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Neuropeptide Y-immunoreactive neurons in the cerebral cortex of humans and other haplorrhine primates

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Abstract

We examined the distribution of neurons immunoreactive for neuropeptide Y (NPY) in the posterior part of the superior temporal cortex (Brodmann's area 22 or area Tpt) of humans and nonhuman haplorrhine primates. NPY has been implicated in learning and memory and the density of NPY-expressing cortical neurons and axons is reduced in depression, bipolar disorder, schizophrenia, and Alzheimer's disease. Due to the role that NPY plays in both cognition and neurodegenerative diseases, we tested the hypothesis that the density of cortical and interstitial neurons expressing NPY was increased in humans relative to other primate species. The study sample included great apes (chimpanzee and gorilla), Old World monkeys (pigtailed macaque, moor macaque, and baboon) and New World monkeys (squirrel monkey and capuchin). Stereologic methods were used to estimate the density of NPY-immunoreactive (-ir) neurons in layers I-VI of area Tpt and the subjacent white matter. Adjacent Nissl-stained sections were used to calculate local densities of all neurons. The ratio of NPY-ir neurons to total neurons within area Tpt and the total density of NPY-ir neurons within the white matter were compared among species. Overall, NPY-ir neurons represented only an average of 0.006% of the total neuron population. While there were significant differences among species, phylogenetic trends in NPY-ir neuron distributions were not observed and humans did not differ from other primates. However, variation among species warrants further investigation into the distribution of this neuromodulator system.

Keywords

Wernicke's area; area Tpt; area 22; evolution; NPY

Introduction

Primates are characterized by an increase in brain size relative to other mammals, particularly of the neocortex [Barton and Harvey, 2000]. It is this expansion in size that has been heralded as the underlying factor supporting an increase in behavioral and cognitive flexibility. However, significant differences in cognitive capacities exist among primates,

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and between human and nonhuman primates, including theory of mind, behavioral inhibition, and language abilities [e.g., Hare et al., 2001, 2007; Herrman et al., 2010; Savage-Rumbaugh et al., 1980]. These differences are not likely to be the result of changes in overall size or encephalization quotients alone [Holloway, 1966]. Broad comparative analyses are needed to understand not only the diversity of neural architecture among species, but also to reveal human-specific adaptations that contribute to our intellectual divergence compared to other species.

Neurotransmitter and neuromodulator systems that regulate the communication among neurons are potential candidates for evolutionary selection due to their critical roles in supporting learning, memory, language, and other higher cognitive functions [Previc, 1999; Raghanti et al., 2008a, b, c, 2009]. Neuropeptide Y (NPY) is a 36-amino acid peptide that is present in high concentrations throughout the central nervous system [Tatemoto et al., 1981] and its actions are mediated by at least four receptor subtypes [Dumont et al., 1998; Michel et al., 1998]. NPY is an evolutionarily conserved peptide which plays a role in basic physiological functions such as the regulation of circadian rhythms, feeding behaviors, and cognitive processes including learning and memory [Lewis et al., 2005; Teramitsu et al., 2004]. In addition, the expression of NPY mRNA, and the distribution of NPY-immunoreactive (-ir) axons and cortical neurons is affected in a variety of neuropathological processes, including depression, bipolar disorder, schizophrenia, schizoaffective disorder, and Alzheimer's disease [Beal et al., 1986; Caberlotto and Hurd, 1999; Kowall and Beal, 1988; Kuromitsu et al., 2001; Morales-Medina et al., 2010; Moris et al., 2009].

NPY-synthesizing neurons are located throughout the cortex and subcortical regions, and within subcortical neuron populations (e.g., locus coeruleus) projecting to the cerebral cortex, hypothalamus and spinal cord [von Bohlen und Halbach and Dermietzel, 2006]. Within the cerebral cortex, NPY is involved in synaptic transmission [Bacci et al., 2002], regulation of cerebral blood flow [Cauli et al., 2004; Estrada and DeFelipe, 1998; Hamel et al., 2002], and inhibition of neuronal excitability [Colmers and Bleakman, 1994]. NPY cortical neurons are mostly GABAergic [Hendry et al., 1984b] and morphologically they appear as bipolar, bitufted, and multipolar types [Hendry et al., 1984b; Kuljis and Rakic, 1989b; Mori, 1996]. These neurons are distributed throughout the layers of the neocortex, but are most numerous in layers II-III and VI [Hendry et al., 1984a; Kubota et al., 1994; Kuljis and Rakic, 1989b]. NPY cortical neurons are categorized as local circuit neurons because their axons do not extend outside of the grey matter [Rakic, 1987]. Kuljis and Rakic [1989b] suggested that the area-specific distributions of NPY-ir neuron subtypes in primate neocortex may reflect adaptations of local circuits for specialized functions. Further, Zaitsev and colleagues [2009] reported that primate NPY-ir interneurons display electophysiological properties unique from those in rodent cerebral cortex. The density and distribution of cortical NPY-ir neurons varies among species and among cortical areas within species [e.g., Butti et al., 2011; Kuljis and Rakic, 1989a, b; Sherwood et al., 2009], suggesting that these neurons may have been recruited to support human- or primate-specific behavioral functions.

