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Neuropeptide Y Receptor Gene Expression in the Primate Amygdala Predicts Anxious Temperament and Brain Metabolism

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Abstract

Background—Anxious temperament (AT) is identifiable early in life and predicts the later development of anxiety disorders and depression. Neuropeptide Y (NPY) is a putative endogenous anxiolytic neurotransmitter that adaptively regulates responses to stress and may confer resilience to stress-related psychopathology. Using a well-validated non-human primate model of AT, we examined expression of the NPY system in the central nucleus (Ce) of the amygdala, a critical neural substrate for extreme anxiety.

Methods—In twenty-four young rhesus monkeys, we measured Ce mRNA levels of all members of the NPY system that are detectable in the Ce using quantitative real time polymerase chain reaction (qRT-PCR). We then examined the relationship between these mRNA levels and both AT expression and brain metabolism.

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Results—Lower mRNA levels of NPY receptor 1 (NPY1R) and NPY receptor 5 (NPY5R), but not NPY or NPY receptor 2 (NPY2R) in the Ce predicted elevated AT; mRNA levels for NPY1R and NPY5R in the motor cortex were not related to AT. *In situ* hybridization analysis provided for the first time a detailed description of NPY1R and NPY5R mRNA distribution in the rhesus amygdala and associated regions. Lastly, mRNA levels for these two receptors in the Ce predicted metabolic activity in several regions that have the capacity to regulate the Ce.

Conclusion—Decreased NPY signaling in the Ce may contribute to the altered metabolic activity that is a component of the neural substrate underlying AT. This suggests that enhancement of NPY signaling may reduce the risk to develop psychopathology.

Keywords

Anxiety; behavioral inhibition; depression; rhesus macaque; stress; prefrontal cortex

INTRODUCTION

Anxious temperament (AT) is a dispositional trait that, when present early in life, increases the risk for the subsequent development of anxiety and depressive disorders (1–3). We have established a non-human primate model of childhood AT, facilitating the identification of the neural mechanisms underlying the development of early life anxiety. In rhesus monkeys, AT is assessed as a composite of threat-induced freezing behavior, inhibition of vocalizations and increased plasma cortisol levels (4, 5). We previously demonstrated that metabolic activity in the central nucleus (Ce) of the amygdala, indexed using high-resolution [^{18}F]-fluorodeoxyglucose-positron emission tomography (FDG-PET), strongly predicts individual differences in AT (6, 7). Moreover, we demonstrated a mechanistic role for the Ce as selective lesions decrease AT (8). To understand the molecular mechanisms in the Ce that underlie the at-risk AT phenotype, we recently performed a transcriptome-wide search for AT-related mRNA expression within the Ce (9). Among our significant results, we found that increased levels of neuropeptide Y receptor 1 (NPY1R) mRNA predict decreased levels of AT. This finding is of particular interest because of the hypothesized role of NPY in anxiety-like responding and as a resilience factor for stress-related psychopathology. The current study expands on our NPY1R mRNA finding by identifying a similar relationship between NPY5R mRNA levels in the Ce and AT. We also provide the first detailed description of NPY1R and NPY5R mRNA distribution in the primate amygdala and associated regions. Lastly, we demonstrate a relationship between NPY1R and NPY5R mRNA levels in the Ce and metabolic activity in cortical brain regions that have the ability to regulate the Ce.

There is growing evidence that neuropeptide Y (NPY), a 36-amino acid peptide, is a stress-modulating resilience factor (10–12). Moreover, alterations in NPY signaling have been linked to anxiety, depressive, eating and substance-abuse disorders (10, 13, 14). NPY is widely expressed in the brain with high levels present in several brain regions which play a role in modulating the response to potential threat, including the amygdala and hippocampus (10). Work in rodents demonstrates that NPY participates in the regulation of anxiety-like responses (15–21) and has marked anti-stress effects (10, 22).

The NPY family includes NPY, which is expressed in the central and peripheral nervous systems as well as pancreatic polypeptide (PP) and peptide YY (PYY), which are expressed in the gut (23). The actions of these peptides are mediated by several G protein-coupled, seventransmembrane domain receptors, including: NPY1R, NPY receptor 2 (NPY2R), NPY receptor 4 (NPY4R), NPY receptor 5 (NPY5R) and NPY receptor 6 (NPY6R) (24). While the NPY6R gene is functional in rabbits and mice, it is absent in rats and considered a pseudogene in primates and pigs (25–27). Receptor signaling is mediated by pertussis toxin-sensitive $G_{i/o}$ proteins and, depending on the cell-type in which they are expressed, can inhibit cAMP formation, alter intracellular Ca^{2+} mobilization, and modulate Ca^{2+} and K^{+} channels (28). There is considerable evidence linking the NPY1R, NPY2R and NPY5R receptors to the effects that central NPY exerts on anxiety-like responding (29–32). The anxiolytic responses to NPY administration are thought to be mediated, in part, by NPY1R and, to a lesser extent, NPY5R, in the amygdala (20, 33), hippocampus (15), septum (34), and locus coeruleus (35). In contrast, activation of NPY2R is thought to produce anxiogenic-like responses (30, 36–38).

