OCULAR INFLAMMATION AND PAIN

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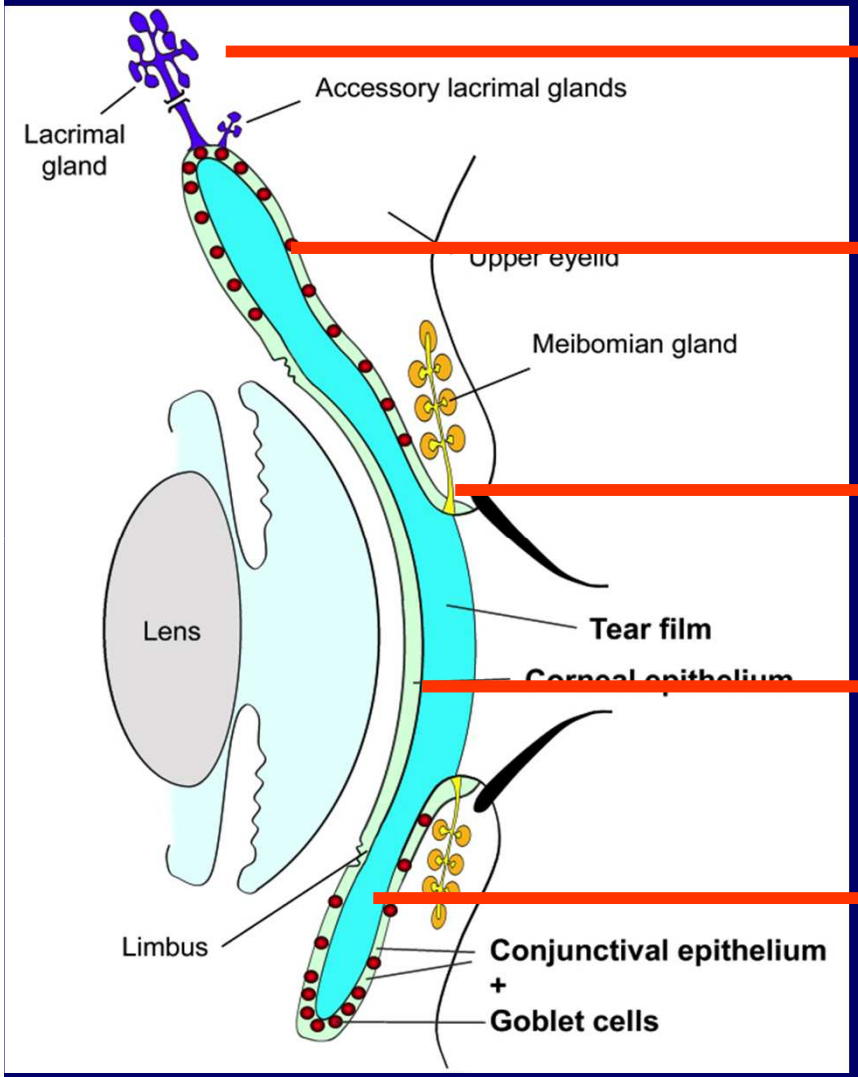
The Ocular Surface



Lacrimal glands

Conjunctival epithelium with goblet cells

Meibomian glands

Corneal epithelium

Tear Film

Ocular surface inflammation

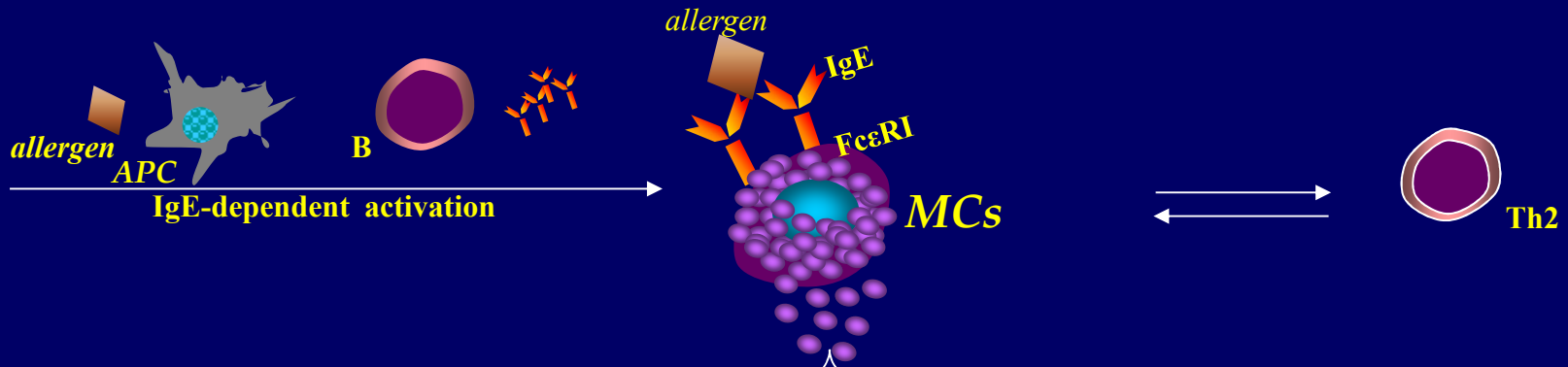
- The ocular surface is a complex structure responsible for visual function and for protection of the eye against external insults.
- Comprising a variety of disorders on cornea, eyelid, conjunctiva, lacrimal apparatus and tear film, there are countless triggers of **ocular surface inflammation**.
- One of the main goals of ocular surface treatment is to control the inflammatory reaction in order to preserve corneal integrity and transparency.

