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ARTICLE

Neuropeptide Y and representation of salience in human nucleus accumbens

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Neuropeptide Y (NPY) produces anxiolytic effects in rodent models, and naturally occurring low NPY expression in humans has been associated with negative emotional phenotypes. Studies in rodent models have also demonstrated that NPY elicits reward behaviors through its action in the nucleus accumbens (NAc), but the impact of NPY on the human NAc is largely unexplored. We recruited 222 healthy young adults of either sex and genetically selected 53 of these subjects at the extremes of NPY expression (Low-NPY and High-NPY) to participate in functional magnetic resonance imaging. Responses of the NAc and surrounding ventral striatum were quantified during a monetary incentive delay task in which stimuli varied by salience (high versus low) and valence (win versus loss). We found that bilateral NAc responses to high-salience versus low-salience stimuli were greater for Low-NPY subjects relative to High-NPY subjects, regardless of stimulus valence. To our knowledge, these results provide the first evidence in humans linking NPY with salience sensitivity of the NAc, raising the possibility that individual differences in NPY expression moderate the risk for disorders of mesoaccumbal function such as addictions and mood disorders. Additionally, we found that head motion was greater among High-NPY subjects, consistent with previous reports linking NPY with hyperactivity. Future studies in animal models are warranted to elucidate the neural mechanisms through which NPY influences NAc function and related behaviors.

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INTRODUCTION

Neuropeptide Y (NPY) is an evolutionarily conserved peptide neurotransmitter that is highly expressed in many parts of the mammalian brain [1–3]. In the amygdala, NPY is released in response to stress and produces anxiolytic-like effects [4, 5]. A number of studies in rodents have also shown that NPY elicits reward behaviors through its action in the nucleus accumbens (NAc). For example, administration of NPY into the NAc produced conditioned place preference [6, 7] and increased ethanol self-administration [8]. Accordingly, application of NPY has been reported to cause dopamine release in the striatum in both slice preparations and intact animals [9–11]. Furthermore, intra-accumbal NPY administration has been reported to decrease neuronal firing in vivo [12]. Although the cellular circuitry of NPY has not been fully elucidated, sources of endogenous NPY in the NAc include at least two functionally distinct classes of interneuron as well as projection neurons from the arcuate nucleus [12, 13].

Consistent with findings in rodents, variation in NPY expression in humans has been associated with stress- and emotion-related phenotypes. For example, higher plasma levels of NPY have been associated with emotional resilience after exposure to stress [14, 15] and lower levels of NPY in cerebrospinal fluid and post-mortem brain have been reported in patients with severe depression [16–18]. An important source of human individual differences in NPY levels is polymorphic variation in the *NPY* gene. Zhou and colleagues [19] described three commonly occurring *NPY* haplotypes and used in vitro, in vivo, and post-mortem evidence to determine that these haplotypes can identify individuals predisposed to low versus high NPY expression (i.e., Low-NPY versus High-NPY subjects). Neuroimaging experiments comparing these two groups showed that Low-NPY individuals had exaggerated amygdala responses to threat stimuli, greater responses of the medial prefrontal cortex to negative emotional stimuli, and blunted release of endogenous opioids in response to pain [19, 20]. Low-NPY status was also associated with higher trait anxiety, more negative emotions during a stress challenge, and major depressive disorder [19, 20].

NPY is among the most highly enriched transcripts in the human NAc (top 1%, Allen Human Brain Atlas [21]). This fact, along with the known effects of NPY on the NAc in rodents, led us to hypothesize that *NPY* genetic variation influences NAc function in humans. The NAc has been conceptualized as a mediator of motivational salience, in which the individual identifies stimuli of interest that demand action in order to attain a goal [22, 23]. Although the NAc is best known for its roles in approach behavior and positive emotion, it also mediates avoidance behavior and negative emotion [24–26]. Thus, the effects of *NPY* may depend on stimulus salience, stimulus valence (positive versus negative), or both.

To address these questions, we genetically selected healthy young adults at the extremes of predicted NPY expression (Low-NPY and High-NPY subjects) to participate in functional magnetic resonance imaging (fMRI). Healthy subjects were studied in order

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to focus on neural mechanisms that may drive genetic risk for development of neuropsychiatric disorders and avoid potential variation that is a consequence of these diseases. Participants performed a monetary incentive delay task that elicited robust hemodynamic responses in the NAc while the salience and valence of stimuli were varied. Based on previous evidence that Low-NPY subjects exhibit greater responsiveness of the amygdala and prefrontal cortex [19, 20] and the inhibitory effects of NPY on nucleus accumbens neurons [12, 27] we predicted that NAc responses to salient stimuli would be greater in Low-NPY subjects. Furthermore, based on NPY's well-established role in counteracting negative emotion in animal models, and previous findings linking Low-NPY status with exaggerated responses to negative-valence stimuli in humans [19, 20], we predicted that NAc responses to negative-valence stimuli (potential loss) versus positive-valence stimuli (potential win) would be greater among Low-NPY subjects.

