ORIGINAL ARTICLE

Emotion Processing, Major Depression, and Functional Genetic Variation of Neuropeptide Y

Brian J. Mickey, MD, PhD; Zhifeng Zhou, PhD; Mary M. Heitzeg, PhD; Elizabeth Heinz, BS; Colin A. Hodgkinson, PhD; David T. Hsu, PhD; Scott A. Langenecker, PhD; Tiffany M. Love, PhD; Marta Peciña, MD, PhD; Tal Shafir, PhD; Christian S. Stohler, DMD; David Goldman, MD; Jon-Kar Zubieta, MD, PhD

Context: Despite recent progress in describing the common neural circuitry of emotion and stress processing, the bases of individual variation are less well understood. Genetic variants that underlie psychiatric disease have proven particularly difficult to elucidate. Functional genetic variation of neuropeptide Y (NPY) was recently identified as a source of individual differences in emotion. Low NPY levels have been reported in major depressive disorder (MDD).

Objective: To determine whether low-expression *NPY* genotypes are associated with negative emotional processing at 3 levels of analysis.

Design: Cross-sectional, case-control study.

Setting: Academic medical center.

Participants: Among 44 individuals with MDD and 137 healthy controls, 152 (84%) had an *NPY* genotype classified as low, intermediate, or high expression according to previously established haplotype-based expression data.

Main Outcome Measures: Healthy subjects participated in functional magnetic resonance imaging while viewing negative (vs neutral) words (n=58) and rated positive and negative affect during a pain-stress challenge (n=78). Genotype distribution was compared between 113 control subjects and 39 subjects with MDD.

Results: Among healthy individuals, negatively valenced words activated the medial prefrontal cortex. Activation within this region was inversely related to genotype-predicted NPY expression ($P=.03$). Whole-brain regression of responses to negative words showed that the rostral anterior cingulate cortex activated in the low-expression group and deactivated in the high-expression group ($P<.05$). During the stress challenge, individuals with low-expression *NPY* genotypes reported more negative affective experience before and after pain ($P=.002$). Low-expression *NPY* genotypes were overrepresented in subjects with MDD after controlling for age and sex ($P=.004$). Population stratification did not account for the results.

Conclusions: These findings support a model in which *NPY* genetic variation predisposes certain individuals to low NPY expression, thereby increasing neural responsivity to negative stimuli within key affective circuit elements, including the medial prefrontal and anterior cingulate cortices. These genetically influenced neural response patterns appear to mediate risk for some forms of MDD.

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Author Affiliations: Molecular and Behavioral Neuroscience Institute (Drs Mickey, Heitzeg, Hsu, Langenecker, Love, Peciña, Shafir, and Zubieta) and Department of Psychiatry (Drs Mickey, Heitzeg, Hsu, Langenecker, Shafir, and Zubieta), University of Michigan, Ann Arbor; and Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, Rockville (Drs Zhou, Hodgkinson, and Goldman and Ms Heinz) and University of Maryland Dental School, Baltimore (Dr Stohler).

THE NEURAL SUBSTRATES OF emotion have been intensely studied in recent years. These studies have identified key brain structures and circuits that underlie affective processing in humans and other mammals, including the prefrontal cortex (PFC), the anterior cingulate cortex (ACC), and the amygdala.[1-3] While much progress has been made in describing the common circuit elements that underlie emotion across individuals, the bases of individual differences in affective processing have received less attention. Among humans, such individual differences are of great importance because they are central to conceptualizations of personality and temperament and they contribute to risk for psychiatric illness. The wide interindividual variation in human affective functioning is partly heritable, with roughly half of the observed variance in emotional traits attributable to genetic factors.[4] Thus, identification of genetic variations that influence affective processing may provide a window into the neurobiology that underlies individual differences in emotion and risk for affective disorders.

A promising candidate gene that has received increasing attention is the gene for neuropeptide Y (*NPY* [GenBank K01911]). The *NPY* gene encodes a prepropeptide that is cleaved to NPY, a 36–amino acid

neurotransmitter that is evolutionarily conserved, widely distributed in the brain, and expressed at high concentrations.[5-8] Neuropeptide Y is coreleased with other neurotransmitters by a variety of neuronal cell types, including γ-aminobutyric acid–ergic interneurons in the cerebral cortex.[9] Experiments in animal models have indicated that stress increases expression and release of NPY in the amygdala and that NPY reduces anxiety-like behavior.[10] Neuropeptide Y also modulates central pain processes in animal models.[11,12] While pain stimuli have been well characterized as universal stressors by physical and emotional responses,[13] NPY's role in pain-related emotional reactivity is not well understood.