In this study, we analyzed the distribution and density of NPY-ir neurons among humans and nonhuman primate species in the superior temporal cortex, Brodmann's area 22. This cortical area (also known as Wernicke's area or area Tpt) is particularly relevant in terms of human-specific abilities as it is involved in the auditory processing of speech [Geschwind, 1967]. Homologous areas have been described in great apes, monkeys, and galagos based on cytoarchitectural characteristics [Poremba et al., 2004; Preuss and Goldman-Rakic, 1991; Spocter et al., 2010]. In humans, there is a leftward asymmetry in the planum temporale, the size of this cortical region, and the length of the adjacent sylvian fissure that is particularly evident in right-handed individuals [Foundas et al., 1994; Galaburda et al., 1978; Naidich et

al., 2001]. A similar leftward asymmetry of the planum temporale and this cortical area volume is also present in chimpanzees [Gannon et al., 1998; Hopkins and Nir, 2010; Spocter et al., 2010]. While macaque monkeys lack the morphological features of the planum temporale, cytoarchitectural asymmetry is evident [Gannon et al., 2008]. Cortical homologues are involved in the processing of species-specific vocalizations in Old World monkeys [Poremba et al., 2004] and chimpanzees [Tagliatela et al., 2009]. This evidence, together with the identification of area Tpt in galagos [Preuss and Goldman-Rakic, 1991], suggest that this cortical area has an evolutionary history extending back 50-60 million years.

Because cortical NPY-ir neurons are local-circuit neurons [Kuljis and Rakic, 1989b] that have species-specific and cortical region-specific distributions, significant differences in this cell population might be expected between humans and nonhuman primates in this cortical area. Specifically, we tested the hypothesis that humans would possess a significantly higher density of cortical NPY-ir neurons relative to other primate species in Wernicke's area (Tpt) to support human language.

Methods

No living animals were used in this study. All postmortem brains from nonhuman primates were acquired from zoological or research institutions. The animals had been maintained in AAALAC- or AZA-accredited facilities and died from either natural causes or from humane euthanization for quality of life. The research presented here is in accordance with the American Society of Primatologists Principles for the Ethical Treatment of Nonhuman Primates. The acquisition and processing of postmortem human and nonhuman brain materials are exempt from the requirement of approval by institutional animal care and human subject use committees.

Specimens

The nonhuman brain specimens included 32 individuals representing seven haplorrhine species (Table I). Human brain specimens were provided by the Northwestern University Alzheimer's Disease Center Brain Bank. All specimens were obtained from adult individuals that were free of neuropathology on gross examination. The human subjects showed no evidence of dementia before death and all individuals received a score of zero for the CERAD senile plaque grade [Mirra et al., 1991] and the Braak and Braak [1991] neurofibrillary tangle stage. The age, sex, and method of fixation for each subject can be found in Table I. Postmortem interval did not exceed 17 hours for any specimen.

The moor macaques were perfused transcardially with 4% paraformaldehyde as part of unrelated experiments following methods described previously [Hof and Nimchinsky, 1992; Hof et al., 1996]. All other brains were fixed by immersion in 10% buffered formalin for at least 10 days, then transferred to a 0.1 M phosphate-buffered saline (PBS, pH 7.4) solution containing 0.1% sodium azide and stored at 4° C until histological processing.

Sample Processing

All samples were from the left hemisphere. Prior to sectioning, samples were cryoprotected in a graded series of sucrose solutions (10%, 20%, and 30%) until saturated. Brain samples were frozen on dry ice and sectioned at 40 μm using a freezing sliding microtome (SM2000R, Leica, Chicago, IL). Each section was placed into an individual centrifuge tube containing freezer storage solution (30% each distilled water, ethylene glycol, and glycerol and 10% 0.244 M PBS), numbered sequentially, and maintained at −20° C until histological or immunohistochemical processing.

A 1:10 series for all samples was stained with 0.5% cresyl violet to reveal cell somata. Nissl-stained sections were used to obtain total neuron densities (Nv) and to define the cytoarchitectural boundaries of Brodmann's area 22 (area Tpt) located in the caudal portion of the superior temporal cortex. Area Tpt has been described for humans [Fullerton and Pandya, 2007], chimpanzees [Bailey et al., 1950; Spocter et al., 2010], macaque monkeys [Fullerton and Pandya, 2007; Gannon et al., 2008; Lewis and Van Essen, 2000; Preuss and Goldman-Rakic, 1991], and galagos [Preuss and Goldman-Rakic, 1991]. This area is distinguished by its well-developed layer II, a columnar appearance of pyramidal neurons in layers III and V, and a thick layer IV (Fig. 1) [Fullerton and Pandya, 2007; Galaburda and Pandya, 1982; Preuss and Goldman-Rakic, 1991].

Immunohistochemistry

A 1:10 series of sections from each individual was immunostained for NPY. Floating tissue sections were immunostained using the avidin-biotin-peroxidase method. Sections were pretreated for antigen retrieval in a 0.05% citraconic anhydride solution (pH 7.4) in a water bath (85° C) for 30 minutes [Alelu-Paz et al., 2008]. Endogenous peroxidase was quenched using a solution of 75% methanol, 2.5% hydrogen peroxide (30%) and 22.5% distilled water for 20 minutes. Sections were preblocked in a solution of 0.1 M phosphate buffered saline (PBS; pH 7.4), 0.6% Triton X-100, 4% normal serum, and 5% bovine serum albumin. Sections were then placed in primary antibody (rabbit anti-NPY polyclonal antibody, ab30914, Abcam, Cambridge, MA, USA) at a dilution of 1:5,000 in PBS for 48 H at 4° C. Following this, sections were incubated in a biotinylated secondary antibody (1:200) in a solution of PBS and 2% normal serum. The sections were then incubated in an avidin-peroxidase complex (PK-6100, Vector Laboratories, Burlingame, CA, USA) followed by a 3, 3′-diaminobenzidine-peroxidase substrate with nickel enhancement (SK-4100, Vector Laboratories). The pattern of NPY-ir neurons and axons obtained using this primary antibody that was generated from the first residue of pig C-terminus were in concordance with that previously described in humans [Kowall and Beal, 1988], chimpanzee [Mori, 1996], and macaque monkeys [Hendry et al., 1984a; Kuljis and Rakic, 1989a, b].