MATERIALS AND METHODS

Participants and design

We studied healthy adults, aged 18–22 years (inclusive), in order to minimize age- and disease-related variability in nucleus accumbens function and thereby optimize the ability to detect genetic effects. The study consisted of two phases. In Phase 1, subjects were recruited to participate in a two- to three-hour visit at a clinical research unit. This visit included informed consent, a structured clinical interview, urine drug screen, urine pregnancy screen, a blood draw by a phlebotomist, questionnaires, and a computerized task (described below). Participants were genotyped and those with pre-specified *NPY* genotypes were invited to participate in a Phase 2 visit. The Phase 2 visit included questionnaires, repeat urine screens, and magnetic resonance imaging (MRI). Further details about the participants and study design are described in the Supplementary Methods.

Behavioral task

We adapted a version of the Monetary Incentive Delay (MID) task used by Cooper and Knutson [28], which independently varied both valence and salience of stimuli. Participants first performed the task outside the MRI scanner during the Phase 1 visit and later performed the task inside the scanner during Phase 2. The five possible trial types were: high-salience and positive-valence (HP); high-salience and negative-valence (HN); low-salience and positive-valence (LP); low-salience and negative-valence (LN); and neutral. The trial type was represented by a cue displayed at the beginning of each trial (W?, L?, W, L, or N; where W and L represent win and loss, the question mark indicates a salient/uncertain outcome, and N is a neutral trial). On high-salience trials, participants had the opportunity to win $1 or avoid losing $1 if they performed well. On low-salience trials, participants won $1 or lost $1 regardless of performance; i.e., the outcome of the trial was presented simultaneously with the cue. On neutral trials, no money was at stake. Reaction time and hit/miss were recorded on each trial. Participants also rated *Arousal* and *Affect* for each cue on a 5-point scale at the end of the Phase 1 task session. See Supplementary Methods for task details.

Genotyping

Six polymorphic markers in the *NPY* gene were determined from genomic DNA. These markers allow assignment of each *NPY* allele to one of three common haplotypes (H1-H3), as well as several additional rare haplotypes, without phase ambiguity [19]. We chose sequencing over other genotyping methods because of low error rates and cost-effectiveness for genotyping in small batches for screening, in addition to the fact that these haplotypes could not be reconstructed from the limited *NPY* coverage on genome-wide arrays. Ancestry was estimated from genome-wide data and principal components analysis. Further genotyping information is provided in the Supplementary Methods.

Image acquisition and processing

Blood oxygenation-level-dependent (BOLD) responses were measured by acquiring T2* weighted images on a 3.0-T Philips Ingenia scanner (Best, Netherlands). Participants completed two task-based fMRI sessions with the MID task. To allow anatomical alignment and non-linear warping, a high-resolution T1-weighted image was also acquired. Pre-processing was performed using SPM software (SPM8, version 4667, RRID: SCR_007037). Because the fMRI method is highly sensitive to motion artifacts [29–31], several procedures were used during image analyses to minimize the influence of head motion. First, subjects with excessive head motion (mean frame displacement > 0.25 mm) were excluded from imaging analyses. Head motion of the subjects included in analyses did not differ by NPY group (see Results). Second, rigid-body least-squares motion-correction was applied during image pre-processing. Third, during first-level modeling, the six realignment parameters from this motion-correction procedure were included as nuisance regressors along with their first derivatives, plus quadratic terms for the original and derivative (24 motion regressors in total). Fourth, subject-level head motion (mean frame displacement) was ruled out as a potential confounder of NPY group effects using linear mixed models (see Supplementary Results). Further image acquisition and processing details are provided in the Supplementary Methods.

Statistical analyses

For hypothesis testing in the primary region of interest, bilateral NAc, we created an anatomical mask of NAc from a standard atlas and dilated by 2 voxels to allow for potential systematic shift in activation peaks [32]. Contrast values extracted from this bilateral NAc region of interest were evaluated using linear mixed models. NAc contrast was modeled as the outcome variable and subject as a random effect. NPY group, stimulus salience, stimulus valence, and their interactions were modeled as fixed effects.

Linear mixed models were also used for exploratory analyses of two other brain regions: a midbrain region of interest [33], since this is the source of dopaminergic innervation of NAc; and a left posterior insula region of interest, based on a previous report of *NPY* effects in this region [34]. Exploratory analyses of other brain regions were performed using SPM8 with whole-brain correction for multiple comparisons. See Supplementary Methods for details about statistical analyses.