Several lines of evidence suggest that variation in NPY expression may be important for emotional processing and affective disorders in humans. Plasma NPY has been positively associated with resilience to psychological stress.[14-17] Conversely, low NPY concentrations in plasma, cerebrospinal fluid, and postmortem tissue have been variably associated with mood disorders.[18-25] Variation in NPY expression appears to be driven in part by variation in the *NPY* gene.[22,26] In particular, at least 1 functional locus that predicted expression in lymphoblastoid cell lines, plasma, and brain was identified within human *NPY* haplotypes.[26] Individuals with low-expression genotypes exhibited greater hemodynamic responses in the amygdala when presented with threat-related stimuli, lower endogenous opioid release during a pain stressor, and greater trait anxiety.[26] Furthermore, a 2004 report linked a single-nucleotide polymorphism in the *NPY* gene with treatment-resistant major depressive disorder (MDD).[22]

These findings suggest a model in which genetic variation in the *NPY* gene predisposes some individuals to low NPY expression within key stress-regulatory neural circuits. Reduced capacity for NPY expression in turn would lead to differential processing of stimuli with negative affective valence and potentially increase the risk of developing affective disorders. We examined the predictive validity of this model at 3 levels. First, we used functional magnetic resonance imaging (fMRI) and an emotional processing task to test the hypothesis that healthy individuals with low-expression *NPY* genotypes exhibit greater cortical activation in response to negative stimuli. Second, we tested the hypothesis that healthy individuals with low-expression *NPY* genotypes have more negative affective experiences during stress. Because pain is a potent, universal stressor that is readily manipulated experimentally, we used moderate levels of sustained pain as a stress challenge. Finally, we tested our hypothesis that low-expression *NPY* genotypes are overrepresented among patients with MDD.

METHODS

NEUROIMAGING

One hundred eleven healthy adults completed an fMRI study of passive affective processing. After screening for quality control (eAppendix, http://www.archgenpsychiatry.com), usable data were available for 93 subjects (mean [SD] age, 29 [9] years; 52% male). Task effects were determined in the sample of 93 individuals. Of the 70 subjects who participated in genotyping, 58 were classified by *NPY* genotype and 12 were unclassified according to a previously established haplotype classification scheme (**Table 1** and the "Genotyping" subsection of the "Methods" section). Sampling and recruitment are described in the "MDD Association" subsection of the "Methods" section. All subjects in the fMRI experiment were right-handed and were fluent English speakers. They were not taking exogenous hormones or medications with central nervous system activity, and they were instructed to abstain from use of all psychoactive substances for 24 hours prior to the study. Written informed consent was obtained and all procedures were approved by the institutional review board at the University of Michigan.

Table 1. *NPY* Classification in Study Subsamples

	Participants, No.				
	NPY Genotype Expression[a]				
Subsample	Low	Intermediate	High	Total Classified	Unclassified
fMRI	8	35	15	58	12
Pain-stress challenge	15	47	16	78	18
MDD association					
Healthy subjects	22	68	23	113	24
Subjects with MDD	15	19	5	39	5
All subjects	37	87	28	152	29

Abbreviations: fMRI, functional magnetic resonance imaging; MDD, major depressive disorder; *NPY*, neuropeptide Y.
[a] As predicted by *NPY* genotype.

As described previously,[27] subjects performed an affective word task during which they silently read emotionally valenced words.[28] The blood oxygenation level–dependent (BOLD) signal was measured in the whole brain using a Signa 3-T MRI scanner (GE Healthcare, Milwaukee, Wisconsin) with a standard radiofrequency coil and T2*-weighted pulse sequence. Images were spatially normalized to standardized space (Montreal Neurological Institute space) and smoothed with a 6-mm gaussian kernel. Spatial coordinates are reported in Montreal Neurological Institute space. Further details are given in the eAppendix.

The BOLD responses were modeled with SPM2 software (Department of Cognitive Neurology, Wellcome Trust Centre for Neuroimaging, London, England) using a general linear model and canonical hemodynamic response function. Statistical analysis proceeded in 2 stages. At the first level, activation maps were derived for individual subjects, including task-related covariates of interest and nuisance covariates (head translation and rotation). At the second level, a random-effects analysis was used to determine group effects, resulting in statistical parametric (t or F) maps. Statistical tests were applied to the 2 primary contrasts of interest, negative – neutral words and positive – neutral words, since these isolated affective processing and controlled for nonspecific lexical and visual processing. Where those contrasts showed significant effects, we also explored responses to word stimuli relative to rest periods (ie, negative – rest and neutral – rest) to aid interpretation. A mask excluded the cerebellum and brainstem below the midbrain because these regions were not well represented. The resulting voxelwise maps ($2 \times 2 \times 2$ mm) were thresholded with 2-sided uncorrected $P < .001$ and extent $k > 55$ voxels (440 mm^3), which protected against overall type I error at $P < .05$ according to Monte Carlo simulations with AlphaSim.[29] All reported P and z values are 2-sided.

For analyses in regions of interest, the average percentage of change in BOLD signal within the region was computed. We used ordinal regression with *NPY* genotype group (low, inter-

Table 2. *NPY* Haplotypes[a]

Marker	Haplotype				
	H1	H2	H3	H4	H5
rs3037354	Ins	Del	Ins	Ins	Ins
rs17149106	G	G	G	G	T
rs16147	C	T	T	C	T
rs16139	T	T	T	T	C
rs5573	A	G	G	A	G
rs5574	T	C	C	C	C

Abbreviation: *NPY*, neuropeptide Y.

