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Gene Expression Analysis of Neuropeptides in Oral Mucosa during Periodontal Disease in Non-human Primates

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Abstract

Background: Neuropeptides (NPs) are innate pivotal regulators of the immunoinflammatory response. Nevertheless, their role in the pathogenesis of the periodontal disease remains unknown. Changes in gene expression of 10 NPs and 16 NP receptors (NPRs) coincident with the initiation, progression and resolution of periodontitis were determined.

Methods: The ligature-induced periodontitis model was used in rhesus monkeys (n=18). Gingival tissue samples were taken at baseline (pre-ligatures), at 2 weeks, at 1 month (initiation), and at 3 months (progression) postligation. Ligatures were removed and samples taken 2 months later (resolution). Total RNA was isolated from tissues and NP/NPR gene expression microarray analysis was performed. Gene expression changes were validated by quantitative polymerase chain reaction and immunohistochemistry.

Results: Unexpectedly, the expression of pro-inflammatory NPs/NPRs did not change during periodontitis or with resolution. However, increased expression of the anti-inflammatory NPs, adrenomedullin (ADM) and galanin (GAL), and the NPRs, calcitonin receptor-like (CALCRL) and receptor activity modifying Protein-2 and 3 (RAMP-2, and -3), were observed during initiation and progression of disease. The expression of the same NPs/NPRs exhibited a significant positive correlation with both molecular (IL-1 β , MMP-9 and RANKL) and clinical measures of gingival inflammation and tissue destruction.

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CONFLICT OF INTEREST STATEMENT

The authors of this manuscript declare no conflict of interest related to this study.

Conclusion: Initiation and progression of periodontitis involve significant overexpression of ADM, GAL, CALCRL, RAMP2, and RAMP3. These anti-inflammatory NPs/NPRs could play a role in the unresolved infection and inflammation that normally drives tissue destruction in periodontitis. Both, ADM and GAL potentially are new candidates to consider as biomolecules associated with periodontal disease.

One-sentence summary:

Initiation and progression of periodontitis involve significant gingival up-regulation of anti-inflammatory neuropeptides and their receptors.

Keywords

Gene expression; inflammation; innate immunity; neuropeptides; periodontitis

INTRODUCTION

Periodontal disease is a highly prevalent oral, chronic, inflammatory disease characterized by the loss of soft tissue and alveolar bone supporting tissues of the teeth¹. It is clear that pathogenic biofilms trigger this persistent inflammatory response; however, it is the unresolved inflammation to these noxious biofilms that causes the tissue damage in periodontitis²⁻⁴. Accordingly, during the last decade a significant effort has been placed on identifying the host cellular and molecular factors involved in the dysregulated inflammation causing periodontitis.⁵

For many years, it has been shown that the neuroendocrine system plays an important regulatory role for immunoinflammatory responses through neuropeptides (NPs), hormones, and other components⁶. Growing evidence indicates that NPs are pivotal regulators (*i.e.*, anti- and pro-inflammatory effects) of the immunoinflammatory response, and some of them can be produced by various innate and adaptive immune cells⁶. In particular, peptide neurotransmitters produce actions on extracellular receptors of target cells including neural and non-neural cells such as macrophages, lymphocytes, and endothelial cells.⁷⁻⁹

Accordingly, differential modulation of cytokine production by NPs has been shown in several immune cell types including antigen presenting cells as well as B and T cells^{6, 10}. In addition to their immunoregulatory role, some NPs (*e.g.*, calcitonin gene-related peptide [CGRP]) have also been shown to be involved in bone metabolism directly affecting osteoblast and osteoclast function through receptor activation, or indirectly enhancing production of pro-inflammatory cytokines.¹¹