RESULTS

Participants

Two-hundred twenty-two young adults were genotyped at the *NPY* locus (see Fig. 1). Subjects in extreme genotype-predicted expression groups (Low-NPY, $n = 31$; High-NPY, $n = 22$) subsequently performed a MID task during fMRI. Nine subjects were excluded during quality-control screening due to excessive head motion and, unexpectedly, 8 of the 9 subjects were members of the High-NPY group. Analyses of head motion and the excluded subjects are discussed below.

Behavioral comparisons

Among the final imaged sample ($n = 44$), the Low-NPY and High-NPY groups did not differ significantly with respect to behavior—reaction time, accuracy, total money earned, affect ratings, and arousal ratings—during the MID task (Table 1). Similarly, we found no significant differences in demographics, genetically estimated ancestry, physiological variables, or questionnaire-based measures, with the exception of a nominally significant difference in respiratory rate (Table S3). Linear mixed models constructed for reaction time, accuracy, arousal ratings, and affect ratings

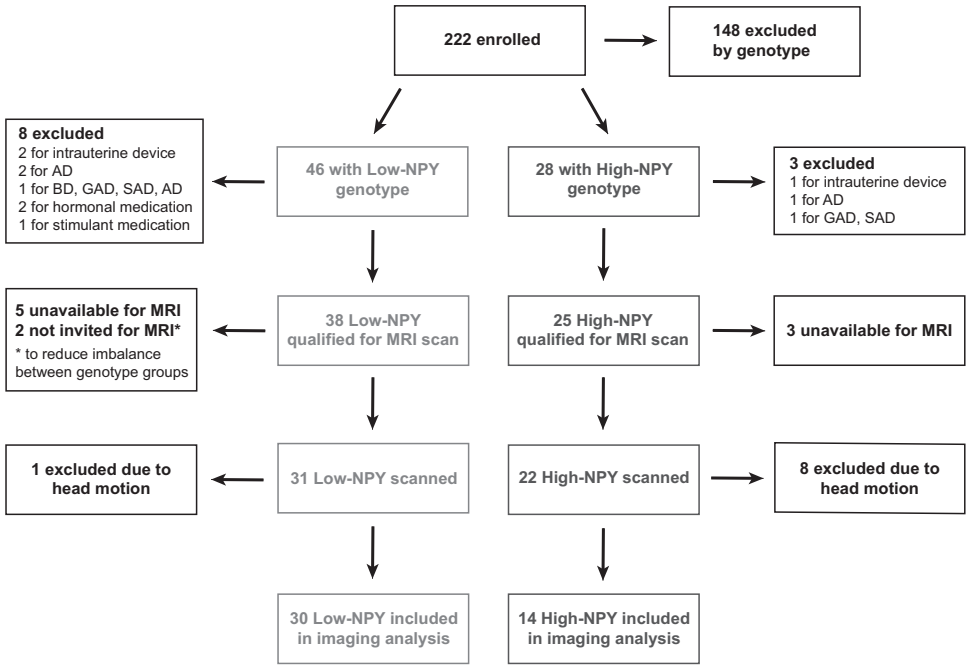

Fig. 1 Subject selection flow figure, where AD = alcohol dependence, BD = bipolar disorder, GAD = generalized anxiety disorder, and SAD = social anxiety disorder. A total of 30 Low-NPY and 14 High-NPY subjects were included in the final analysis

confirmed that there were no significant main effects of group or interactions of salience or valence with group ($p > 0.05$; data not shown). After excluding subjects during quality-control procedures, head motion during scanning did not differ between the groups ($p = 0.36$, Wilcoxon rank sum test).

Neural responses to salience and valence

Analyses of our primary region of interest, bilateral NAc, showed that hemodynamic responses to incentive cues were sensitive to both stimulus salience (high versus low) and stimulus valence (positive versus negative, i.e., win versus loss). Using the linear mixed model, significant effects were evident for salience ($p < 2 \times 10^{-16}$) and valence ($p = 2.4 \times 10^{-6}$), with no significant salience-by-valence interaction ($p = 0.08$). The NAc responded more strongly to high-salience stimuli compared to low-salience stimuli, and to positive-valence stimuli compared to negative-valence stimuli (Fig. 2). This analysis established that the task engaged the NAc as expected and confirmed a previous report [28] that NAc responses are sensitive to both salience and valence. Robust associations were also observed between NAc activation and several behavioral measures, including reaction time, accuracy, and arousal ratings (Table S4). Beyond the NAc, regions that showed whole-brain-significant task effects ($p < 0.05$, FWE-corrected) included the midbrain, visual cortex, anterior cingulate cortex, and cerebellum (Tables S5, S6).