Data collection and analysis

Nissl-stained sections and sections immunostained for NPY were analyzed using an Olympus BX-51 photomicroscope equipped with a Ludl X,Y motorized stage, Heidenhain z-axis encoder, StereoInvestigator software (MBF Bioscience, Williston, VT, USA, version 9), and a digital camera that projects images onto a 24-inch LCD flat panel monitor.

NPY-ir neuron densities (Nv) were calculated using the optical disector as described previously [Raghanti et al., 2009]. Cortical NPY-ir neuron density (within layers I-VI of area 22) was calculated separate from the density of NPY-ir neurons in the white matter (i.e., interstitial elements). Adjacent Nissl-stained sections were used to obtain total neuron densities (total Nv) using the optical disector with a fractionator sampling scheme [Raghanti et al., 2009]. The ratio of NPY-ir Nv to total Nv represents the percentage of cortical neurons expressing NPY and was used to evaluate the density of NPY-ir neurons in the context of species differences in overall neuron density. The ratio of NPY-ir Nv to total Nv within area Tpt and interstitial NPY-ir Nv were compared among species using one-way ANOVAs. Tukey's HSD post hoc tests were used to evaluate significant findings. An α level was set at 0.05 for all statistical tests.

Photomicrographs were processed using Adobe Photoshop (version 12.1, San Jose CA, USA). Brightness and contrast were adjusted to obtain images that most closely resemble the appearance of histological features as seen through the objectives of the microscope.

Additional figures were produced by obtaining photomontage images of the cortex at low magnification and tracing NPY-ir neurons using Adobe Photoshop software.

Results

NPY-ir cortical and interstitial neurons were present in all species examined (Figs. 2 and 3). The morphological cell types and distributions were in general accordance with previous results in macaque [Hendry et al., 1984a; Kuljis and Rakic, 1989a; Zaitsev et al., 2009], chimpanzee [Mori, 1996], and human [Delalle et al., 1997; Kowall and Beal, 1988; Suarez-Sola et al., 2009], with bipolar and multipolar interneuron subtypes represented in all species (Fig. 4).

Overall, NPY-ir neurons represented on average 0.006% (SD = 0.003, range = 0.002 – 0.01%) of the total cortical neuronal population in area 22 in haplorrhines. NPY-ir Nv was significantly correlated with total Nv in the cortex (Pearson correlation: $r = 0.61$, 1 df, $P < 0.001$, $N = 36$) and in the white matter (Pearson correlation: $r = 0.57$, 1 df, $P < 0.001$, $N = 36$). There were significant differences among species in both the percentage of NPY-ir neurons within the cortex (Brown Forsythe: $F_{7, 5.96} = 3.87$, $P < 0.05$, $N = 36$; Fig. 5) and interstitial NPY-ir Nv (Brown-Forsythe: $F_{7, 14.76} = 3.42$, $P < 0.05$, $N = 36$; Fig. 6). Tukey's post hoc analyses did not reveal differences among species that clustered according to phylogeny and there were no significant differences between humans and any of the nonhuman primate species in this sample. The non-significant Tukey's post hoc P values ranged from 0.05 to 1.00. In the white matter, gorillas had significantly fewer NPY-ir Nv than both capuchins ($P < 0.05$) and moor macaques ($P < 0.05$). Within the cortex, squirrel monkeys had a great a greater percentage of NPY-ir neurons than capuchins ($P < 0.05$), pigtailed macaques ($P < 0.01$), baboons ($P < 0.05$), chimpanzees ($P < 0.01$), and gorillas ($P < 0.05$).

For the New World monkeys (capuchin and squirrel monkey), the majority of NPY-ir neurons were found in layer VI and the subjacent white matter (see Fig. 2 A, B). NPY-ir neurons were relatively sparse or absent in layers I-V. In Old World monkeys (moor macaque, pigtailed macaque, and baboon), neuron density was highest in layers II-III, VI, and in the white matter (see Fig. 2 C, D, E). A similar pattern was also observed in humans and chimpanzees (Fig. 2 G, H). In gorillas, NPY-ir neurons were more scattered throughout the layers of the cortex and white matter (Fig. 2 F).

Discussion

The present analysis represents the first large-scale quantitative analysis of NPY-expressing neurons in the neocortex of human and nonhuman primates and the first description of NPY-ir cortical neurons in several of these species. The distribution of NPY-ir cortical interneurons is of major interest due to reported interspecies differences, differential expression among cortical areas, and their susceptibility in a variety of human-specific neuropathological processes [e.g., Beal et al., 1986; Kuljis and Rakic, 1989a, b; Morales-Medina et al., 2010; Moris et al., 2009]. NPY-ir interneurons were present in all species examined here, with differences among species in the densities and distribution within area 22 and subjacent white matter. However, contrary to our hypothesis, humans did not possess a significantly higher density of cortical NPY-ir neurons relative to other primate species. Further, the differences did not accord with phylogenetic relationships nor did a human-specific pattern emerge.