Consistent with the role of NPs as important regulators of the innate and adaptive immune responses, as well as bone metabolism, variations in the levels of NPs have been reported in chronic inflammatory disorders such as rheumatoid arthritis, psoriasis and periodontitis.¹²⁻¹⁴ In particular, variations in the levels of a limited number of NPs have been reported in gingival crevicular fluid (GCF) and gingival biopsies from periodontal lesions, whereby levels of substance P (SP) and neurokinin A (NKA) were increased with the clinical diagnosis of periodontitis,¹⁵⁻¹⁷ and levels of CGRP and Vasoactive Intestinal Peptide (VIP)

were decreased with disease.^{18, 19} Importantly, these altered levels of NPs returned to quantities detected in healthy samples following periodontal therapy.^{15, 16, 18}

Overall, evidence from limited cross-sectional studies indicates that variation in the levels of some NPs could be associated with established periodontal lesions¹⁷; however, the role that NPs play in the pathogenesis of periodontitis remains unknown. Growing evidence supporting an immunoregulatory role of NPs makes this group of molecules an interesting and innovative target for elucidating their potential contributions to the progression and resolution phases of periodontal disease. In the current report the authors sought to evaluate the gene expression levels of a subset of pro- and anti-inflammatory NPs and their receptors during initiation, progression, and resolution of experimental periodontal disease and their correlation with clinical parameters of periodontitis using the non-human primate model.

MATERIALS AND METHODS

Animals and diet

Rhesus monkeys (*Macaca mulatta*) (n = 18; 10 females and 8 males) housed at the Caribbean Primate Research Center (CPRC) at Sabana Seca, Puerto Rico, were used in these studies. The age range was 12 to 23 years old (mean=16.1±3.7). The non-human primates were fed a 20% protein, 5% fat and 10% fiber commercial monkey diet[#]. The diet was supplemented with fruits and vegetables, and water was provided *ad libitum* in an enclosed corral setting.

Ligature-induced periodontitis and gingival tissue sample collection

The ligature-induced periodontitis model was used in healthy rhesus monkeys as previously described following a protocol approved by the Institutional Animal Care and Use Committee of the University of Puerto Rico.²⁰ Periodontal health was defined by mean Pocket Depth (PD) ≤ 3.0 mm and mean Bleeding on Probing (BOP) ≤ 1 (0–5 scale) in a full mouth examination excluding 3rd molars and canines.²⁰ Clinical measures of BOP and PD, as well as gingival tissue samples were taken at baseline (preligatures), at 2 weeks and 1 month (initiation), and at 3 months (progression) post-ligation. Ligatures were then removed after sampling at the 3rd month, and samples were taken 2 months later (resolution). Tissues were maintained in RNA stabilization solution prior to processing^{**}.

Total RNA was isolated from tissues using trizol reagent^{††}. After cleaning with a commercially available kit^{‡‡}, the isolated RNA was quantified with spectrophotometric analysis and pooled into equal amounts from each sample for an individual animal. Gene expression was evaluated for 26 NP genes and their receptors with pro- and anti-inflammatory activity (see Supplementary Table 1 in online *Journal of Periodontology*).

[#]Commercial monkey diet 8773, Harlan Teklad, Agricultural Exports Inc. Doylestown, PA, USA.

^{**}RNAlater Soution, ThermoFisher Scientific, Invitrogen, Carlsbad, CA, USA

^{††}ThermoFisher Scientific, Invitrogen, Carlsbad, CA, USA

^{‡‡}Qiagen RNeasy mini kit. Qiagen, Valencia, CA, USA

Microarray analysis and quantitative reverse transcription-polymerase chain reaction (qRT-PCR).