To assess the contribution of the NPY system to early-life AT in primates, we focused on mRNA expression levels in the Ce, a key component of the AT neural circuit. Our aim was to extend our earlier microarray findings by examining relations between expression levels of all members of the NPY system in the Ce, AT, and brain metabolism, indexed using FDG-PET. To understand the selectivity of the effects of the Ce NPY system in relation to AT, we also assessed the relationship between NPY system gene expression in a region that is not a core component of the neural circuit underlying AT, the motor cortex (6, 7). Moreover, because there is no detailed description of NPY1R and NPY5R mRNA levels in either the human or nonhuman primate amygdala, we also used *in situ* hybridization to characterize expression patterns for NPY1R and NPY5R mRNA across the major amygdala nuclei and adjacent brain regions. Lastly, to define potential neural circuits that underlie the influences of the NPY system on AT, we looked at metabolic activity throughout the brain to identify regions outside of the Ce where variation in metabolism is correlated with NPY1R or NPY5R mRNA levels in the Ce.

MATERIALS AND METHODS

Overview

Methods were similar to those previously described in detail and are only briefly summarized here (7, 12). A detailed description of the subjects as well as select methods that were not employed in prior work by our group is provided in the Supplemental Information. We assessed individual differences in the AT phenotype and brain metabolic activity using the well-validated, widely used No-Eye Contact (NEC) condition of the human intruder paradigm and high-resolution FDG-PET. The AT phenotype was defined as a composite score of behavioral (increased freezing and decreased coo vocalizations) and hormonal measures (increased plasma cortisol) in response to the mildly-threatening NEC challenge. A magnetic resonance imaging (MRI) scan was acquired in a separate session to aid in image registration. At the end of the study, subjects were sacrificed and brain tissue was obtained from the Ce and motor cortex for RNA extraction and quantification by microarray

analysis and quantitative real-time polymerase chain reaction (qRT-PCR). A series of regression models was used to test relations between AT and mRNA expression levels for members of the NPY family that were detectable in the Ce (NPY, NPY1R, NPY2R and NPY5R); it was not possible to reliably quantify PP, PYY or NPY4R. In cases where expression levels predicted AT (NPY1R and NPY5R), we used *in situ* hybridization to assess the pattern of expression across the amygdala and neighboring regions. We also used whole-brain FDG-PET to test whether mRNA expression levels in the Ce (*ex vivo*) are correlated with metabolism in distal regions of the brain (*in vivo*).

RESULTS

1) Elevated NPY1R and NPY5R mRNA levels in the Ce selectively predict decreased AT

We previously demonstrated that metabolic activity in the rhesus Ce strongly predicts individual differences in AT (7) (Figure 1A–C), and that the NPY1R receptor was one of 139 genes that had mRNA expression levels in the Ce as determined by microarray analysis that predicted significant variation in the AT phenotype (FDR $q < 0.05$) (9). Given the known role of NPY system in anxiety-like responding, in the present study we sought to define the relationship between AT, Ce metabolism and the expression of all members of the NPY family of genes. As shown in Figures 1D and 1E, analyses of qRT-PCR-determined gene expression levels in the Ce revealed significant negative correlations between AT and both NPY1R ($t = -2.28$; $p = 0.035$) and NPY5R ($t = -2.55$; $p = 0.020$). Interestingly, there was a trend for NPY1R and NPY5R mRNA levels to be correlated with each other in the Ce ($r = 0.35$, $p = 0.11$). In contrast, AT was unrelated to variation in the expression of NPY ($t = 0.6$, $p = 0.553$) and NPY2R ($t = -1.56$, $p = 0.135$) in the Ce. It was not possible to reliably quantify PYY or NPY4R, likely due to very low levels of expression in the Ce. The NPY6R mRNA was not included in our analysis as it is considered a non-functional pseudogene in primates (27). Collectively, these results replicate our published gene chip finding on the NPY1R receptor and extend these results to the NPY5R receptor by demonstrating an anatomically-selective relationship between mRNA expression levels in the Ce and AT expression.

To assess the regional selectivity of the correlation between NPY1R mRNA and AT as well as NPY5R mRNA and AT, we examined mRNA expression levels for these two genes in a region of the motor cortex that is not a core component of the neural substrate underlying AT (7). Gene expression analysis using qRT-PCR did not reveal a significant correlation between AT and motor cortex expression levels for either NPY1R mRNA ($t = 0.18$, $p = 0.856$) or NPY5R mRNA ($t = -1.2$, $p = 0.245$). Additionally, there was no significant association between NPY1R mRNA levels in the motor cortex and Ce ($r = 0.37$, $p = 0.084$) or NPY5R mRNA levels in the motor cortex and Ce ($r = 0.18$, $p = 0.41$). The motor cortex is a brain region involved in the expression of locomotion (9), and highlighting the anxiety-specific nature of these results, there was no significant correlation between motor cortex NPY1R or NPY5R mRNA levels and locomotion (NPY1R; $t = 0.05$, $p = 0.957$ and NPY5R; $t = 0.23$, $p = 0.824$).

2) Expression pattern for NPY1R and NPY5R mRNA in the non-human primate amygdala and neighboring regions

To assess the regional expression of NPY1R and NPY5R mRNA in the rhesus amygdala, *in situ* hybridization was performed using tissues slices obtained through the same region of the Ce that was used for PCR analysis in the other hemisphere. The pattern of NPY1R and NPY5R hybridization signals are shown in Figure 2. For NPY1R mRNA, hybridization signals are seen throughout the entire extent of the amygdala. Qualitatively, the highest levels of expression are found in the lateral and medial nuclei, amygdalopiriform cortex transition area and the ventral cortical amygdala nucleus. Moderate levels of expression are seen in the Ce and parvicellular division of the basomedial nucleus. For the NPY5R mRNA, a diffuse signal throughout the extent of the amygdala was observed. This signal was considerably weaker than the NPY1R signal necessitating a significant longer exposure time on the phosphor screen (13 days versus 1 day for NPY1R mRNA). However, it is evident that there are relatively high levels of expression in the medial amygdala, moderate levels of expression in the La and relatively low levels of expression in the Ce (Figure 2). Interestingly, because half the samples were obtained from each hemisphere it was possible to use our qRT-PCR data to assess hemispheric differences in expression within the Ce. The levels of NPY1R mRNA did not differ between hemispheres (left 0.80 ± 0.08; n = 11 vs. right 0.67 ± 0.04; n =12; t = 1.47; p = 0.16), but NPY5R mRNA levels were 20% higher in the left hemisphere compared to the right hemisphere (left 2.18 ± 0.14; n = 11 vs. right 1.81 ± 0.08; n = 12; t = 2.34; p = 0.029).