Inhibitory interneurons perform key functions within cortical circuits. Disinhibition, due to reduced numbers of inhibitory interneuron or loss of function, has been associated with

cognitive deficits in a variety of diseases, including Alzheimer's disease and schizophrenia [Beal et al., 1986; Kowall and Beal, 1988; Lewis et al., 2005]. Butti and colleagues [2011] recently reported a comparable pattern and density of cortical NPY-ir neurons in the striped dolphin, manatee, harp seal, Atlantic walrus, and African elephant, with a sparse distribution that was concentrated in layers V-VI and the underlying white matter. A similar pattern was also found for Xenarthra and Afrotheria [Sherwood et al., 2009]. It appears that primates deviate from this more common pattern among other mammals in having an increased density of NPY-ir cortical neurons overall and alterations in their distributions among species, with NPY-ir interneurons present throughout most layers of the cortex.

As with NPY-ir cortical interneurons, the patterns of distribution for other interneuron populations also display species- and cortical area-specificity [Glezer et al., 1993, 1998; Sherwood et al., 2004, 2007, 2009, 2010]. In comparison to the relatively small numbers of NPY-ir interneurons, however, other cortical interneuron subtypes as defined by expression of calcium-binding proteins and other neuropeptides together comprise approximately 20-30% of the total neuron population [DeFelipe et al., 2002]. Previous comparative analyses of cortical inhibitory interneuron subpopulations as defined by the calcium-binding proteins parvalbumin, calretinin, and calbindin in haplorrhine primate prefrontal cortex (area 9), for example, revealed that the density of these cortical cells hyperscales relative to neuron density [Sherwood et al., 2010].

NPY-ir neurons are derived from a progenitor cell population that are likely comprised of thyroid transcription factor 1-positive (TTF1, also called NKX2) cells in the ventral forebrain that migrate dorsally into the cortex [Fertuzinhos et al., 2009]. Developmental and functional constraints may limit the degree to which this population of neurons can be modified, as supported by the current results demonstrating only a limited degree of phylogenetic variation in the distribution of cortical NPY-ir neurons in area 22 and the underlying white matter. While these constraints may not allow for large changes in neuron number, there is evidence that the electrophysiological properties of NPY-ir interneurons differ between rodents and primates [Zaitsev et al., 2009]. Such differences may have dramatic effects on the processing of local cortical circuits by influencing synaptic plasticity or modulating the signal-to-noise ratio within or between minicolumns. This could translate into differences in cognitive and behavioral plasticity, but its true functional significance remains speculative.

The developmental trajectory of NPY-ir cortical neurons in human prefrontal cortex has been outlined and includes a high density of NPY-ir neurons in the white matter before 1 year of age, an increased density in cortical layers after 1 year, a peak density of cortical NPY-ir neurons occurring between 4 and 7 years, and the adult pattern of a relatively low density occurring around 8 years of age [Delalle et al., 1997]. Interestingly, NPY-ir neurons are rare in layers I-V in the New World monkeys included in this sample. Neurons in these upper layers are younger in terms of development as cells migrate from the subplate outwards [Rakic, 1990]. Developmental analyses are needed to determine if the distribution of NPY-ir neurons in New World monkeys is the result of cell migration or adult patterning of neuron types.

The present data contribute to our understanding of human and nonhuman inhibitory cortical circuits, revealing a component of the cerebral cortex that exhibits species-specificity in terms of localization and densities. Despite their susceptibility in human neuropathologies and the involvement of this cortical area in human language, our data indicate that a relative increase in the number of NPY-ir cortical or interstitial neurons does not distinguish area 22 of humans from that of other haplorrhine primates. The variation present within this sample is of interest, but because the range of phylogenetic differences is very small and the overall

percentage of NPY-ir interneurons in the cortex is minimal, this variation is difficult to characterize in terms of function. Comparisons between the left and right hemispheres and analyses of additional cortical areas may contribute further to an understanding of how these neurons contribute to cortical function and an interpretation of species-specific patterns. Further research is also needed to determine if other components of cortical NPY innervation (e.g., electrophysiological properties, axon collaterals, or receptor distributions) differ among human and nonhuman primates.