Total RNA from each gingival tissue (n=18) was isolated using a standard procedure, as we have described, and submitted to the microarray core to assess RNA quality and analyze the transcriptome^{§§}.^{21, 22}

For qRT-PCR analysis, messenger RNA (mRNA) samples for each time point were pooled in two groups (nine samples/group) and gene expression analyzed in triplicates (n=6). The complementary DNA (cDNA) synthesis was carried out starting with 1 µg of pooled mRNA, and expression of specific transcripts for NPs and NPRs analyzed^{***}. For each gene the following primers were used: adrenomedullin (ADM): forward: GGTGTGAACCATGAGAAGTATGA, reverse: GAGTCCTTCCACGATACCAAAG; galanin (GAL): forward: GACGACGTGAAACCAGGAA, reverse: GCCTCTTTGAGATGCAAGAAAG; calcitonin receptor-like (CALCRL): forward: GTACGTGTTCTCATCACCAGT, reverse: AGCAAGGGCACCAAGATAAG; receptor activity-modifying protein-2 (RAMP2): forward: GAGACTCACCAGATCCACTTT, reverse: GATCATGGCCAGGAGTACAT; RAMP3: forward: ACAGGCAGTTCTTCTCCAAC, reverse: GACAGTCAGAACAACAGGGATAA; vasoactive intestinal peptide receptor 1 (VIPR1): forward: GAAGAGTGACAGCAGTCCATAC, reverse: GTCAGGAAAGAAGGCGAACA; interleukin (IL)-1B: forward: GACAGGATCTGGAGCAACAA, reverse: CCCAAGGCCACAGGTATTT; matrix metalloproteinase 9 (MMP9): forward: CGTCTTCCAGTACCAAGAGAAA, Reverse: GGATGTCATAGGTCACGTAGC; receptor activator of nuclear factor kappa-B ligand (RANKL): forward: GGGAGACCTAGCTACAGAGTAT, reverse: TGGTGCTTCCTCCTTTTCATC, and GADPH: forward: GGTGTGAACCATGAGAAGTATGA, reverse: GAGTCCTTCCACGATACCAAAG. Concentration ratios for the target genes were calculated by normalizing to the housekeeping gene GADPH.

Immunohistochemistry

Immunohistochemical staining was performed on 4-µm sections of fixed paraffin embedded gingival tissues taken at baseline (BL), two weeks (2W), one month (1M), three months (3M) and five months (5M) post ligation. The sections were placed on charged microscope glass slides and dried at 65°C for 60 min. The slides were immersed in xylene (5 minutes, 2x), then 100% ethanol (3 minutes, 2x), followed by 90% and 70% ethanol (3 minutes each) before moving them into water. Endogenous peroxide was blocked using peroxide blocking solution^{†††} for 10 minutes at room temperature. Heat-induced epitope retrieval (HIER) was performed by heating the slides once with HIER buffer (Diva Decloaker) in a pressure chamber^{†††} for 5 minutes at 21 psi, followed by 45 minutes cool-down time. Slides were then washed in phosphate-buffered saline three times and placed in humidity chamber. The NPs were detected using the following antibodies: rabbit polyclonal GAL antibody diluted

§§GeneChip® Rhesus Gene 1.0 ST Array. Affymetrix, Santa Clara, CA, USA

***LightCycler 480, Roche, Basel, Switzerland.

†††Biolegend, San Diego, CA, USA.

†††Biocare Medical, Pacheco, CA, USA.

1:300, mouse monoclonal RAMP2 antibody diluted 1:150, or rabbit polyclonal CALCRL antibody diluted 1:400 ^{§§§}. All antibodies were diluted in blocking solution (Power Block) ^{****} and incubated for 1 hour at room temperature. Signal was developed using a biotin-free horseradish peroxidase polymer detection system for use with polyclonal and monoclonal antibodies made in rabbits and mice ^{††††}. The kit was used in accordance with the manufacturer's instructions. DAB (3, 3'-diaminobenzidine) ^{††††} was used as a chromogen, applied for 5–10 minutes, followed by counterstaining with modified Harris hematoxylin ^{§§§§} for 30 seconds. Finally, the slides were submerged for 2 seconds in a saturated solution of lithium carbonate. The sections were then dehydrated and mounted for microscopic analysis ^{*****}. Images were acquired on a microscope ^{†††††}.