These tissue sections provided us with the opportunity to examine mRNA expression patterns in other brain regions that are present in the same anterior/posterior plane as the amygdala. For NPY1R mRNA, the strongest signals are present throughout the cortex. These cortical signals tended to be laminar-specific, and in general the signals were strongest in the superficial and deep cortical layers and less intense in the middle layers, although this pattern was less evident in the temporal cortex. In addition, the cortical signals were most intense in the ventral half of the tissue section and include the somatosensory cortex, superior temporal sulcus, insular cortex, temporal cortex, and entorhinal cortex. There is also significant expression in the anterior cingulate and ventral medial region of the head of the caudate nucleus. While the full extent of the claustrum contained in this section has moderate levels of NPY1R expression, the strongest expression is seen at the ventral tip, and represents some of the strongest expression in the entire section. There was also significant expression seen in several midline structures including the stria terminalis, retrochiasm of the supraoptic nucleus, arcuate nucleus, anterior paraventricular region of the thalamus and the septohippocampal nucleus. For NPY5R mRNA, there is a diffuse signal throughout the extent of the section including regions of the temporal cortex and somatosensory cortex that was less laminar-specific in comparison to NPY1R mRNA. There are also signals in the ventral tip of the claustrum, internal capsule and a band across the central portion of the putamen. The most intense mRNA signals are in midline structures including the optic tract, retrochiasm of the supraoptic nucleus and the medial division of the arcuate nucleus.

3) Assessing the relationship between NPY1R and NPY5R gene expression and metabolism throughout the brain

We used whole-brain voxel-wise regressions to identify brain regions where NPY1R or NPY5R mRNA levels significantly predict metabolism ($p < 0.005$, uncorrected; for detailed results, see Tables 1–2). As shown in Figure 3, individuals with higher levels of NPY1R mRNA in the Ce were characterized by increased metabolism in the right dorsolateral prefrontal cortex (dlPFC) and decreased metabolism in the pregenual anterior cingulate cortex (pgACC). As shown in Figure 4, individuals with higher levels of NPY5R mRNA in the Ce were characterized by increased metabolism in the dorsal prefrontal cortex (PFC; area 8). In terms of Ce metabolic activity, the mean FDG-PET signal extracted from the 95% confidence interval most predictive of AT (see Figure 1) showed a weak trend toward a significant negative correlation with NPY5R mRNA levels as assessed by qRT-PCR analysis ($t = -1.63$, $p = 0.12$). There was no significant correlation with NPY1R mRNA ($t = -0.01$, $p = 0.99$).

DISCUSSION

The current work links the NPY system to AT by examining the expression of NPY system genes in the Ce. Specifically, we demonstrate that individuals with increased expression of NPY1R or NYP5R mRNA in the Ce are characterized by lower levels of the anxious phenotype. We expanded on these finding to describe the distribution of NPY1R and NPY5R mRNA in the rhesus amygdala and surrounding regions. Lastly, we identify several brain regions where metabolic activity is predicted by the Ce expression of the NPY1R or NPY5R genes.

The inverse relationship between AT and NPY1R and NPY5R mRNA levels suggests that lower anxiety levels are accompanied by increased NPY receptor expression in the Ce, which is consistent with studies indicating that NPY1R and NPY5R receptors mediate the anxiolytic-like effects of NPY in the brain (29, 31, 32). Because extreme and stable AT is a risk factor for the development of psychopathology, the current results are in agreement with the postulated role of the NPY neurotransmitter system as a resilience factor that decreases the risk to develop stress-related psychopathology (39). It should be noted that the studies reported here assessed NPY receptor mRNA levels; confirmation of the mRNA findings at the protein level would provide further confidence in the results. While there is often strong agreement between variations in mRNA and protein levels, ultimately our findings need to be confirmed at the protein level using immunodetection or *in vitro* autoradiography.

Consistent with a Ce-specific regulation of NPY-receptor gene expression in AT, we failed to find a significant relationship between AT and NPY1R and NPY5R mRNA expression in motor cortex, which is not a core component of the AT-circuit. Consistent with an AT-specific relationship, the levels of NPY1R and NPY5R mRNA in the motor cortex were not correlated with locomotion even though motor cortex metabolic activity was correlated with locomotion. Moreover, NPY1R and NPY5R mRNA levels in the motor cortex were not significantly correlated with expression in the Ce. These results indicate that the relationship between NPY1R and NPY5R mRNA levels and AT is not general across brain regions and

that the correlations are not simply non-specific markers for brain metabolism or other behaviors.