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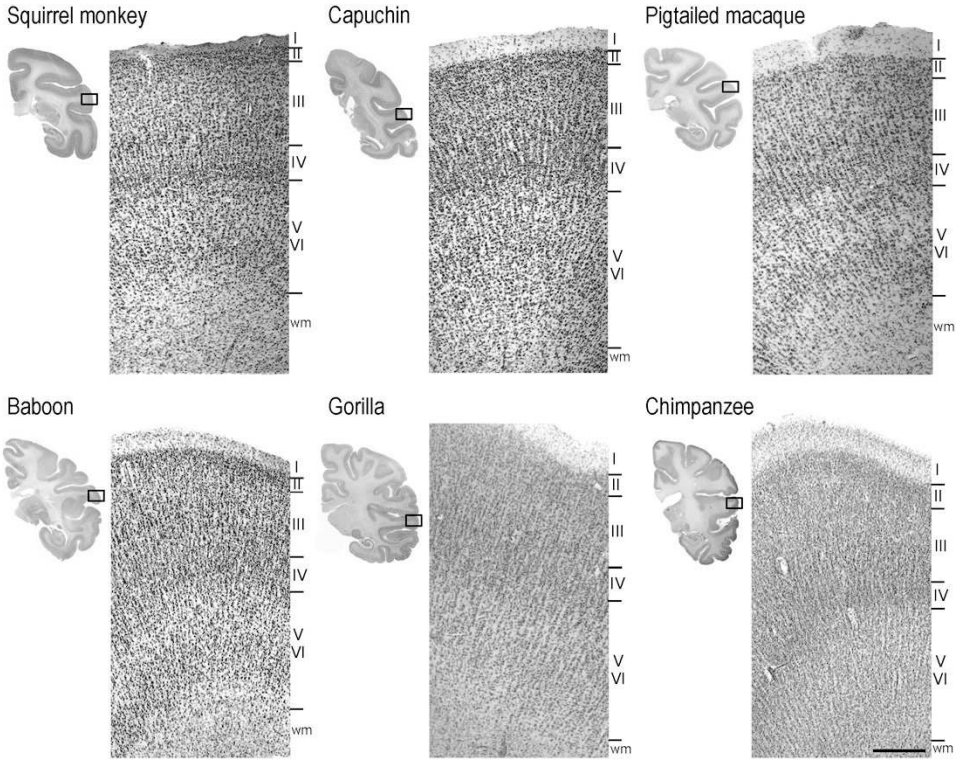

Figure 1.

Coronal Nissl-stained sections with the sampled area outlined with open rectangles and high magnification photomicrographs of area Tpt in representative species. Cortical layers are indicated by roman numerals, wm = white matter. Scale bar for photomicrographs = 500 μm.

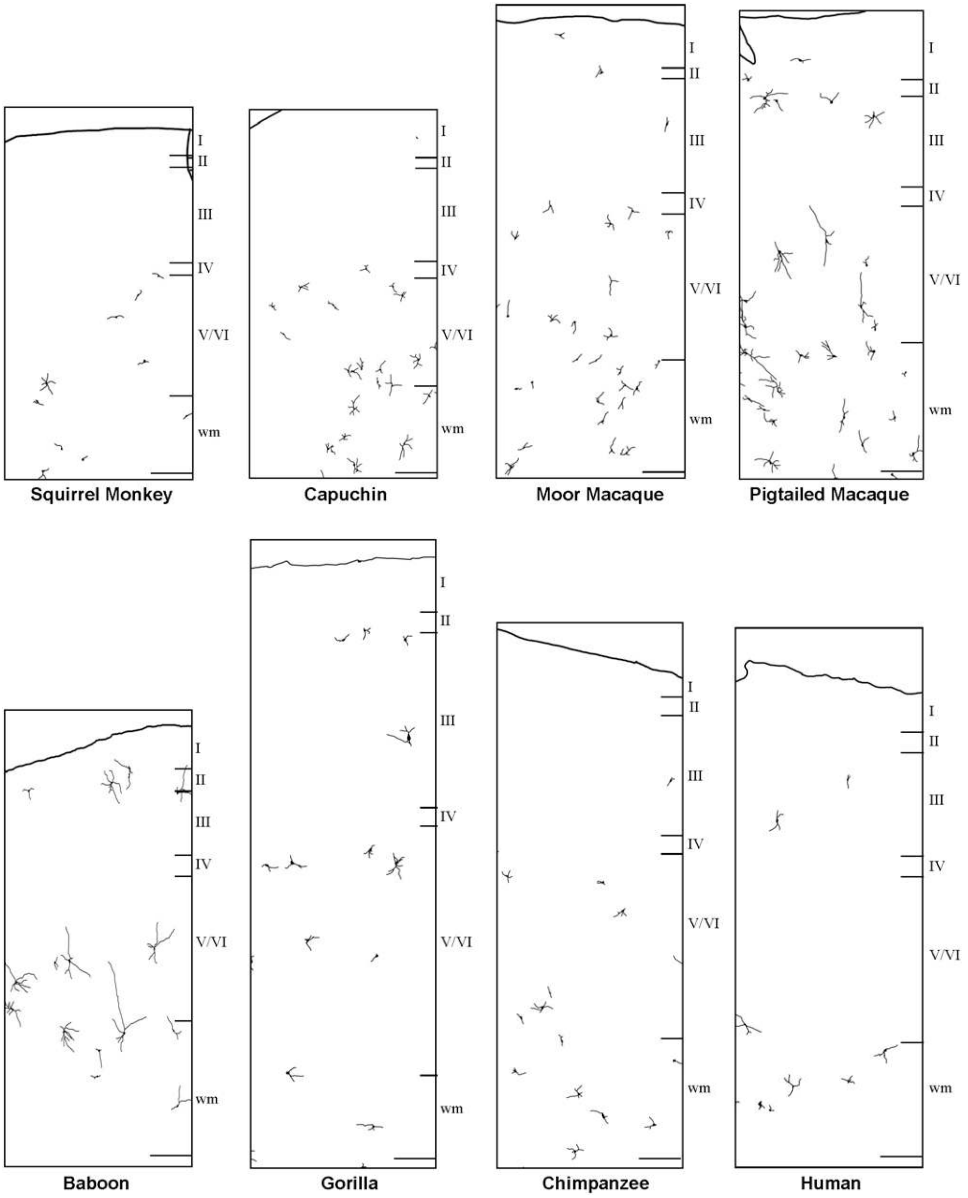

Figure 2.
NPY-ir neuron tracings in area 22 from each species. Cortical layers are indicated by roman numerals. Scale bars = 250 μm.