Data analysis

The expression intensities for all genes across the samples were estimated using the robust multi-array average (RMA) algorithm with probe-level quintile normalization, as implemented in a Genomics software ^{†††††}. Differences in gene expression at different time points were tested using repeated measures with Benjamin-Hochberg adjustment for multiple comparisons; post hoc paired t tests comparing other time points to baseline followed the significant repeated measures tests. We also determined correlations between the expression of NP and NPR genes and molecular and clinical measures of tissue inflammation and tissue destruction during the course of the disease using a Pearson's correlation analysis. Statistical significance was considered by a *P* value 0.05.

RESULTS

Pocket depths were significantly increased following ligation through the third month and returned to basal levels at 5 months after removing the ligatures (see Supplementary Table 2 in online *Journal of Periodontology*). BOP scores were elevated during the initiation phase of disease (i.e., 2W and 1M) and returned to basal levels by 3 and 5 months (see Supplementary Table 2 in online *Journal of Periodontology*).

Figure 1 shows the expression levels of NPs and their receptors described as exhibiting pro-inflammatory activities. There were no significant changes in the gingival expression of these genes during ligature-induced periodontitis. In contrast, there was a significant increase in the expression of the anti-inflammatory NPs ADM and GAL during the initiation (2W, 1M) and progression (3M) of periodontitis, followed by a return to baseline expression levels with resolution of disease (5M) (Figure 2A). Expression of the anti-inflammatory receptors CALCRL, RAMP2 and RAMP3 was also significantly increased during initiation and progression of disease, followed by a return to baseline expression levels with

^{§§§}Novus Biologicals, Littleton, CO, USA. Anti-CALCRL (Cat#NLS6755), anti-RAMP2 (Cat#NBP2-01853), anti-Galanin (Cat#NBP2-33582).

^{****}Biogenex Laboratories, San Ramon, CA, USA.

^{††††}ACUTITY Advanced kit, Roche Life Science, Madison, WI, USA.

^{††††}Fisher Scientific, Fair Lawn, NJ, USA.

^{§§§§}Richard-Allan Scientific, San Diego, CA, USA.

^{*****}Cytoseal 280, Richard-Allan Scientific, San Diego, CA, USA

^{†††††}Nikon, Tokyo, Japan.

^{†††††}Partek, St. Louis, MO, USA.

resolution. VIPR1 was the only one of these NPs or receptors that showed a significant reduction during the course of periodontitis (Figure 2B). Significant changes in gene expression observed by microarray analyses were validated by qRT-PCR demonstrating similar trends of expression during the progression of periodontitis (Figure 3).

Gene expression changes of molecular markers of inflammation, as well as genes related to both soft and bone tissue destruction during the progression of periodontitis are shown in Figure 4. There was a significant increase in the expression of these disease markers during initiation (2 weeks/1month) and/or progression (3 months) of periodontitis. Return to normal expression levels was rapid for IL1B by 1 month, and with MMP-9 and RANKL the up-regulated levels, although lower after removal of ligatures during the resolution phase of the disease, remained significantly elevated even at 5 months at a time-point of apparent clinical resolution of disease.

Expression analysis of RAMP2, CALCRL and GAL through immunochemistry suggested that the oral epithelium was positive for all them at baseline, but changes in epithelial expression were not significantly different after ligature induced disease (data not shown). An increased staining intensity was noted in epithelial cells located at the basal layer. Nevertheless, an increase in inflammatory cells infiltrating the connective tissue positive for neuropeptides expression were normally seen throughout all disease process time-points (2W, 1M, 3M) and a decrease in inflammatory cells two months later after removing the ligatures (i.e., 5 months) was also observed (Figure 5).