Effects of NPY genotype group in nucleus accumbens

A linear mixed model of extracted bilateral NAc contrast values revealed a significant group-by-salience effect ($p = 2.6 \times 10^{-5}$, $\chi^2 = 17.65$, df = 1; see Fig. 3). The group-by-salience interaction was due to a greater response to high-versus-low-salience stimuli among subjects in the Low-NPY group relative to the High-NPY group. The Hedges' effect size for this group difference in salience contrast was 1.13 (95% CI = 0.66,1.59). The main effect of NPY group, group-by-valence interaction, and group-by-salience-by-valence interaction were all nonsignificant ($p > 0.05$). As described in the Supplementary Results, a series of control analyses showed that the effect of NPY group on NAc response remained significant when potential confounding factors were incorporated into the linear mixed model, including task behavior, stimulus ratings, genetically estimated ancestry, head motion, anxiety, and depression. Furthermore, the shape of the NAc hemodynamic response function was similar between groups.

Effects of NPY genotype group in other brain regions

Because of the strong reciprocal mesolimbic connections between midbrain and NAc, we tested the effect of NPY group on hemodynamic response in a midbrain region of interest. Similar to findings in the NAc, a linear mixed model of midbrain contrast revealed a significant interaction between group and salience ($p = 0.012$). The main effect of NPY group, group-by-valence interaction, and group-by-salience-by-valence interaction were all nonsignificant ($p > 0.05$).

Based on a previous report that a rare *NPY* gene duplication influenced neural responses in the left posterior insula during anticipation of large losses during a monetary incentive delay task [34], we attempted to confirm the finding in Low-NPY versus High-NPY subjects. A linear mixed model of insula contrast values revealed no significant effects of NPY group or interactions of group with salience or valence ($p > 0.05$)

Exploratory analysis using whole-brain correction for multiple comparisons showed no significant main effects of NPY group or interactions with group in other brain areas ($p > 0.05$, FWE-corrected, Fig. S1).

Head motion

We found an unexpected association between NPY group and head motion during fMRI. As shown in Fig. 4, High-NPY subjects showed significantly greater head motion than Low-NPY subjects (Wilcoxon-rank sum test, $p = 0.007$). Data from 8 of 22 (36%) of the High-NPY subjects failed quality-control screening due to head motion (mean frame displacement > 0.25 mm) whereas only 1 of 31 (3%) of the Low-NPY subjects demonstrated this level of movement in the scanner. The High-NPY subjects excluded due to excessive movement were similar to other subject groups with respect to behavioral measures during the MID task, except that they reported lower arousal during the low-salience loss condition (Table 1). However, excluded High-NPY subjects did show

Table 1. Behavioral measures from the monetary incentive delay task

	All subjects	Low-NPY	High-NPY	Excluded High-NPY
	n = 53	n = 30	n = 14	n = 8
Total money earned ($)	10 (4)	11 (3)	10 (4)	11 (4)
Reaction Time (ms)				
LN	192 (28)	193 (25)	185 (24)	194 (39)
LP	180 (33)	189 (28)	172 (51)	174 (23)
HN	167 (39)	170 (24)	158 (44)	166 (100)
HP	173 (37)	175 (32)	173 (56)	137 (65)
neutral	193 (27)	196 (25)	185 (39)	189 (28)
Accuracy				
LN	0.55 (0.20)	0.58 (0.19)	0.58 (0.28)	0.55 (0.15)
LP	0.60 (0.20)	0.63 (0.20)	0.60 (0.20)	0.63 (0.16)
HN	0.75 (0.10)	0.75 (0.15)	0.75 (0.15)	0.78 (0.11)
HP	0.80 (0.15)	0.80 (0.19)	0.75 (0.09)	0.80 (0.06)
neutral	0.50 (0.15)	0.53 (0.10)	0.58 (0.24)	0.50 (0.11)
Arousal Ratings				
LN	2.00 (1.50)	2.00 (1.56)	2.00 (1.34)	1.50 (0.75)a,b
LP	3.25 (1.25)	3.25 (1.25)	3.88 (2.00)	3.50 (1.50)
HN	4.25 (1.00)	4.38 (1.00)	4.00 (1.06)	4.40 (1.00)
HP	5.00 (0.50)	5.00 (0.50)	5.00 (0.56)	5.00 (0.63)
neutral	2.00 (2.00)	1.63 (1.56)	2.88 (2.00)	2.38 (1.81)
Affect Ratings				
LN	1.00 (0.75)	1.25 (0.94)	1.00 (0.19)	1.25 (0.63)
LP	5.00 (0.25)	4.88 (0.50)	5.00 (0.00)	5.00 (0.06)
HN	2.75 (1.25)	3.00 (1.94)	2.75 (1.00)	2.25 (0.81)
HP	4.00 (0.75)	4.00 (0.94)	4.00 (0.75)	4.25 (0.50)
neutral	3.00 (0.50)	3.00 (0.69)	3.00 (0.00)	2.88 (0.81)

Values represent median (inter-quartile range)
LN, low salience, negative valence; LP, low salience, positive valence; HN, high salience, negative valence; HP, high salience positive valence
a: $p < 0.05$ compared to included Low-NPY (Wilcoxon rank sum test for group difference)
b: $p = 0.057$ compared to included High-NPY (Wilcoxon rank sum test for group difference)

significant differences from other subject groups with respect to emotional state and trait measures, including higher positive affect, lower neuroticism, higher agreeableness, and greater motivational drive (Table S3).