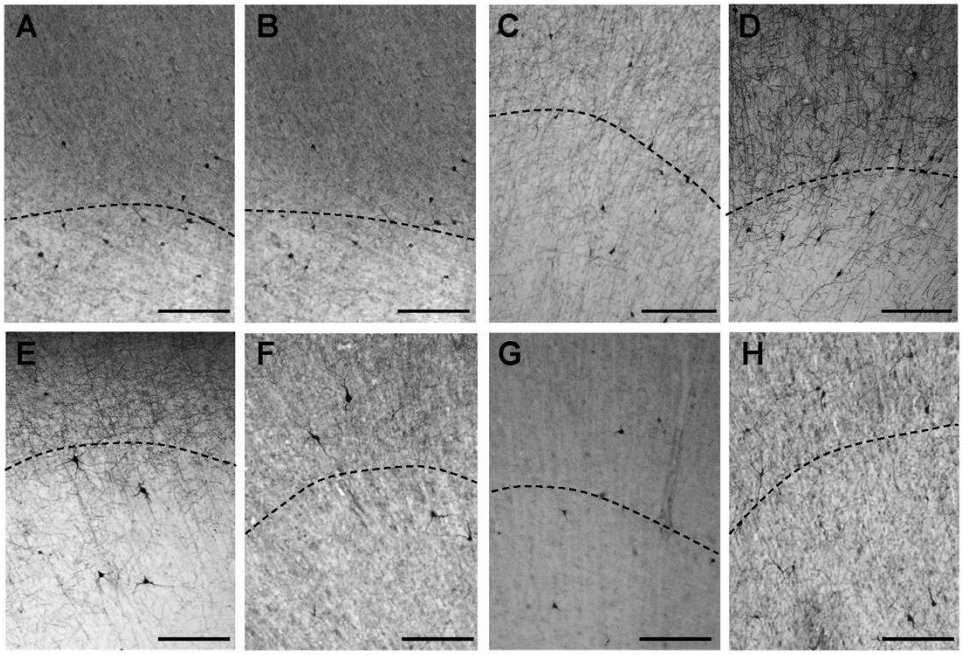

Figure 3.
Low magnification photomicrographs of the infragranular layers and subjacent white matter in squirrel monkey (A), capuchin (B), moor macaque (C), pigtailed macaque (D), baboon (E), gorilla (F), chimpanzee (G), and human (H). The dotted lines represent the border between the cortex and white matter. Scale bars = 250 μm.

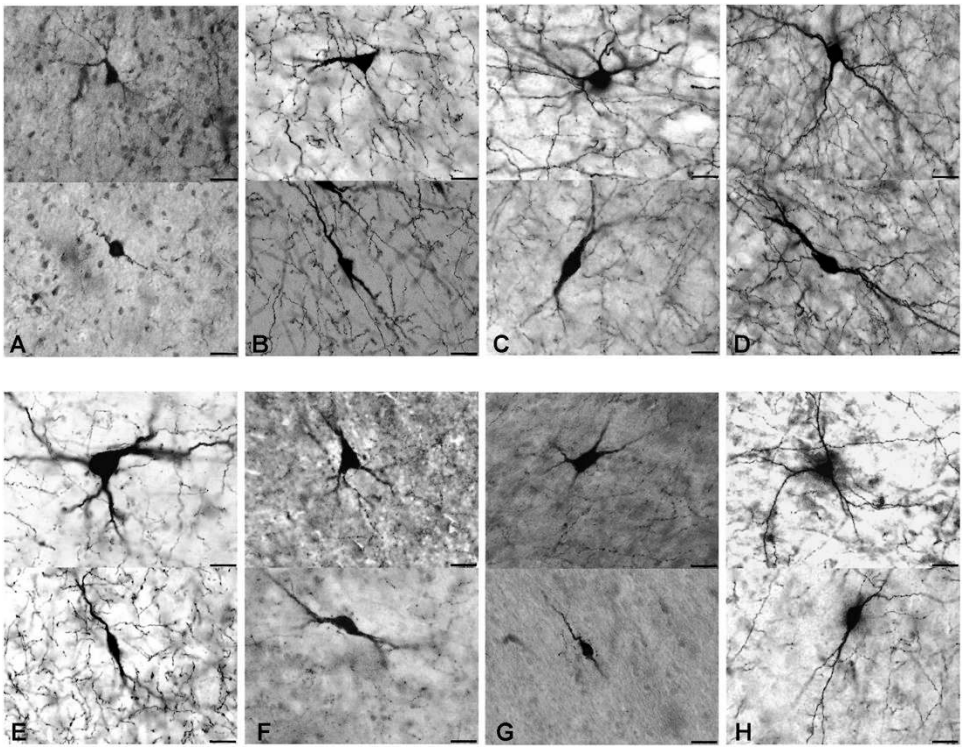

Figure 4.
Examples of NPY-immunoreactive interneurons in squirrel monkey (A), capuchin (B), moor macaque (C), pigtailed macaque (D), baboon (E), gorilla (F), chimpanzee (G), and human (H). Scale bars = 25 μm.