Correlation analysis of NPs and their receptors with molecular and clinical measures of inflammation and tissue destruction are shown in Table 1. Consistent with their pro-inflammatory role, the NP/NPR genes TAC4, NK1/TACR1 and NK3/TACR3 showed a significant positive correlation with the expression of IL1B and RANKL; however, the same NP/NPR genes did not correlate with clinical measures of BOP or PD. In contrast, and consistent with the expression kinetics observed during progression of periodontitis, the anti-inflammatory NP/NPR genes ADM, GAL, CALCRL, RAMP2 and RAMP3 showed a significant positive correlation, and VIPR1 showed a significant negative correlation with both molecular and clinical measures of inflammation and tissue destruction.

DISCUSSION

Growing evidence indicates that NPs are central regulators of the innate and adaptive immune responses. An imbalance of these mediators can result in persistent inflammation or unresolved infection, both characteristics of periodontal disease. In this study the expression of a subset of NP and NPR genes was evaluated in gingival tissues from non-human primates during initiation, progression and resolution of periodontitis to better understand their potential role in the pathogenesis of this oral chronic inflammatory disorder. Unexpectedly, there were no significant changes in expression of the pro-inflammatory NP and receptor pairs with progression of the disease; however, a significant increase in the anti-inflammatory NP and NPR genes ADM, GAL, CALCRL, RAMP2 and RAMP3 was observed.

Adrenomedullin (ADM) is a NP that is produced by neural, endothelial, epithelial, fibroblast, macrophage and smooth muscle cells constitutively or in response to oxidative stress, TNF α and lipopolysaccharide (LPS).²³ Cellular activation by ADM requires its interaction with two different receptors, CALCLR and RAMP2/RAMP3).²³ There is evidence indicating that ADM inhibits inflammation by decreasing pro-inflammatory cytokine production, and T-cell proliferation; however, ADM also has potent vasodilation properties²⁴. Hence, increased ADM in gingival tissues during progression of periodontitis could involve an increase in the number of recruited inflammatory cells into the gingival milieu due to its vasodilatory properties. However, coincident with this activity ADM could also block the expression of important inflammatory mediators by these recruited immune cells to control the infection. Thus, up-regulation in gingival tissue ADM levels could result in unresolved inflammation and persistent infection. Moreover, it has also been shown that ADM has antimicrobial properties²⁵. Of note, a major periodontal pathogen *Porphyromonas gingivalis* increases the expression of ADM by human oral epithelial cells but is resistant to its antimicrobial actions^{26, 27}, and GCF increased levels of ADM with periodontal disease have also been reported.²⁸ Therefore, increased ADM levels induced by periodontal pathogens such as *P. gingivalis* could be involved not only in unresolved inflammation but also contribute to the dysbiosis as an early event involved in the pathogenesis of periodontitis²⁸.

The other NP that was significantly elevated during periodontitis was GAL. Limited evidence indicates that GAL is a component of the innate immune system through its capacity to prevent excess inflammation that could be deleterious to the host.²⁹ Variations in GAL expression during periodontal disease have not been previously reported. Therefore, the role of increased levels of GAL and its receptors, noted in progressing periodontitis needs to be further evaluated.

Importantly, the same subset of anti-inflammatory NP/NPR genes that were significantly elevated during periodontitis showed a positive correlation with molecular and clinical measures of inflammation and tissue destruction. It is clear that the immuno-inflammatory responses against pathogens in the complex microbial ecology need to be balanced in order to successfully manage the pathogens without causing collateral tissue damage. These regulated responses involve a balanced expression of both pro- and anti-inflammatory mediators. Thus, an increased expression of anti-inflammatory NP/NPR genes in the gingival tissues during periodontitis could help to tip the balance and foster an excess of down-regulation of protective inflammation that could impair the normal resolution of infection and disease. Interestingly, the main changes in gingival expression of these anti-inflammatory NPs/NPRs seem to be associated with an increase in the number of inflammatory cells positive for those NPs/NPRs, rather than expression changes in the oral epithelium.