DISCUSSION

This study had three major findings. First, we found a large effect of *NPY* genetic variation on NAc responses to salient stimuli. As predicted, NAc responses to salience were greater among Low-NPY subjects. Second, counter to predictions, we found no effect of *NPY* on sensitivity of NAc to stimulus valence. Finally, and unexpectedly, we found that the High-NPY group exhibited excessive head movement during brain imaging.

To our knowledge, these findings provide the first evidence in humans linking *NPY* with salience sensitivity of the NAc. Our results build upon a previously reported association of NAc function with a rare familial *NPY* gene duplication associated with higher NPY levels [34]. In that study, 4 subjects with duplication of *NPY* exhibited reduced responses in the left NAc during anticipation of monetary reward—a positive-valence stimulus [34]. Our study of commonly occurring *NPY* variation confirms lower responsiveness of NAc among High-NPY subjects. We additionally found reduced NAc response to monetary loss (a negative-valence stimulus) among High-NPY subjects, demonstrating that the effects of *NPY* are not valence-specific. Lesch and colleagues did not find an effect of *NPY* variation on response to loss. We believe the most likely reason for this discrepancy is low power related to the limited sample size available for this rare gene-duplication event.

Our finding of an *NPY* effect for negative-valence stimuli adds to findings from a previous positron emission tomography study that employed a sustained pain stimulus [19]. That study found more endogenous opioid release in left NAc (among other regions) in 6 High-NPY subjects versus 8 Low-NPY subjects, suggesting that this endogenous homeostatic mechanism was diminished in Low-NPY subjects. In a parallel observation, we found an *NPY* effect on bilateral NAc responses to potential monetary loss, suggesting that the influence of *NPY* genetic variation on NAc function extends across stimuli in different sensory and behavioral contexts.

The valence-independent effect of *NPY* that we observed in the NAc differs from previous fMRI findings in the amygdala and medial prefrontal cortex. Zhou and colleagues found greater amygdala responses to angry or fearful faces among Low-NPY relative to High-NPY subjects [19]; they did not report effects with positive-valence faces. Similarly, Domschke and colleagues [35] reported that amygdala responses to emotional faces were associated with a SNP (rs16147) in the *NPY* promoter: amygdala responses to angry or sad faces were greater among C-allele homozygotes (most of whom would be categorized as Low-NPY) than T-allele homozygotes (most of whom would be High-NPY). They found no significant effect of this polymorphism on response to positive-valence faces. In a study of medial prefrontal cortical activation with emotional words, we found greater responses to negative words among Low-NPY subjects relative to High-NPY subjects, and no *NPY* effect on responses to positive words [20]. These imaging findings, along with extensive evidence from rodent models linking NPY with amelioration of negative emotional states [36–40] led us to hypothesize that the effects of *NPY* in the human NAc would be greater for negative-valence than for positive-valence stimuli.

Our findings implicate NPY in the more general NAc function of salience detection, regardless of emotional valence. Anatomically segregated negative-valence and positive-valence NAc circuits have been described in rodents, raising the possibility that these parallel NAc circuits mediate distinct modes of behavior—approach for appetitive stimuli and avoidance for aversive stimuli [22, 41]. While the neurochemical and anatomical details of these circuits are still being elucidated [24], our findings suggest that individual differences in NPY could influence approach and avoidance behaviors via actions on both kinds of circuitry.

Our findings raise the question of the molecular and cellular mechanisms through which variation in NPY expression influences function of the NAc. There are at least three known sources of NPY in the NAc—neurogliaform interneurons, low-threshold spiking interneurons, and GABAergic projection neurons from the arcuate nucleus [12, 13, 42]—but the relative contributions of these sources are unclear. NAc function may differ between High-NPY and Low-NPY groups because of differences in basal NPY expression, phasic release, or both. A difference between groups in phasic NPY release in response to salient stimuli might produce measurable changes in the BOLD signal. Alternatively, if NPY levels are consistently lower or higher throughout a lifetime, this could result in long-term structural and functional changes in NAc circuitry that are revealed during fMRI. The BOLD signal correlates most strongly with the local summation of synaptic potentials, rather than with spiking output or with neurotransmission via any particular transmitter [43, 44]. Floresco has therefore suggested that the BOLD signal in the NAc reflects afferent signals processed