It is interesting that mRNA levels for both the NPY1R and NPY5R receptors show an inverse correlation with AT. In humans, the genes for these two receptors are located on the same region of chromosome 4 in opposite orientations and share common transcriptional control regions (40). A search of the rhesus genome reveals a similar organization with these two genes located on chromosome 5 in opposite orientations. It is possible that in the Ce there are shared transcription factors that control the amount of expression of these two genes, which is consistent with the observed trend for a correlation between NPY1R and NPY5R mRNA levels in the Ce. Understanding the regulation of the transcription factors that control expression of these two genes in the Ce sets the stage for identifying novel targets for pharmacological manipulation of NPY receptor expression in relation to anxiety.

It is possible that the variations in NPY receptor mRNA levels are determined by DNA sequence variation in the promoter region of the NPY1R and NPY5R genes. In fact, there is evidence that a polymorphism in the promoter region of the human NPY gene (SNP rs16147) regulates the level of NPY mRNA and protein in the brain and is associated with differences in stress responsiveness and anxiety symptoms (41, 42). Moreover, polymorphisms in the NPY1R and NPY5R genes have been linked to drug addiction and variations in diet (43–45). In future studies it will be of value to determine if these variations exist in the rhesus monkey and if they influence the function of the brain NPY system in relation to anxiety.

The rhesus monkey model of extreme AT is associated with persistently elevated levels of anxiety as well as chronically elevated Ce metabolism (7). Although NPY receptor expression is not related to Ce metabolism in this study, it is possible that the decreased levels of NPY receptor expression impair NPY signaling resulting in extreme AT. Conversely, there may be an increase in NPY signaling in an attempt to compensate for the extreme AT phenotype that then results in downregulation of Ce NPY1R and NPY5R receptor expression.

Few previous reports have described the role of the NPY5R in anxiety-like responding, in part, because of the lack of selective ligands that differentiate between the various NPY receptor subtypes. Furthermore, outside of hypothalamic regions, NPY5R receptors are expressed at significantly lower levels compared to the NPY1R receptor. The present study highlights the important role of the NPY5R receptor in AT, and anxiety in general, suggesting that future studies aimed at more fully delineating the role of the NPY5R receptor in the anxiety-like responding are likely to prove fruitful.

This study is the first to provide a detailed description of the expression of NPY1R and NPY5R mRNA in the amygdala and neighboring regions in non-human primates. The signal for NPY1R mRNA was strong and widespread in several cortical areas, consistent with prior reports in humans (46–48). While there are no published studies describing the mRNA distribution of NPY1R in the rhesus brain, a PET and *in vitro* autoradiography study employing ^{18}F-Y1-973 revealed patterns of receptor localization that are in general

agreement with our observations (49). The widespread distribution throughout the cortex is also seen in the rat and mouse brain where NPY1R mRNA is detectable in essentially all cortical fields (50). In all of these species, there is a layer specific pattern to the NPY1R mRNA expression with the layers of highest expression varying between cortical regions and between species. Regarding the expression pattern of NPY1R mRNA in the amygdala, only limited information is available for the primate brain, with moderate expression levels being reported in the human amygdala, but the report did not assess the differential distribution across the various amygdala nuclei (47, 48). In the rat amygdala, the highest mRNA expression is seen in the amygdalohippocampal transition area, amgydalopiriform transition area, anterior basomedial nucleus (BMe) and posteroventral medial nucleus, while a small number of intensely labelled cells are present in the Ce, and weakly labeled cells were scattered throughout the BMe, basolateral (Bla) and lateral nuclei (La). Similar mRNA expression patterns are seen in the mouse (50–52). The mRNA expression patterns are in general agreement with two detailed studies examining NPY1R immunoreactivity in rat brain (51, 53). The expression pattern in the rodent amygdala is very similar to that described in the present study for the rhesus amygdala, and suggests there is significant cross-species conservation in the expression of the NPY1R transcript.

The expression of the NPY5R mRNA as determined by *in situ* hybridization tended to be less robust and more localized to hypothalamic and amygdala regions compared to the NPY1R mRNA expression. There have been several published reports describing the mRNA distribution of NPY5R in the rodent brain (54–58). The results from these studies are in general agreement with the mRNA expression pattern reported here and previously reported in the human brain (58, 59). There were regions of common expression between the NPY1R and NPY5R, and these included the Ce, La and Me nuclei of the amygdala as well as supraoptic nucleus, arcuate nucleus, and temporal and somatosensory cortex. This is consistent with studies in the rat brain, where the presence of NPY5R mRNA always corresponded with the presence of NPY1R mRNA, but not vice versa (54). This may arise from the overlapping structure of these genes on the chromosome and the shared transcriptional control elements. The significantly higher Ce expression of NPY5R mRNA in the left hemisphere compared to the right is noteworthy in light of evidence describing hemispheric difference in the control and expression of emotion via the amygdala (60).

In terms of NPY1R and NPY5R mRNAs in relation to metabolic activity of the Ce region, there was no significant correlation with glucose metabolism in the Ce. Nevertheless, whole-brain voxelwise analyses revealed several other regions where NPY1R or NPY5R mRNA expression predicted metabolism. Brain regions that had metabolic activity that were significantly correlated with NPY1R expression included the dlPFC and pgACC. Similarly, for the NPY5R receptor mRNA, regions with metabolic activity that significantly positively correlated with mRNA levels in the Ce included the dorsal PFC. These prefrontal cortical regions have previously been shown to be part of the circuit that regulates the activity of the amygdala (61, 62). Thus, our data suggest that NPY1R and NPY5R mRNA levels in the Ce may be regulated by the influences of prefrontal cortex on the NPY1R and NPY5R expressing neurons. Alternatively, NPY1R- or NPY5R-expressing Ce neurons could modulate metabolism in these brain regions via direct or indirect mechanisms.