[a]The 3 major haplotypes (H1, H2, and H3) defined the *NPY* expression classification of each subject (low expression, H1/H1; intermediate expression, H1/H2, H1/H3, or H3/H3; and high expression, H2/H2 or H2/H3) based on expression levels previously determined in vitro and in vivo.[26]

mediate, or high expression) as the dependent variable and percentage of signal change as a covariate (SPSS version 17.0 statistical software; SPSS Inc, Chicago, Illinois). Parameter estimates β (ordered log odds) and 95% confidence intervals are reported. We tested our a priori hypothesis of an *NPY* genotype effect in a single region (medial PFC), identified as the single cluster activated by this task (negative – neutral words). This hypothesis was based on the following: (1) prior reports that low-expression *NPY* genotypes are associated with greater amygdala activation specifically to negative (vs neutral) stimuli[26,30]; and (2) the proposed role of this region in emotion processing[1-3] and depression.[31-35] The task also produced deactivations in other regions (neutral – positive, 2 clusters; neutral – negative, 4 clusters) (eTable). To characterize the regional and valence-related specificity of the *NPY* effect, these clusters were also tested for an effect of genotype using a Bonferroni correction based on the number of clusters per contrast to account for multiple comparisons.

PAIN-STRESS CHALLENGE

Ninety-six healthy adults (mean [SD] age, 25 [4] years; 66% male) participated in a pain-stress challenge described previously.[36,37] Sampling and recruitment are described in the "MDD Association" subsection of the "Methods" section. Seventy-eight of the 96 subjects were classified by *NPY* genotype and 18 were unclassified (Table 1). Fifty-one of these participants also completed the fMRI affective word task. Each individual underwent a standardized pain paradigm in which hypertonic saline was infused intramuscularly into the masseter muscle, resulting in deep sustained muscle pain for 20 minutes at a level that was individually calibrated to a level of approximately 40% of "the most pain imaginable." Subjects provided affective ratings at baseline and immediately after the pain protocol. Written informed consent was obtained and all procedures were approved by the institutional review board at the University of Michigan.

Participants rated affective experience before and after pain with the 60-item Positive and Negative Affective Schedule (PANAS),[38,39] which includes 2 main pseudoindependent subscales: negative affect and positive affect. At both times, the positive affect subscale scores were approximately normally distributed, but the negative affect subscale scores were severely skewed toward low values. For that reason, we analyzed PANAS responses in 2 ways. First, we used a composite measure (the difference of positive affect and negative affect scores), which was readily interpreted, normally distributed, and appropriate for hypothesis testing using repeated-measures analysis of variance and Tukey post hoc tests (SPSS version 17.0 statistical software). Five individuals who were missing baseline data were excluded from that analysis. Second, we used nonparametric Spearman correlation to test for associations between *NPY* genotype and individual PANAS subscale scores before and after pain.

MDD ASSOCIATION

We genotyped 44 individuals with MDD who were recruited for 2 separate studies in the Department of Psychiatry, University of Michigan[40,41] (39 classified by *NPY* genotype, 5 unclassified) (Table 1). Participants were recruited through local advertisement for neuroimaging studies of MDD. Recruitment criteria were identical between the 2 studies except that one recruited women only,[40] whereas the other recruited both sexes.[41] Major medical illness and other Axis I disorder diagnoses were excluded except generalized anxiety disorder, social anxiety disorder, and specific phobia. Subjects were diagnosed as having MDD and a current moderate-to-severe depressive episode using the Structured Clinical Interview for *DSM-IV*[42] administered by an experienced psychiatric research nurse, and diagnosis was confirmed with a clinical interview by a psychiatrist. The healthy comparison sample consisted of 137 healthy control subjects (113 classified by *NPY* genotype, 24 unclassified) (Table 1). Participants were recruited through local advertisement for neuroimaging studies of MDD or pain processing.[36,37,41] Subjects were screened to exclude major medical illness, psychiatric disorder, or substance use disorder. Written informed consent was obtained and procedures were approved by the institutional review board at the University of Michigan.

We tested a single a priori hypothesis that low-expression *NPY* genotypes are overrepresented in the MDD sample. Ordinal regression (SPSS version 17.0 statistical software) was used with *NPY* genotype group (low, intermediate, or high expression) as the dependent variable and diagnostic group as an independent factor. Sex and age were not well matched between groups and were therefore entered as covariates. Because we tested a single hypothesis using a haplotype-based classification scheme validated in prior work,[26] no correction for multiple comparisons was indicated.[43,44] Other association tests were exploratory and aimed at ruling out confounders.

GENOTYPING

Seven polymorphisms within and near the *NPY* gene, including 6 single-nucleotide polymorphisms and a 2-nucleotide in/del, were genotyped with a 5′ nuclease assay as previously described.[26] Each marker was in Hardy-Weinberg equilibrium (all $P > .30$, Pearson χ^2 test). Six polymorphisms composed 5 major haplotypes, H1 through H5 (**Table 2**). Each subject was assigned to a genotype group (low, intermediate, or high expression) based on protein and messenger RNA expression levels previously established in vitro and in vivo (Table 2).[26] Because definitive expression data are not available for the 2 minor haplotypes H4 and H5 (allele frequency 3%-5%), individuals carrying those haplotypes (16% of our sample) were not included in genetic analyses (unclassified individuals in Table 1).