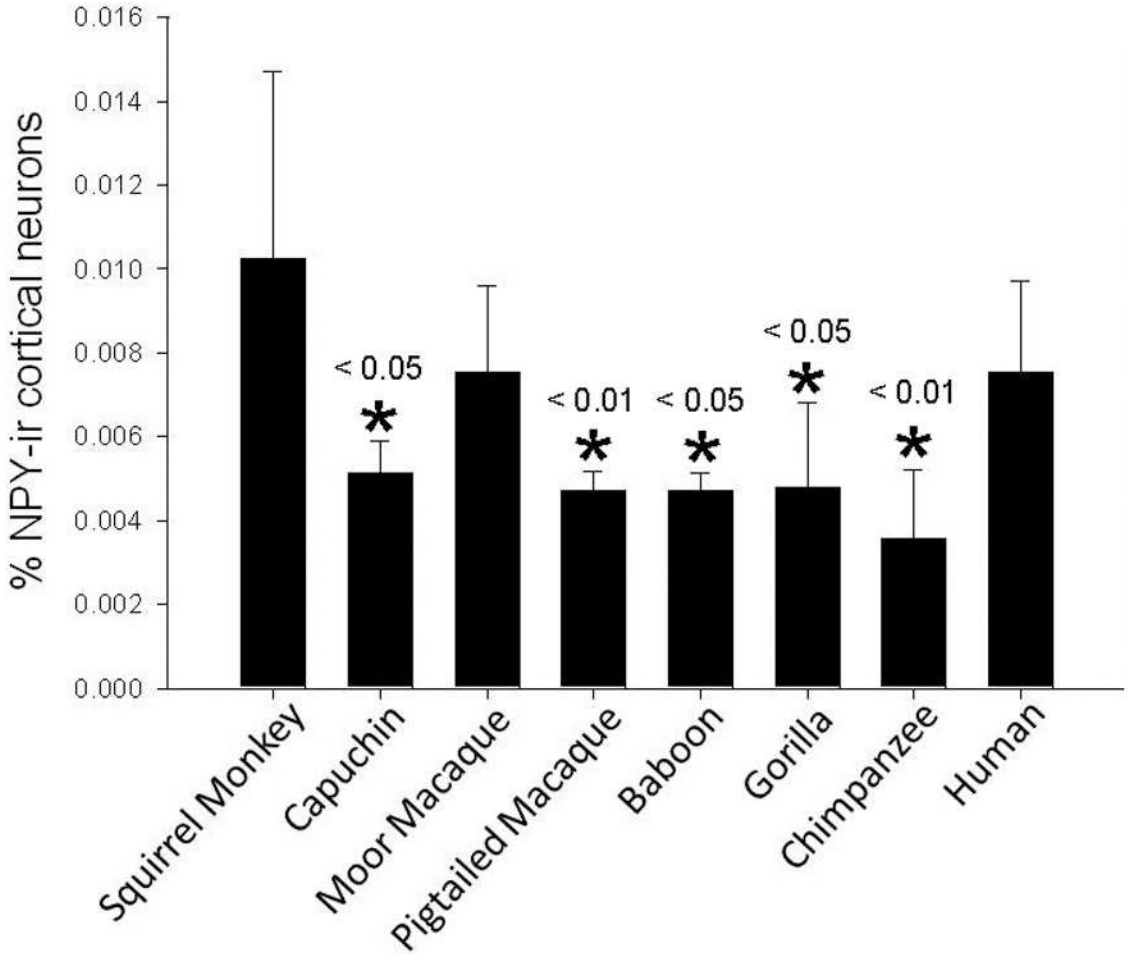

Figure 5.
Percentage of NPY-immunoreactive neurons within the cortex of each species. Error bars represent SD. Asterisks indicate a significant difference relative to squirrel monkeys. The numbers above the asterisks represent the P values associated with Tukey's post hoc tests.