We also observed that VIPR1 expression was significantly reduced during periodontitis, and this anti-inflammatory NP receptor also showed significant negative correlations with both molecular and clinical measures of inflammation and tissue destruction. VIPR1 is activated by VIP, which has been shown to inhibit TNF- α , IL-6, and IL-12 production by LPS-activated macrophages^{30, 31}. VIP also stimulates the production of the potent anti-

inflammatory cytokine, IL-10, and suppresses T-cell proliferation³². In healthy gingival tissues, VIP is at higher levels compared with levels in GCF from periodontal disease sites.¹⁸ Consistent with this evidence, our results support that in addition to reduced levels of VIP in periodontitis, the anti-inflammatory effects mediated by VIP could also be significantly altered through a reduction in the expression of one of its primary receptors, *i.e.*, VIPR1.

Significant variations in the expression of the pro-inflammatory NP/NPR genes were not observed with progression of periodontal disease. Previous evidence showed that the GCF levels of some pro-inflammatory NPs such as SP and NKA, are increased in established periodontitis lesions.¹⁵ Production of both SP and NKA involves post-transcriptional modifications (*e.g.*, alternative splicing) from the same TAC1 (preprotachykinin-1) transcript.³³ Thus, variations in the levels of these NPs with periodontitis may involve changes in posttranscriptional regulatory mechanisms rather than specific TAC1 gene expression changes. In addition, correlational analyses demonstrated that the NP/NPR genes TAC4, NK1/TAC1R and NK3/TACR3 showed significant positive correlations with the expression of IL1 β and RANKL during the disease process. These results are consistent with previous evidence indicating that the NPs/NPRs encoded by these genes are increased in chronic inflammatory disorders and may contribute to osteoclastogenesis and bone resorption. For example, Hemokinin-1, derived from the TAC4 gene, has been shown to increase inflammation in the late phase of adjuvant-induced arthritis³⁴, the SP receptors NK1/TAC1R are also up-regulated in bone resorption³⁵, and NK3/TACR3 is involved in osteoclast formation and bone resorption by cellular activation through Neurokinin B binding.³⁶

CONCLUSIONS

It was demonstrated that initiation and progression of periodontal disease involves significant variation in the gene expression of reported anti-inflammatory neuropeptides ADM and GAL, and receptors CALCRL, RAMP2, RAMP3, and VIPR1 in nonhuman primates. These disease-related changes in NP/NPR expression seem to be mainly associated with variations in inflammatory cells exhibiting lymphocyte and macrophage-like morphological features. Of note, these variations showed significant correlations with molecular markers and clinical measures of periodontitis. While acknowledging that there are limitations in animal models of periodontitis as used in this study, the results support a consideration of additional pathways and molecules that could be potential new candidates to consider within the complex mechanisms of this disease process, or as new potential biomarkers associated with periodontal disease activity in future clinical studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Pro-Inflammatory NP Receptors

Figure 1.

Expression of pro-inflammatory (A) NPs, and (B) NP receptors during initiation (2 weeks [2W] and 1 month [1M]), progression (3 months [3M]), and resolution (5 months [5M]) of periodontitis. Data are expressed as means $\pm$ standard deviations (n=18). BL, baseline.

Anti-Inflammatory Receptors

Figure 2.

Expression of Anti-inflammatory (A) NPs, and (B) NP receptors during initiation (2 weeks [2W] and 1 month [1M]), progression (3 months [3M]) and resolution (5 months [5M]) of periodontitis. Data are expressed as means $\pm$ standard deviations (n=18). *p < 0.05 when baseline values were compared to the different time points. BL, baseline.

Figure 3.

Gene expression levels of ADM, GAL, CALCRL, RAMP2, RAMP3 and VIPR1 determined by qRT-PCR during periodontitis. Total mRNA samples (n=18) from each time point were pooled in six groups of three samples per group and gene expression was analyzed. Data are expressed as means $\pm$ standard deviations. *p 0.01 and † p 0.05 when baseline values were compared to the different time points. BL, baseline; 2W, 2 weeks; 1 M, 1 month; 3 M, 3 months; 5 M, 5 months.