Population stratification was evaluated as a potential confounder using ancestry-informative markers as described previously.[26] In brief, 186 highly informative markers were genotyped using a GoldenGate assay (Illumina, Inc, San Diego, California). Factor analysis resulted in a 7-factor solution that yielded ethnic factor scores for each individual. To test for population stratification in the neuroimaging and pain-stress challenge experiments, we performed Spearman correlations between ethnic factor scores and percentage of BOLD signal change or PANAS composite scores, respectively. For the MDD association study, ancestry-informative markers were unavailable for 9

healthy control subjects and 25 patients with MDD. Therefore, we estimated Caucasian, African, or Asian ancestry based on a European, African, or Asian factor score greater than 0.5 when available (n=118) and used self-reported Caucasian/white, African American, Asian, or other race/ethnicity otherwise (n=34).

RESULTS

HEMODYNAMIC RESPONSES TO AFFECTIVE STIMULI

From the 93 healthy subjects who completed the fMRI affective word task, 58 were genotyped for *NPY* and classified as having an *NPY* genotype of low, intermediate, or high expression. Twelve additional unclassified individuals carried uncommon haplotypes that lack definitive expression data, so they were not included in genotype analyses (Table 1).

For the key contrast of interest, negative vs neutral words, this task activated the medial PFC (corrected $P<.05$; n=93; SPM2 1-sample *t* test; peak coordinates=−2,56,22; $z=4.3$; cluster size=2184 mm^3) (**Figure 1**A-C). We extracted responses within this task-related cluster and tested it as a region of interest. Neither sex nor age was associated with *NPY* genotype ($P=.82$ [sex], $P=.31$ [age], ordinal regression) or percentage of signal change in the medial PFC ($P=.75$ [sex], $P=.71$ [age], linear regression). Similarly, ancestry-informative markers were not associated with *NPY* genotype or percentage of signal change (all $P>.10$, Spearman correlations). Consistent with our primary hypothesis, medial PFC responses to negative (vs neutral) words were inversely related to predicted NPY expression level ($P=.03$; β=−2.00 [95% confidence interval, −3.80 to −0.20]; n=58; ordinal regression) (Figure 1D). Comparison with a resting condition indicated that the effect was driven by greater hemodynamic responses to negative words and a lack of response to neutral words among the low-expression group (Figure 1E).

We followed up on this finding by performing a complementary whole-brain linear regression on *NPY* genotype with the negative–neutral contrast. This analysis revealed an effect of genotype in the rostral ACC (corrected $P<.05$; peak coordinates=14,38,0; $z=3.7$; cluster size=592 mm^3) (**Figure 2**A-C). The low-expression group showed rostral ACC activation to negative (vs neutral) words, whereas the high-expression group showed deactivation (Figure 2D). Notably, activation of the rostral ACC was not evident as a task effect (Figure 1A-C) because responses were oppositely directed in the different genotype groups. Comparison with the resting condition suggested that hemodynamic responses in the rostral ACC decreased with negative words among individuals in the high-expression group and decreased with neutral words among those in the low-expression group (Figure 2E).

The *NPY* genotype effects were further examined in brain regions where other task effects were found. There was no significant activation for the positive–neutral contrast, but task effects were observed in the bilateral parietal and left temporal cortices with the neutral–negative contrast and in the left ventrolateral frontal cortex with the neutral–positive contrast (eTable). Percentage of signal change within these regions was not associated with *NPY* genotype (all $P>.30$, ordinal regression, n=58). Thus, the effect of *NPY* genotype appeared to be specific to the medial frontal cortex and to negative stimuli.

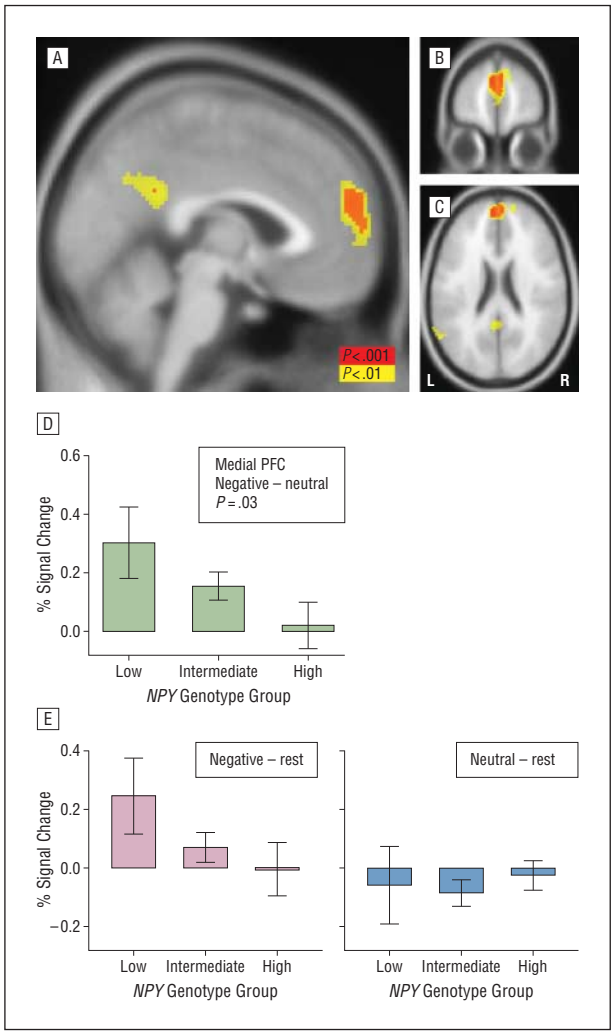
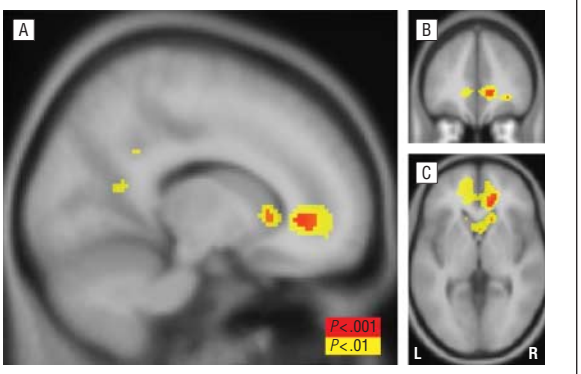