It is very relevant that variations in Ce NPY receptor mRNA expression that are associated with AT are also associated with variations in the metabolic activity of the pgACC and right dlPFC. This is because alterations in both of these brain regions have been associated with anxiety disorders. For example, generalized anxiety disorder (GAD) has been linked to impaired functional connectivity between the pgACC and the amygdala (63). In addition, adolescents with GAD show greater activation to fearful faces in a distributed network centered on the ACC (64). With regard to the right dlPFC, high-frequency (10-Hz) repetitive transcranial magnetic stimulation of this region has been shown to decrease anxiety symptoms in PTSD (65).

In conclusion, NPY1R and NPY5R mRNA expression levels within the Ce are negatively correlated with AT and predict altered metabolic activity in prefrontal regions that are thought to regulate the amygdala. Higher levels of expression of these receptor subtypes would be expected to increase the capacity for NPY signaling in this region. NPY in the amygdala has been hypothesized to suppress anxiety-like responding and NPY has a putative role as a resilience factor. It is possible that children that express more NPY receptors in the Ce region may have a lowered anxiety response to threatening situations which may protect against the development of stress-related psychopathologies such as anxiety and depression. Moreover, these data suggest a potential link between prefrontal metabolic mechanisms and Ce molecular mechanisms that may underlie resilience. Future studies aimed at genetic manipulation of the NPY system in the Ce in rodent as well as primate species will provide additional evidence to support this hypothesis. Because the present findings are directly relevant to at-risk early anxious dispositions, treatment strategies targeting the NPY system may have therapeutic benefit in the prevention of stress-related anxiety disorders in at-risk children. It will also be of interest to determine if behavioral treatments that promote resilience impact the NPY system.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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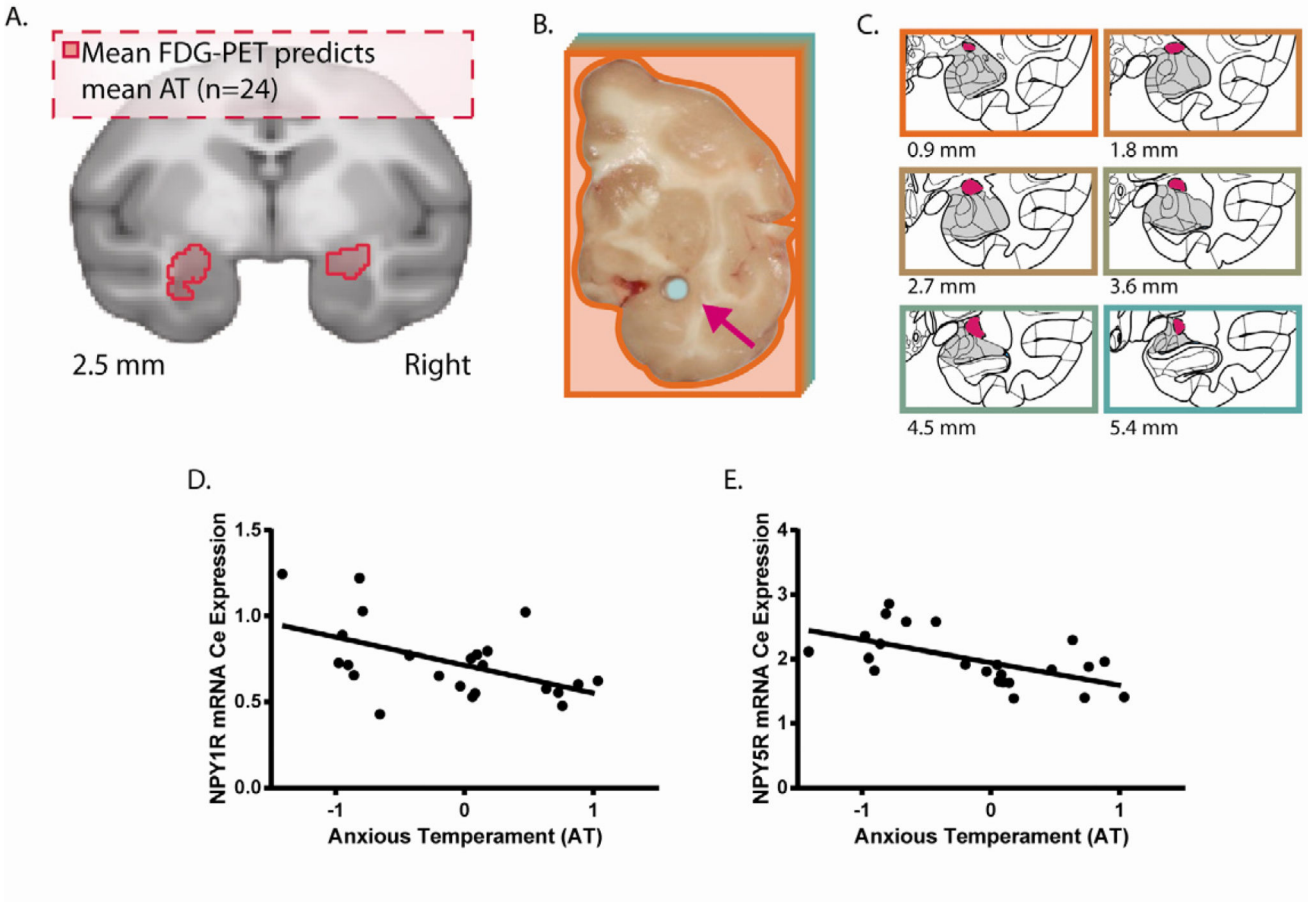