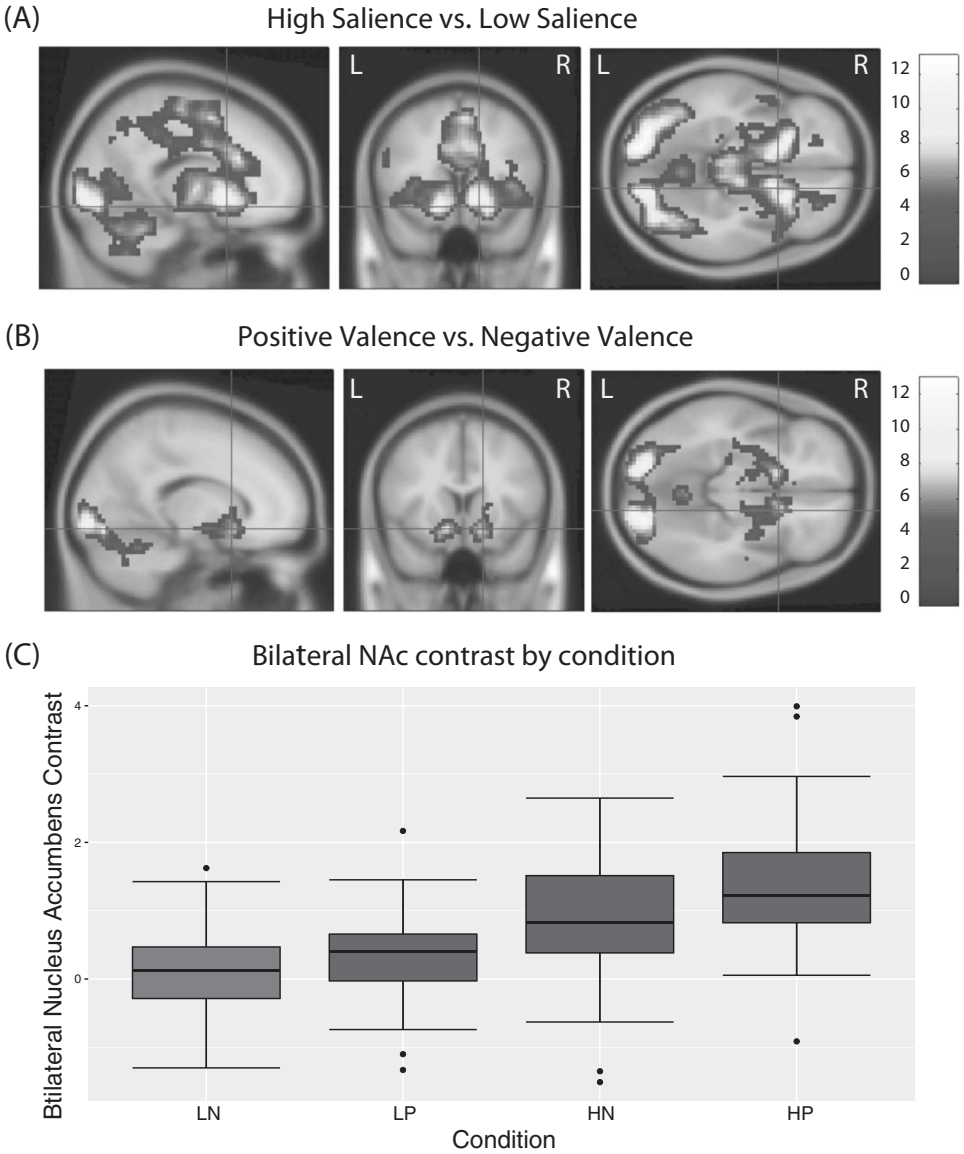

Fig. 2 Effects of MID task, where **a** is the main effect of salience, (display threshold: $p < 0.001$ uncorrected), **b** is the main effect of valence, (display threshold: $p < 0.001$ uncorrected), and **c** is the extracted contrast from the dilated bilateral NAc mask, shown by condition. In **a** and **b**, Gradient bars represent the *t* statistic, and statistical maps are overlaid on T1-weighted sagittal ($x = 16$), coronal ($y = 15$), and axial ($z = -11$) sections. In **c**, whisker length is 1.5× the inter-quartile range, the lower and upper hinge are the 25% and 75% quartile, respectively, outliers represented by dots are any data points beyond the previously stated quartiles, and the central line represents the median

by the NAc, rather than NAc output signals that result from that processing [45]. Because NAc synapses use a diversity of neurotransmitters (GABA, glutamate, dopamine, acetylcholine, NPY, and other neuropeptides), and the BOLD signal does not distinguish among them, the neurochemical basis of our findings remains unknown. Genetic studies in animal models are needed to determine how genetically driven variation in NPY expression influences NAc function at the cell and circuit level.