Figure 1. Effect of neuropeptide Y (*NPY*) genotype on medial prefrontal cortex (PFC) responses to negative words. The task effect in the medial PFC with the negative–neutral word contrast is shown in 3 sections: sagittal at x=−2 (A), coronal at y=56 (B), and horizontal at z=22 (C). Red and yellow areas indicate uncorrected 2-sided $P<.001$ and .01, respectively. L indicates left; R, right. This cluster was extracted as a region of interest to test for the effect of *NPY* genotype. D, Effect of *NPY* genotype group on mean percentage of signal change in the medial PFC region of interest shown in A through C ($P=.03$, ordinal regression). E, Mean percentage of signal change for negative–rest and neutral–rest contrasts. Error bars indicate standard error.

AFFECTIVE EXPERIENCE DURING STRESS

Ninety-six healthy adults who had completed the experimental pain-stress challenge were genotyped for *NPY*.[36,37] Seventy-eight individuals were classified as having low, intermediate, or high NPY expression; 18 additional individuals were unclassified (Table 1).

Self-rated affect was associated with *NPY* genotype before and after the pain challenge (**Figure 3**). Neither sex



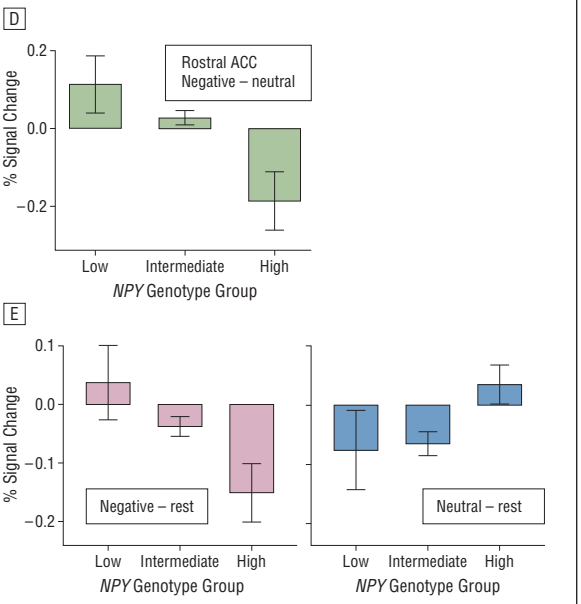


Figure 2. Effect of neuropeptide Y (*NPY*) genotype on rostral anterior cingulate cortex (ACC) responses to negative words. The effect of *NPY* genotype in the right rostral ACC with the negative–neutral word contrast is shown in 3 sections: parasagittal at x=14 (A), coronal at y=38 (B), and horizontal at z=0 (C). Red and yellow areas indicate uncorrected 2-sided $P<.001$ and .01, respectively. L indicates left; R, right. D, Effect of *NPY* genotype group on mean percentage of signal change in the rostral ACC region identified in A through C. E, Mean percentage of signal change for negative–rest and neutral–rest contrasts. Error bars indicate standard error.

nor age was associated with *NPY* genotype ($P=.52$ [sex], $P=.33$ [age], ordinal regression) or PANAS ratings ($P=.14$ [sex], $P=.54$ [age], main effect in repeated-measures analysis of variance). Similarly, factor weights of ancestry-informative markers were not associated with *NPY* genotype or PANAS ratings (all $P>.15$, Spearman correlations), indicating that population stratification is unlikely to account for the association. Repeated-measures analysis of variance on the PANAS composite rating indicated an effect of *NPY* genotype ($P=.002$; $F_{2,70}=6.84$), an effect of pain ($P<.001$; $F_{1,70}=13.44$), and no genotype × pain interaction ($P=.16$; $F_{2,70}=1.89$). Post hoc tests demonstrated more negative affect ratings in the low-expression group compared with the other 2 groups ($P=.002$ for low vs intermediate expression, $P=.01$ for low vs high expression, and $P=.99$ for intermediate vs high expression; Tukey test). Examination of subscale scores before and after pain suggested that the effect of