Figure 4:

Expression levels of genes related with inflammation and tissue destruction during initiation (2 weeks [2W] and 1 month [1M]), progression (3 months [3M]), and resolution (5 months [5M]) of periodontitis by qRT-PCR. Data are expressed as means $\pm$ standard deviations from six groups of three pooled samples for each time point. *p < 0.01 when baseline values were compared to the different time points.

Figure 5.

Immunohistochemical staining of gingival tissue from non-human primates for RAMP2, CALCRL, and GAL at baseline, 2 weeks, 1 month, 3 months and 5 months postligation. Each panel shows representative samples for each time point at 10X (top) and 40X (bottom) magnification. Small-scale bar=100µm; large scale bar=50µm.

Table 1.

Correlation analysis of the expression of NPs and their receptors with molecular (IL-1B, MMP9, and RANKL) and clinical (BOP and PD) measures of inflammation and tissue destruction.

		CYTOKINES & TISSUE DESTRUCTION GENES						CLINICAL MEASURES			
		IL1B		MMP9		RANKL		BOP		PD	
		R	p-value	R	p-value	R	p-value	R	p-value	R	p-value
PRO-INFLAMMATORY											
Neuropeptides	TAC1	0.053		-0.217	0.041	0.113		-0.185		-0.232	0.029
	TAC3	-0.013		0.024		0.182		-0.039		-0.087	
	TAC4	0.417	4.8E-05	0.1828		0.490	1.1E-06	0.001		-0.038	
	NPY	0.082		0.195		0.105		0.031		0.065	
	NK1/TACR1	0.262	0.013	0.198		0.212	0.046	-0.009		0.065	
Receptors	NK2/TACR2	0.085		-0.089		0.005		0.243	0.022	0.179	
	NK3/TACR3	-0.042		-0.075		0.224	0.035	-0.076		-0.042	
	NPY1R	-0.247	0.020	-0.301	0.004	-0.076		-0.102		-0.179	
	NPY5R	-0.081		-0.101		0.193		-0.045		0.035	
ANTI-INFLAMMATORY											
Neuropeptides	VIP	-0.123		-0.116		0.231	0.029	-0.050		0.074	
	CALCB	-0.165		-0.148		0.072		-0.066		-0.027	
	POMC	0.181		0.135		0.312	0.003	0.167		0.079	
	ADM	0.261	0.013	0.465	4.4E-06	0.071		0.393	1.4E-04	0.368	3.9E-04
	GAL	0.460	5.8E-06	0.631	0.000	0.355	0.001	0.389	1.6E-04	0.374	3.1E-04
Receptors	SST	0.200		0.066		0.387	1.8E-04	0.041		-0.014	
	VIPR1	-0.250	0.018	-0.473	2.9E-06	-0.334	0.001	-0.380	2.4E-04	-0.373	3.2E-04
	VIPR2	0.267	0.011	-0.007		-0.001		-0.052		-0.079	
	CALCRL	0.441	1.5E-05	0.629	0.000	0.302	0.004	0.348	0.001	0.252	0.017
	MC2R	0.117		0.059		0.231	0.029	0.037		-0.072	0.017
	RAMP2	0.170		0.546	3.0E-08	0.336	0.001	0.411	6.3E-05	0.601	0.008
	RAMP3	0.230	0.030	0.319	0.002	0.223	0.036	0.432	2.4E-05	0.629	0.005
	GALR1	-0.083		0.165		0.308	0.003	0.079		0.002	
	GALR2	-0.024		0.116		0.013		0.184		0.063	
	GALR3	0.168		-0.135		0.177		-0.058		-0.145	
	SSTR1	0.018		-0.160		0.290	0.006	-0.186		-0.083	
	SSTR2	0.106		0.113		0.305	0.004	0.045		0.028	

Significant positive correlations are highlighted in red and negative correlations in blue.