Figure 1. Higher levels of AT are associated with reduced NPY1R and NPY5R mRNA expression in the primate Ce as assessed by qRT-PCR

A. Metabolic activity in the Ce strongly predicts variation in AT. The bilateral regions identified by the red trace correspond to the 95% confidence interval for the maximal voxelwise correlation between amygdala metabolic activity and AT. ***B***. The functionally-defined location of the Ce punch (see *Materials and Methods*). ***C***. Atlas plates corresponding to the 3 mm tissue punch (adapted with permission from a published atlas (66)). The Ce is depicted in red, and the numbers indicate distance posterior to the anterior commisure. ***D***. and ***E***. Correlational analyses between AT and NPY1R and NPY5R mRNA levels determined by qRT-PCR analysis. Scatter plots depicting the significant correlations between AT and the expression of NPY1R ($t = -2.28$, $p = 0.035$, n = 23) and of NPY5R ($t = -2.55$, $p = 0.020$, n = 23) as detected by qRT-PCR. Panels A–C are adapted from our previously published figure (7, 66).

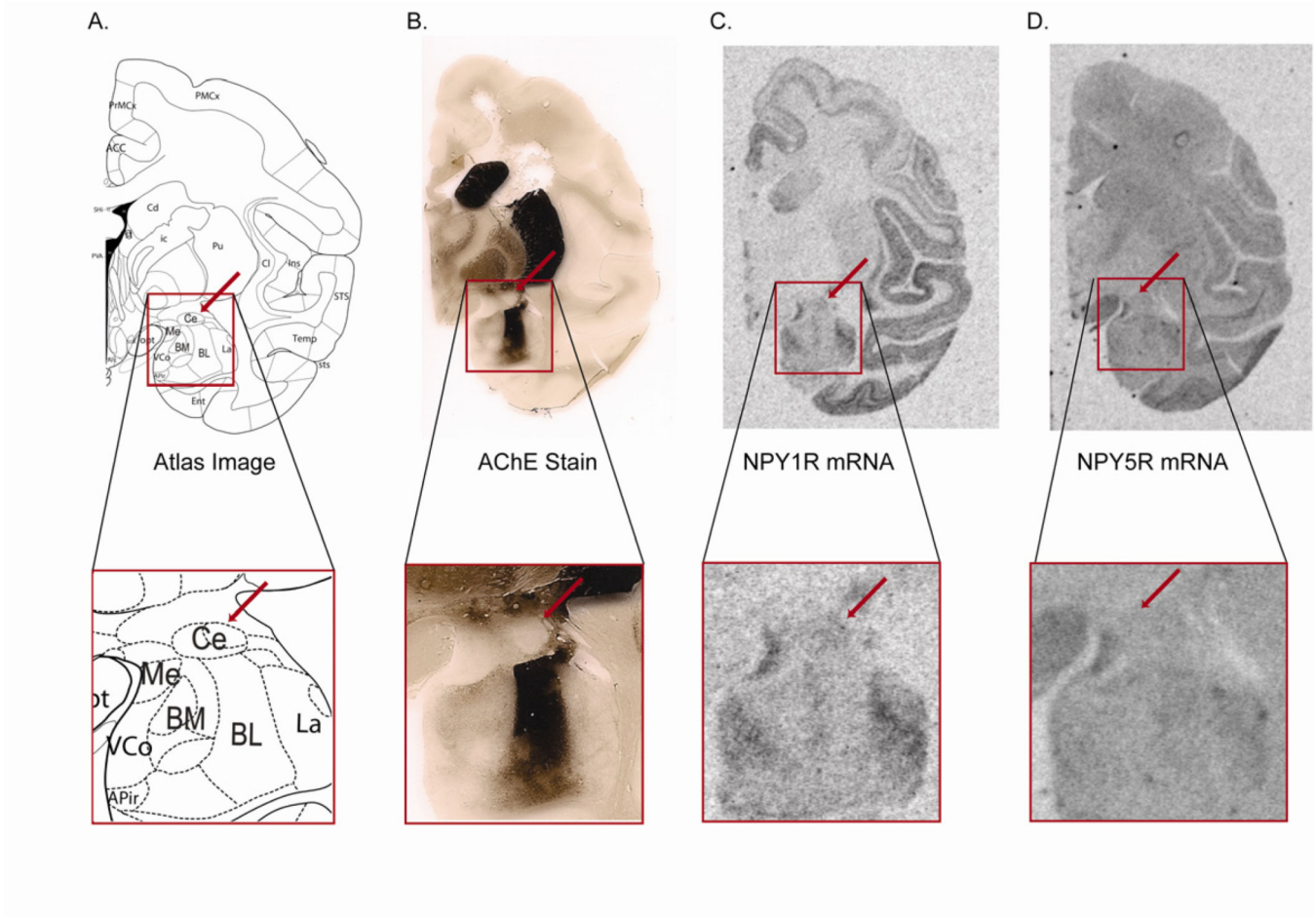

Figure 2. NPY1R mRNA and NPY5R mRNA expression in the amygdala region assessed by *in situ* hybridization

A. Atlas image of the rhesus brain at the level of 2.25 mm posterior to the anterior commisure (−5.85 mm bregma) to identify regions discussed in the Results section. Adapted with permission from a published atlas (66). ***B***. AChE stain of an adjacent section used to identify the structure of the rhesus amygdala. ***C***. Signal for NPY1R mRNA. ***D***. Signal for NPY5R mRNA in coronal brain sections at the level of the amygdala. The red arrow indicates the location of the Ce. **Abbreviations** – ACC: anterior cingulate cortex; APir: amygdalopiriform cortex; Arc: arcuate nucleus; BL: basolateral amygdala; BM: basomedial amygdala; Cd: caudate nucleus; Ce: central amygdala; Cl: claustrum; ic: internal capsule; Ins: insular cortex; La: lateral amygdala; Me: medial amygdala; PMCx: primary motor cortex; PrMCx: premotor cortex; Pu: putamen; PVA: anterior paraventricular region of the thalamus; SHi: septohippocampal nucleus; SOR: retrochiasm of the supraoptic nucleus; STS: somatosensory cortex; sts: superior temporal sulcus.