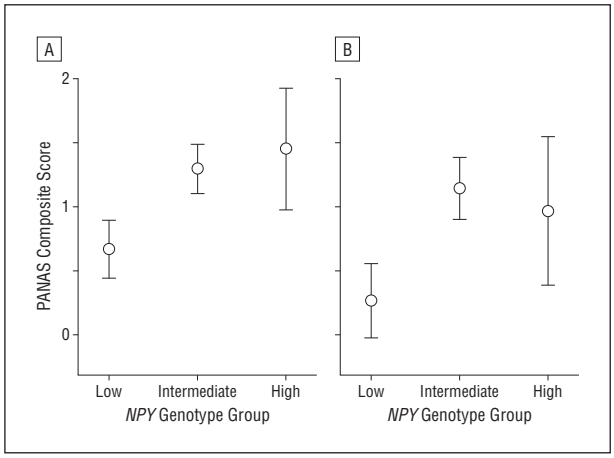


Figure 3. Effect of neuropeptide Y (*NPY*) genotype on affect self-report during stress. The composite Positive and Negative Affective Schedule (PANAS) score, defined as positive affect–negative affect, is shown as a function of *NPY* genotype group (mean and 95% confidence intervals). The PANAS ratings were more negative in the low-expression group before (A) and after (B) a standardized pain-stress challenge (repeated-measures analysis of variance).

NPY genotype was greater on the negative affect subscale scores ($P=.08$, $\rho=-0.21$, n=73 before pain; $P=.02$, $\rho=-0.26$, n=78 after pain; Spearman correlations) than on the positive affect subscale scores ($P=.13$, $\rho=0.18$, n=73 before pain; $P=.74$, $\rho=0.04$, n=78 after pain; Spearman correlations). Among individuals who participated in both neuroimaging and stress-challenge studies (n=51), we found no association between PANAS ratings and activation of the medial PFC or rostral ACC ($P=.27$ and .29, respectively, Pearson correlations).

ASSOCIATION WITH MDD

Thirty-nine individuals with moderate-to-severe MDD and 113 healthy comparison subjects were classified by *NPY* genotype (Table 1). Demographic and clinical characteristics are shown in **Table 3**.

Genotype distributions are shown in **Figure 4**. We confirmed that *NPY* genotype was not associated with sex or age ($P=.54$ [sex], $P=.48$ [age], ordinal regression). However, patients in the MDD sample were older ($P<.001$, 2-sample *t* test) and more often female ($P<.001$, Fisher exact test). We addressed this imbalance by entering age and sex as covariates in the ordinal regression model. An association between the MDD diagnosis and *NPY* genotype was present before adjustment, and it strengthened after adjusting for age and sex ($P=.004$) (**Table 4**).

Two follow-up analyses were performed to further explore age and sex as potential confounders. Because most patients were female, we tested women only and found the association after adjusting for age ($P=.005$) (Table 4). In addition, we performed a restricted analysis of only those healthy control subjects who had been recruited for the MDD studies, which resulted in a small, well-matched control sample (sex: $P=.15$, Fisher exact test; age: $P=.71$, $t_{71}=0.37$, 2-sample *t* test) that did not differ from other healthy control subjects in *NPY* genotype distribution ($P=.51$, ordinal regression). Within this un-

Table 3. Demographic and Clinical Characteristics of Subjects With Major Depressive Disorder and Control Subjects

Characteristic	Subjects With MDD (n=39)	Control Subjects (n=113)
Female, No. (%)	34 (87)	44 (39)
Age, mean (SD), y	36 (11)	27 (7)
Race/ethnicity, No. (%)		
White	27 (69)	77 (68)
African American	7 (18)	20 (18)
Asian	0	8 (7)
Mixed or other ancestry	5 (14)	8 (7)
17-item Hamilton Scale for Depression score, mean (SD)	23 (5)	...
Atypical features, No. (%)[a]	7 (18)	...
Melancholic features, No. (%)[a]	11 (29)	...
Comorbid anxiety disorder, No. (%)[a]	12 (32)	...
First episode, No. (%)[a,b]	11 (29)	...
Age at onset, mean (SD), y[c]	25 (13)	...
Duration of episode, mean (SD), mo[c]	21 (25)	...

Abbreviations: MDD, major depressive disorder; ellipses, not applicable.
[a] Data unavailable for 1 subject.
[b] As opposed to a recurrent episode.
[c] Data unavailable for 4 subjects.

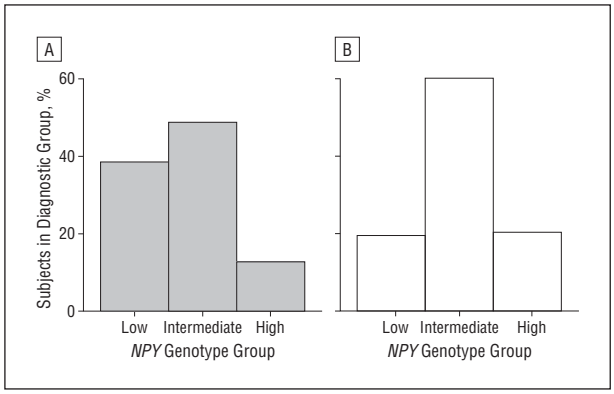


Figure 4. Association of neuropeptide Y (*NPY*) genotype with major depressive disorder (MDD) diagnosis. The percentage of subjects within each diagnostic group is shown vs *NPY* genotype group for patients with MDD (A) and healthy control subjects (B). Low-expression genotypes are more prevalent in the MDD group (ordinal regression).

derpowered sample, we found a trend ($P=.06$) (Table 4) toward overrepresentation of low-expression *NPY* genotypes in the MDD group.