Because depression has been associated with both low-NPY levels and low reward circuit function, our finding that Low-NPY subjects had greater NAc response may seem counterintuitive. However, we believe our findings are consistent with studies of schizophrenia, depression, and trait anhedonia which suggest that striatal hypoactivation may be characteristic of anhedonia rather than major depressive disorder per se [46, 47]. Based on those studies, one would expect NAc function to track with anhedonic symptoms, returning to baseline after a depressive episode resolves. Because our participants were not currently depressed, their NAc function was presumably near baseline. Our findings suggest that Low-NPY status could heighten the risk of a depressive episode by increasing baseline NAc responsiveness during the non-depressed state. Consistent with this idea, striatal hypersensitivity has been reported among individuals with major depressive disorder after remission of the depressive episode [48]. This hypersensitivity to salient stimuli may also put those with Low-NPY status at a higher risk for anxiety disorders.

We discovered that High-NPY subjects moved their heads more during scanning relative to Low-NPY subjects, suggesting a hyperactive phenotype for High-NPY subjects. To our knowledge, objective measures of movement have not been previously reported in human NPY studies. Intriguingly, in the aforementioned familial gene-duplication study [34], *NPY*

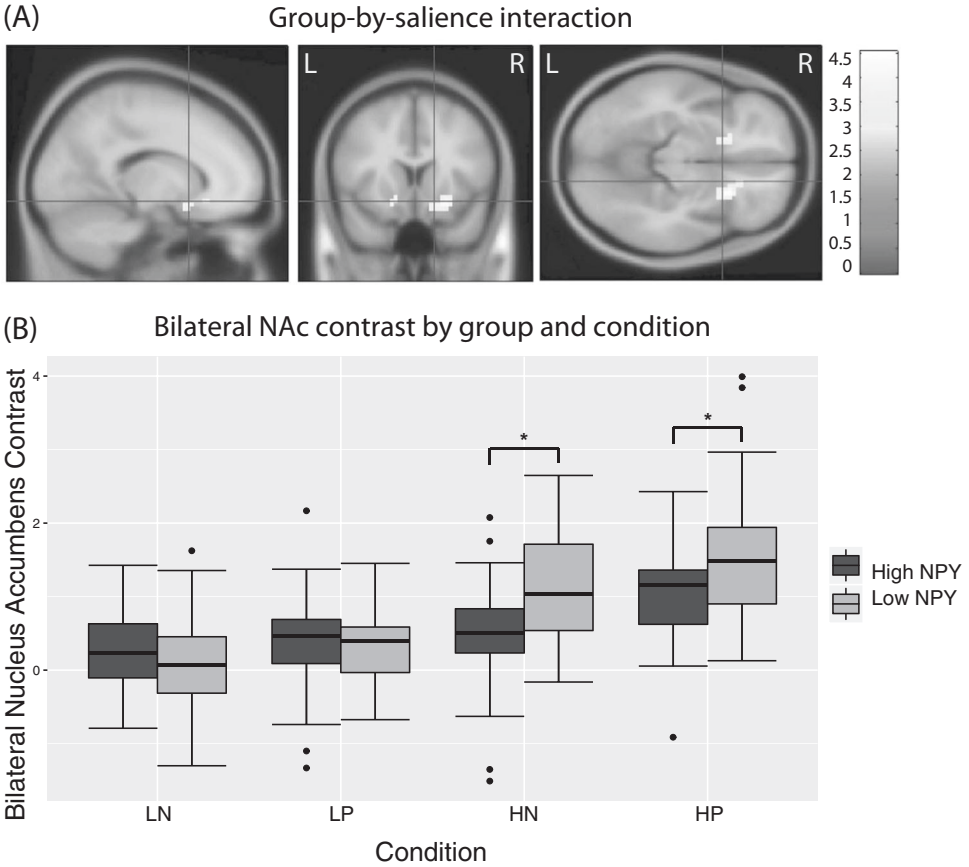

Fig. 3 Effects of MID task as differentiated by NPY group. **a** The between-group contrast (Low-NPY greater than High-NPY) for the high-versus-low-salience contrast (display threshold: $p < 0.001$ uncorrected). **b** The extracted bilateral NAc contrast values for each task condition (LN, LP, HN, HP) per group. In **a**, gradient bar represents the *t* statistic, and statistical maps are overlaid on T1-weighted sagittal ($x = 16$), coronal ($y = 15$), and axial ($z = -11$) sections. In **b**, whisker length is 1.5× the inter-quartile range, the lower and upper hinge are the 25% and 75% quartile, respectively, outliers represented by dots are any data points beyond the previously stated quartiles, and the central line represents the median. *$p < 0.05$