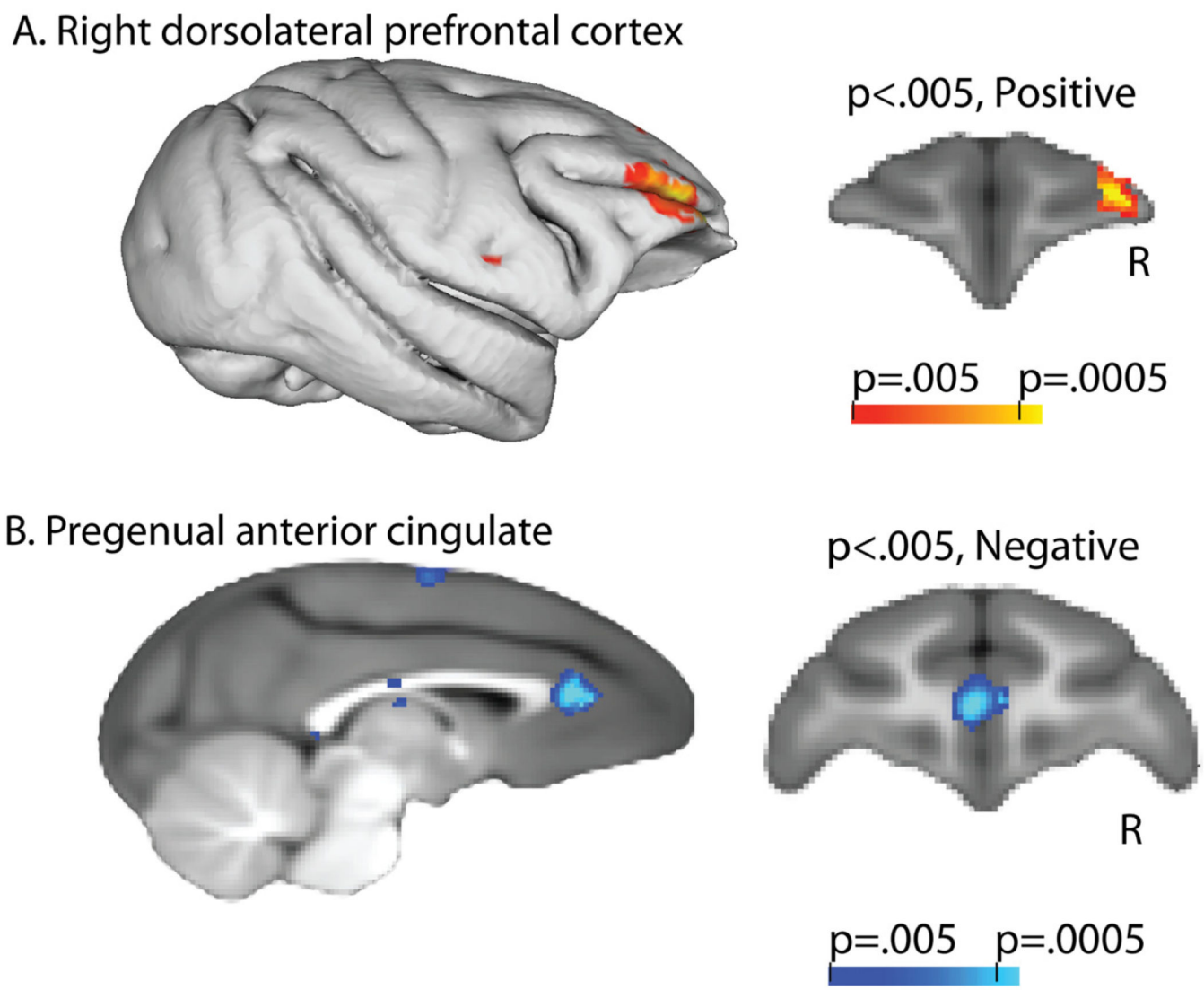

Figure 3. Ce NPY1R mRNA levels predicted metabolism in the prefrontal and cingulate cortices

Voxel-wise analysis revealed that Ce NPY1R mRNA levels predicted ***A***. increased metabolism in the right dlPFC and ***B***. decreased metabolism in the pgACC. Color variation represents level of statistical significance as defined in horizontal color bars with shades of red through yellow for positive correlations and shades of blue for negative correlations.