Further control analyses indicated that population stratification (ie, racial/ethnic stratification) was unlikely to account for the apparent association between *NPY* genotype and MDD. First, *NPY* genotype was not associated with white, African American, or Asian race/ethnicity ($P=.45$, .14, and .79, respectively, ordinal regression). Second, race/ethnicity did not differ between patients with MDD and control subjects ($P=.27$, $\chi^2_3=3.88$, Pearson χ^2 test). Third, we performed an additional association test between MDD diagnosis and *NPY* genotype adjusting for white, African American, and Asian status in addition to age and sex and found the same result ($P=.007$) (Table 4). Fourth, because most participants were white, we verified that the association was present in white participants only ($P=.03$) (Table 4).

COMMENT

Our results implicate genetically driven NPY expression in emotional functioning at 3 levels of analysis. At the neural circuit level, we found that low-expression *NPY* genotypes were associated with greater hemodynamic responses in the medial PFC and rostral ACC in healthy individuals viewing negative words. At the level of psychological experience, individuals with low-expression *NPY* genotypes reported more negative affect during a stressor involving sustained, moderate pain over 20 minutes. At the level of syndromal, categorical diagnosis, we found that low-expression *NPY* genotypes were more prevalent among patients with MDD. These convergent findings support a model in which genetically driven low NPY expression predisposes certain individuals to hyperresponsivity to negative stimuli within key affective circuit elements, including the medial PFC, the rostral ACC, and, based on prior work,[26,30] the amygdala. The association of these same low-expression *NPY* genotypes with negative affect during stress and with MDD suggests that these *NPY*-associated neural response patterns may mediate risk for at least some forms of depression.

The association we found with activation of the medial PFC and rostral ACC builds on prior neuroimaging studies that have implicated *NPY* genotype in amygdala function. With the same haplotype groupings that we use here, Zhou et al[26] used fMRI with threat-related stimuli (fearful and angry faces) and reported that low-expression *NPY* genotypes were associated with increased hemodynamic responses in the right amygdala and hippocampus. Domschke et al[30] used fMRI while subliminally presenting emotional faces to patients with MDD. Analyzing a single-nucleotide polymorphism in the *NPY* gene (rs16147, –399T/C), they found that amygdala responses to angry faces (and, to a lesser extent, sad faces) were greater among individuals with the CC genotype, which would include the low-expression group in our analyses.[30] We detected no task or genotype effects in the amygdala. We attribute this result to our use of a different fMRI task, one that involves reading emotionally valenced words and does not generally engage the amygdala.[27,33,35,45,46] Thus, we view our findings as complementary to (rather than in conflict with) findings of previous studies of amygdala responses to threat-related facial stimuli. By using an emotion word task, we demonstrate for the first time to our knowledge that *NPY* genotype has effects on the function of the medial PFC and rostral ACC, core circuit elements that have been multiply implicated in normal emotion processing, regulation of emotion, and MDD pathophysiology.[1-3,31-35] In particular, we found that low- and high-expression genotypes were associated with activation and deactivation, respectively, in the rostral ACC. This cortical region has been consistently implicated in normal emotion processing and depression.[3,31,47] Thus, our fMRI findings add substantially to previously described central effects of *NPY* genotype to include key emotional circuits in the frontal cortex. These findings also suggest that NPY expression in the frontal cortex[5,19,23,24] may have important functional consequences.

Table 4. Ordinal Regression Analysis of *NPY* Genotype and Major Depressive Disorder

Subjects	Subjects, No.		β (95% CI)	*P* Value
	Control	MDD		
All subjects, adjusted for age and sex	113	39	1.24 (0.39 to 2.09)	.004
All subjects, unadjusted	113	39	0.83 (0.11 to 1.55)	.02
All subjects, adjusted for age, sex, and race	113	39	1.20 (0.33 to 2.07)	.007
Women only, adjusted for age	44	34	1.39 (0.43 to 2.35)	.005
White subjects only, adjusted for age and sex	77	27	1.14 (0.12 to 2.16)	.03
Age- and sex-matched samples	25	39	0.94 (–0.05 to 1.93)	.06

Abbreviations: CI, confidence interval; MDD, major depressive disorder; *NPY*, neuropeptide Y.

