genetic data for the CHDWB cohort. GG and APW performed the genetic replication study. TJ designed the study of fear conditioning and analyzed the neurophysiology data. APW and TSW performed the GCTA heritability analysis. GT contributed to the miRNA data analysis. YL and PJ contributed to the generation of miRNA data. EBB contributed to the generation of GWAS data. BB contributed to the generation of positive affect data.

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