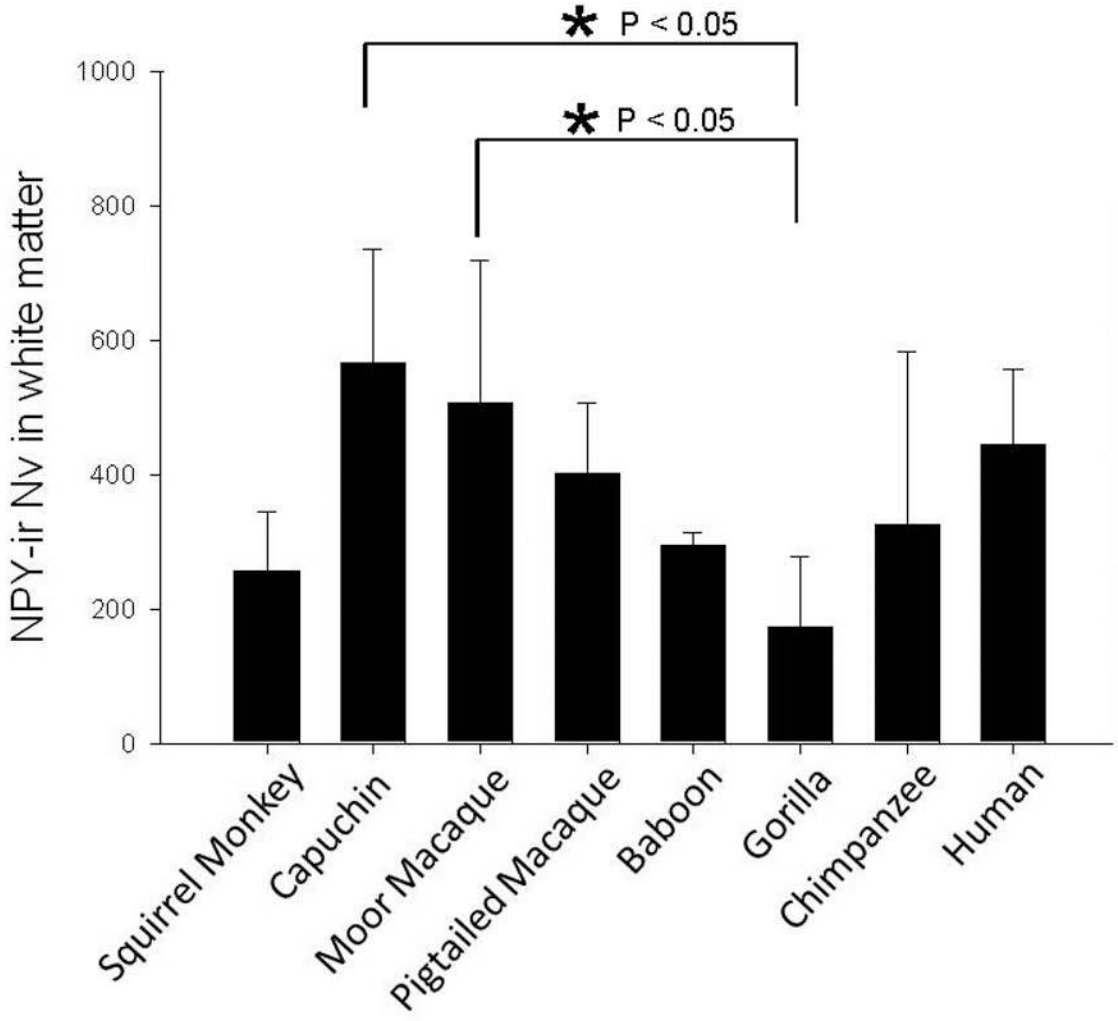

Figure 6.
NPY-immunoreactive neuron densities present in the white matter of each species. Error bars represent SD. Significant differences detected using Tukey's post hoc tests (P values) are indicated by asterisks and brackets, with gorillas having fewer interstitial cells relative to capuchins and moor macaques.

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Table I

Group	Species	Common Name	Sex	Age	% NPY-ir cortex	NPY-ir Nv WM
Humans	*Homo sapiens*	Human	M	54	0.0086	507.4
	Homo sapiens	Human	M	56	0.0071	484.2
	Homo sapiens	Human	F	40	0.0097	278.2
	Homo sapiens	Human	F	43	0.0047	513.9
Great Apes	*Pan troglodytes*	Chimpanzee	F	44	0.0032	228.6
	Pan troglodytes	Chimpanzee	M	25	0.0059	709.7
	Pan troglodytes	Chimpanzee	M	17	0.0031	174.1
	Pan troglodytes	Chimpanzee	M	19	0.0021	190.4
	Gorilla gorilla	Gorilla	M	13	0.0097	203.9
	Gorilla gorilla	Gorilla	M	21	0.0150	359.6
	Gorilla gorilla	Gorilla	F	50	0.0061	208.4
	Gorilla gorilla	Gorilla	M	49	0.0032	85.0
OWM	*Macaca nemestrina*	Pigtailed macaque	M	15	0.0048	404.7
	Macaca nemestrina	Pigtailed macaque	F	15	0.0048	379.2
	Macaca nemestrina	Pigtailed macaque	F	9	0.0045	378.4
	Macaca nemestrina	Pigtailed macaque	M	7	0.0046	424.1
	Macaca nemestrina	Pigtailed macaque	M	6	0.0054	218.5
	Macaca nemestrina	Pigtailed macaque	M	5	0.0051	570.9
	Mcaca nemestrina	Pigtailed macaque	M	5	0.0040	446.0
	Macaca maura	Moor macaque	M	8	0.0054	257.0
	Macaca maura	Moor macaque	F	5	0.0078	662.8
	Macaca maura	Moor macaque	F	7	0.0087	754.4
	Macaca maura	Moor macaque	F	7	0.0099	657.0
	Macaca maura	Moor macaque	F	8	0.0088	424.0
	Macaca maura	Moor macaque	M	10	0.0047	291.9
	Papio anubis	Baboon	F	9	0.0042	296.1
	Papio anubis	Baboon	F	5	0.0050	276.6
	Papio anubis	Baboon	M	6	0.0049	315.4
NWM	*Saimiri boliviensis*	Squirrel monkey	F	12	0.0075	325.4

Group	Species	Common Name	Sex	Age	% NPY-ir cortex	NPY-ir Nv WM
	Saimiri boliviensis	Squirrel monkey	F	9	0.0052	131.8
	Saimiri boliviensis	Squirrel monkey	F	9	0.0033	147.2
	Cebus apella	Capuchin	F	17	0.0041	489.0
	Cebus apella	Capuchin	F	18	0.0056	566.7
	Cebus apella	Capuchin	M	15	0.0055	638.7
	Cebus apella	Capuchin	M	16	0.0046	346.3
	Cebus apella	Capuchin	F	12	0.0060	798.5

OWM = Old World monkeys; NWM = New World monkeys. Age is in years. M = male; F = female. NPY-immunoreactive Nv (neuron density) in WM (white matter) is expressed as density per mm^3.