Our finding of associations between *NPY* genotype, affect under stress, and MDD diagnosis are consistent with growing evidence that implicates NPY in both normal emotion regulation and affective disorders.[10,48] Plasma NPY concentration has been positively associated with resilience to psychological stress,[14-17] and expression of NPY in the central nervous system has been suggested as a general resilience mechanism.[49,50] Conversely, low NPY levels have been implicated in affective illnesses. Low-expression *NPY* haplotypes were associated with greater trait anxiety and undifferentiated anxiety disorders.[26] Low plasma NPY concentrations were found among currently depressed patients with MDD[21] but not among patients with remitted MDD.[20] Postmortem studies have variably reported low NPY levels in the frontal cortex of patients with MDD and bipolar disorder.[19,23,24] Early studies of cerebrospinal fluid concentrations of NPY in patients with MDD were discrepant,[18,25] but a more recent study reported robust reductions among patients with treatment-resistant MDD.[22] Furthermore, the latter study found a greater prevalence of the –399C allele (rs16147) among those same patients with MDD.[22] Because our low-expression group includes individuals who are –399*C*/*C* homozygotes, our study represents a quasi replication of that finding with a less treatment-resistant sample. Furthermore, our findings from healthy subjects during the pain-stress challenge suggest that *NPY* genotype influences an individual's affective experience under stress, even before the onset of illness. Taken together, the evidence suggests that genetic predisposition to low NPY expression increases risk for MDD (and possibly other affective disorders) by increasing sensitivity to negative stimuli at the psychological and neural circuit levels and possibly at the cellular and molecular levels as well.

We tested this model of NPY function in affective processing using a functional genomics strategy that differs from conventional approaches in important ways. Conventional molecular genetic association studies are more susceptible to false-positives because the total number of statistical comparisons (and therefore the extent to which type I error should be corrected) is not always apparent, leading to "hypothesis creep."[43,44] Furthermore, a nonfunctional locus may be more prone to spurious replication because the direction of the effect is ambiguous.[44] We have avoided these pitfalls by testing a single a priori hypothesis using a haplotype-based classification previously validated with in vitro and in vivo NPY expression data.[26] This functionally informed strategy increases statistical power by avoiding the multiple-comparison problem and by targeting genetic variation that has a functional effect. This functional genomics approach may also be compared with conventional measurements of peripheral NPY levels. Such measures may approximate the variables of most interest (eg, synaptic NPY levels), but unlike genotype, they are subject to other sources of variability such as peripheral sympathetic activation,[22] clinical state (depressed vs remission),[20] and random measurement error. Thus, our strategy improves on the classic statistical genetics approach by leveraging prior measurements of peripheral and central NPY levels. Our confidence in these results is further strengthened by the coherent directionality of the haplotype-driven effect across 3 levels of analysis. Nonetheless, independent replication of these results and meta-analyses of larger pooled samples will be essential to validate these findings.

Several limitations of this study are noteworthy. First, we have interpreted these findings as being supportive of a causative model in which (1) genetically driven variation in NPY expression causes neural hyperresponsiveness in key circuit elements and (2) hyperresponsive circuits cause negative affect and increase risk of developing MDD. Given the correlative nature of these experiments, however, our findings can only suggest causality, and other models are certainly possible. Experimental interventions in animal models are needed to test causal mechanisms. Second, our subject sample was one of convenience and may not be representative of the general population or of patients with MDD who are encountered in usual clinical practice. For example, our sample was limited to individuals who were willing to volunteer for neuroimaging experiments and genotyping, which could bias certain personality traits of the sample. Third, because definitive expression data were unavailable for minor *NPY* haplotypes, we were unable to include about 16% of subjects in our analyses. We felt that this limitation was outweighed by the benefits of functionally validated haplotype classification. The role of *NPY* genotype among those individuals will require characterization of in vivo and in vitro expression data for minor haplotypes. Fourth, about two-thirds of our subjects were of European ancestry, so the extent to which these findings apply to individuals of other genetic backgrounds remains to be seen. Similarly, because our MDD sample was 84% female, we were unable to test for association with *NPY* genotype among men. Control analyses indi-

cated that the association with MDD survived (and actually strengthened) after controlling for sex, but sexual dimorphism in the NPY system deserves to be explored. Fifth, the design of this study did not allow us to characterize the degree to which *NPY* genotype might contribute differentially to the risk of MDD vs anxiety. We favor a model of shared risk, but this remains to be tested. Sixth, the sample sizes used here were limiting in some ways. For example, only 58 subjects were classified in the neuroimaging study, and only 8 had a low-expression genotype. Limited statistical power may have prevented us from detecting brain regions besides the medial PFC and rostral ACC that are truly modulated by *NPY* genotype, and parametric statistical tests become less valid for subgroups that contain fewer observations.

Our findings may eventually have clinical implications. The heterogeneity of MDD represents a major barrier to improving our understanding of its etiology, pathophysiology, and optimal treatment. Based on the NPY system's established role in anxiety and stress responses in experimental animals and the increasing evidence for its dysregulation in affective disorders, the NPY system may be an excellent target for MDD subtyping and treatment selection. Along those lines, a recent report suggested that response to antidepressant medication varies with *NPY* genotype.[30] The greatest potential for NPY-based biological markers may lie in guiding development of novel antidepressant agents for the many individuals who fail to respond to currently available treatments.

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Correspondence: Brian J. Mickey, MD, PhD, Molecular and Behavioral Neuroscience Institute, University of Michigan, 205 Zina Pitcher Pl, Ann Arbor, MI 48109-5720 (bmickey@umich.edu).
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