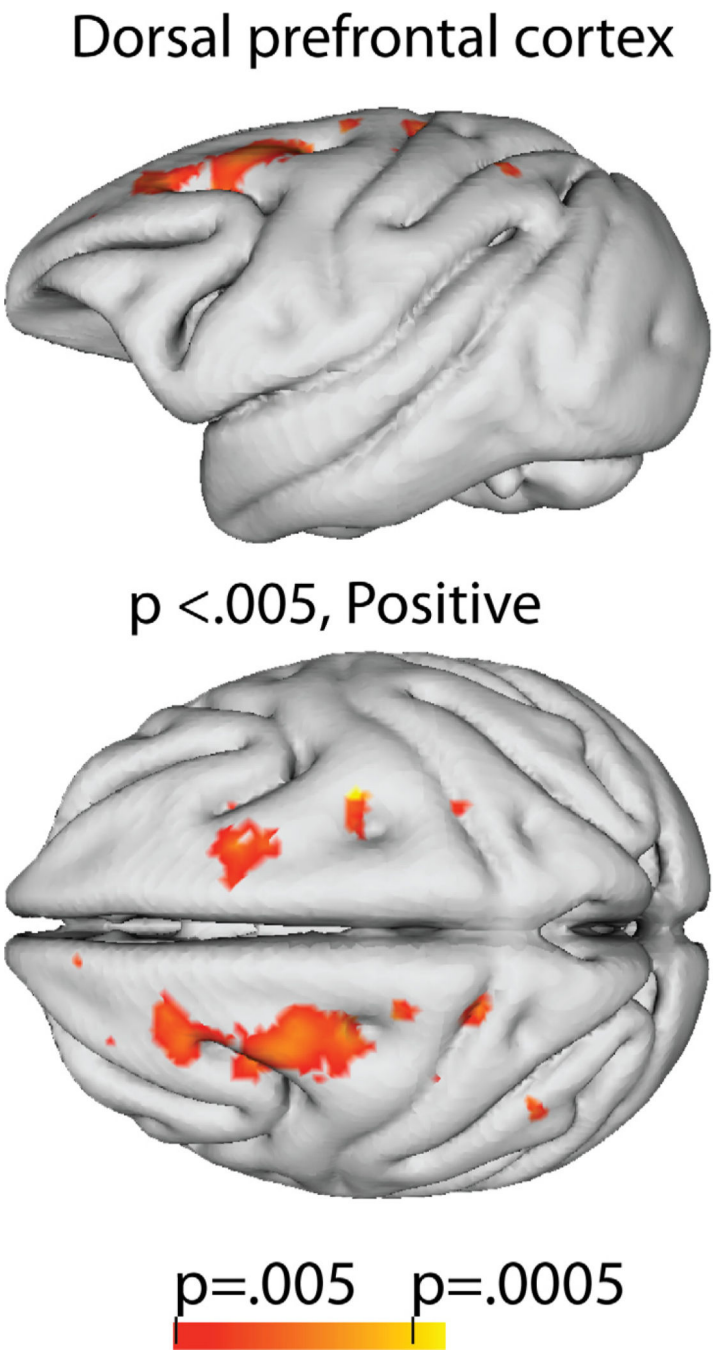

Figure 4. Ce NPY5R mRNA levels predicted increased metabolism in the dorsal PFC

Voxel-wise analysis revealed that Ce NPY5R mRNA levels predicted increased metabolism in the dorsal PFC. Color variation represents level of statistical significance as defined in horizontal color bar.

Table 1

NPY1R-related brain regions

Direction of Relationship	**Hemisphere**	**Cluster**	**Cluster volume (mm^2)**				***Location relative to anterior commisure (mm)***		
				Local maxima within region	**t-value**	**p-value**	**x**	**y**	**z**
Positive	Right	Dorsolateral Prefrontal Cortex	78.8574	Area 46/Area 47	6.67	5.42×10^{-6}	13.750	23.750	8.750
Negative	Right	Thalamus/ Caudate	51.0254	Anterodorsal Thalamus/Caudate	−6.37	9.22×10^{-6}	4.375	−5.000	7.500
	Left	Pregenual Anterior Cingulate	73.9746	Area 32	−6.75	4.69×10^{-6}	−2.500	16.875	7.500
	Right	Motor Cortex	31.0059	Area 4	−5.55	4.44×10^{-5}	0.625	−2.500	23.750

Regions with a significant correlation ($p < 0.05$, two-tailed uncorrected) between metabolic activity and NPY1R mRNA expression as assessed by qRT=PCR.

Table 2

NPY5R-related brain regions

Direction of Relationship	Hemisphere	Cluster	Cluster volume (mm^2)				*Location relative to anterior commisure (mm)*		
				Local maxima within region	**t-value**	**p-value**	**x**	**y**	**z**
Positive	Left	Dorsal Prefrontal Cortex	24.1699	Area 8	4.52	3.51×10^{-4}	−7.500	11.875	18.125
	Left	Visual Cortex	23.4375	V1	4.66	2.64×10^{-4}	−2.500	−43.750	2.500
	Left	Dorsal Prefrontal Cortex	27.0996	Area 8	5.03	1.24×10^{-4}	−11.875	5.625	12.500
	Left	Premotor Cortex	51.2695	Area 6	5.24	8.08×10^{-5}	−8.125	−1.250	21.250
	Right	Motor Cortex	15.8691	Area 4	5.85	2.47×10^{-5}	13.750	−1.875	21.250
Negative	Right	Thalamus	78.3691	Anterior Ventral Thalamus	−8.07	4.96×10^{-7}	4.375	−3.750	3.750
	Left	Somatosensory Cortex	13.6719	Area S2	−4.67	2.54×10^{-4}	−16.875	−8.125	8.125
	Right	Putamen	17.5781	Putamen	−4.17	7.22×10^{-4}	15.000	−8.125	4.375
	Left	Posterior Cingulate	8.5449	Area 30/Area 29	−4.15	7.58×10^{-4}	−3.125	−13.750	8.750

Regions with a significant correlation ($p < 0.05$, two-tailed uncorrected) between metabolic activity and NPY5R mRNA expression as assessed by qRT=PCR. For details see the legend to Table 1.