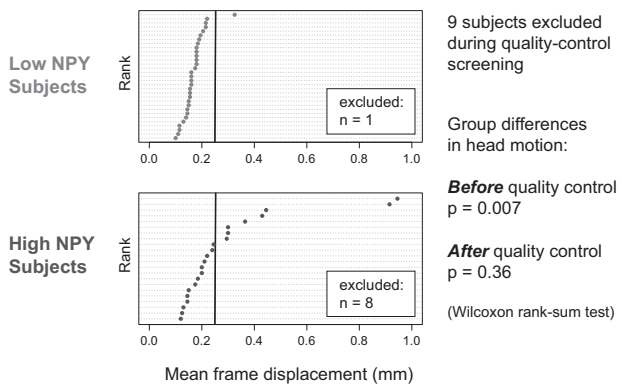

Fig. 4 Cleveland dot plot representing mean frame displacement for each subject by NPY group. Subject dots to the right of the vertical exclusion line (>0.25 mm) were excluded from further analysis to satisfy quality-control threshold. A total of 9 subjects were excluded during quality-control screening, including 1 Low-NPY subject and 8 High-NPY subjects

duplication segregated with severe attention-deficit hyperactivity disorder (ADHD) in a multigenerational pedigree. Thus, our results with High-NPY individuals are consistent with that finding and further support *NPY* as a candidate gene for hyperactivity. Interestingly, we found significant differences between this group and other subjects with respect to traits such as neuroticism (lower), agreeableness (higher), behavioral inhibition (lower), and motivational drive (higher). Because those differences were found in post hoc exploratory analyses, they need to be replicated. However, the data suggest that naturally occurring high expression of NPY may cause (in at least some people) greater body movement while at rest, more positive emotional traits, and greater motivational drive.

Our study has several important limitations. First, the NPY groups we used were not based on directly measured brain NPY levels, but rather on genetic variants in the *NPY* gene previously shown to affect NPY levels in brain tissue, plasma, and in vitro gene reporter assays [19]. A complementary study design would involve randomized administration of NPY or placebo to human subjects, but substantial practical issues currently limit the feasibility of that approach. Future human studies incorporating measurements of NPY levels in post-mortem brain tissue or cerebral spinal fluid will also be helpful, as will the development of radiotracers that can specifically label molecular components of the NPY system using positron emission tomography. A second limitation is that the MID task performed in this study activates a limited set of brain regions. The absence of group differences observed outside of those regions should not be interpreted as the absence of a true effect, because it is likely that our power to detect group differences was limited. Furthermore, various reward tasks differ in important ways, and different results might be

expected if we had chosen an alternative task. Third, our subject age range was restricted to 18–22, limiting the generalizability of our findings to other age groups. Fourth, the excessive head motion among High-NPY subjects necessitated the exclusion of 8 High-NPY members, leaving just 14 High-NPY subjects available for analysis. It is possible that High-NPY subjects that moved excessively differed from other High-NPY subjects in a systematic way with respect to neural function. In support of that idea, the excluded High-NPY subjects did show more positive emotional traits, suggesting that they exhibited the most extreme traits of this group. If the excluded group also had a more extreme neural phenotype, then we may have underestimated neural differences between NPY groups.

While we identified clear effects of *NPY* variation on NAc responses, we did not observe substantial *NPY* group differences in behavior during the monetary incentive delay task. Furthermore, a wide variety of biological and psychological measures collected were not found to be significantly different between groups. The lack of differences in these traits may indicate that individual differences in NPY expression have a subtle role in healthy individuals, with divergent phenotypes only emerging under salient or stressful (whether positive or negative) situations. Alternatively, the exclusion of participants with psychiatric disorders such as depression and anxiety may have obscured some group differences in behavior or psychological traits. Differences in personality traits have been found in other studies. For example, Low-NPY subjects were reported to have higher trait anxiety as measured by the Tridimensional Personality Questionnaire [19] and more negative emotions in the context of an experimental pain challenge [20]. These self-report measures likely incorporate many sources of variance, including variation in genetics and brain function, but also in such factors as previous life experiences and experimental context. Consequently, our study may lack the necessary power to detect group differences in many self-report measures.

The NAc, situated at the interface between environmental input and motivated behaviors, appears to be important for a number of human neuropsychiatric disorders, including addictions, depression, and ADHD. Our findings that genetically driven variation in NPY expression influences NAc function in healthy humans may therefore be clinically relevant. Low-NPY expression could increase risk of addiction or depression through excessive NAc reactivity to salient stimuli. Similarly, High-NPY expression and NAc hypofunction could increase risk of ADHD. These hypotheses should be addressed in prospective human studies. Finally, our findings suggest that genotyping of *NPY* could be useful to identify biological subtypes of neuropsychiatric disorders that respond differentially to different treatments, thus enhancing our understanding of these disorders and improving individual outcomes.

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ADDITIONAL INFORMATION

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