Types of inflammation

- Allergic inflammation: cytokines, kemokines, histamine, etc…
- Inflammation in dry eye: mediators, hyperosmolar stress, etc…
- Ocular surface toxicity: medications, preservatives…

➔ Neurogenic inflammation: neuropeptides (SP, CGRP, NPY, VIP)

Allergic Inflammation

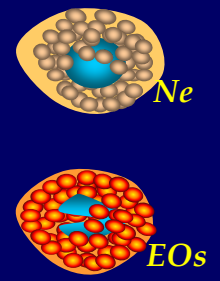


Early phase response
↑Ca^{2+} and degranulation

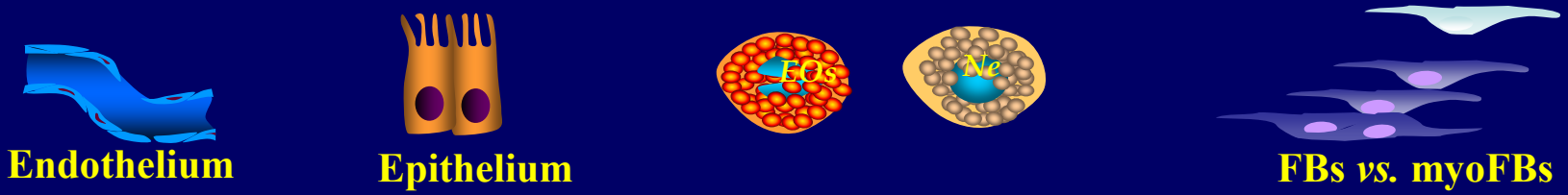
Histamine, tryptase, chymase,
TNFα
TGFβ1, NGF

Late phase response
PKT down-stream
***(de novo* synthesis)**

Leukotrienes, tromboxanes,
IL6, IL8, IL5, IL10,
TNFα, TGFβ1, NGF
VEGF

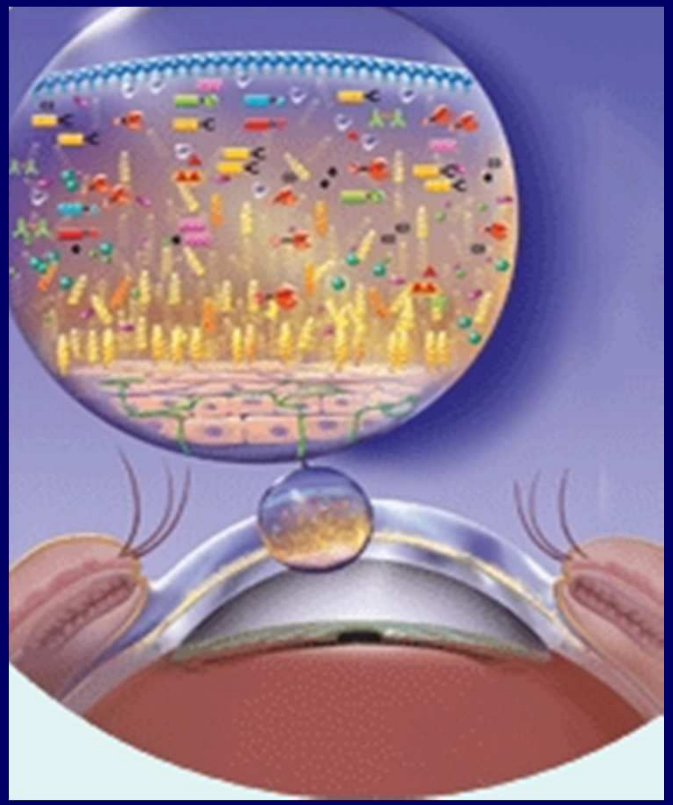


Chronic Inflammation and Tissue remodeling ocular surface damage

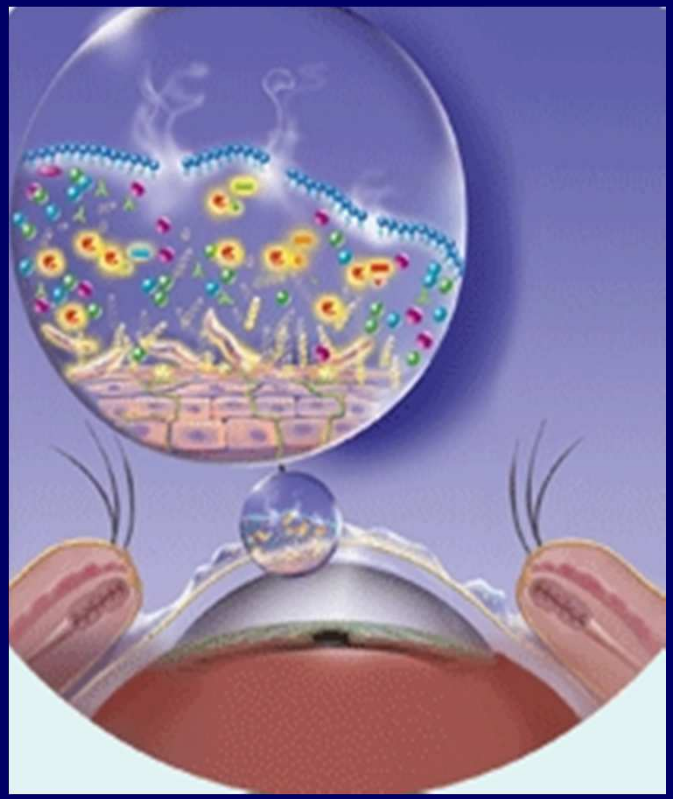


Inflammation in Dry Eye

Healthy tear film

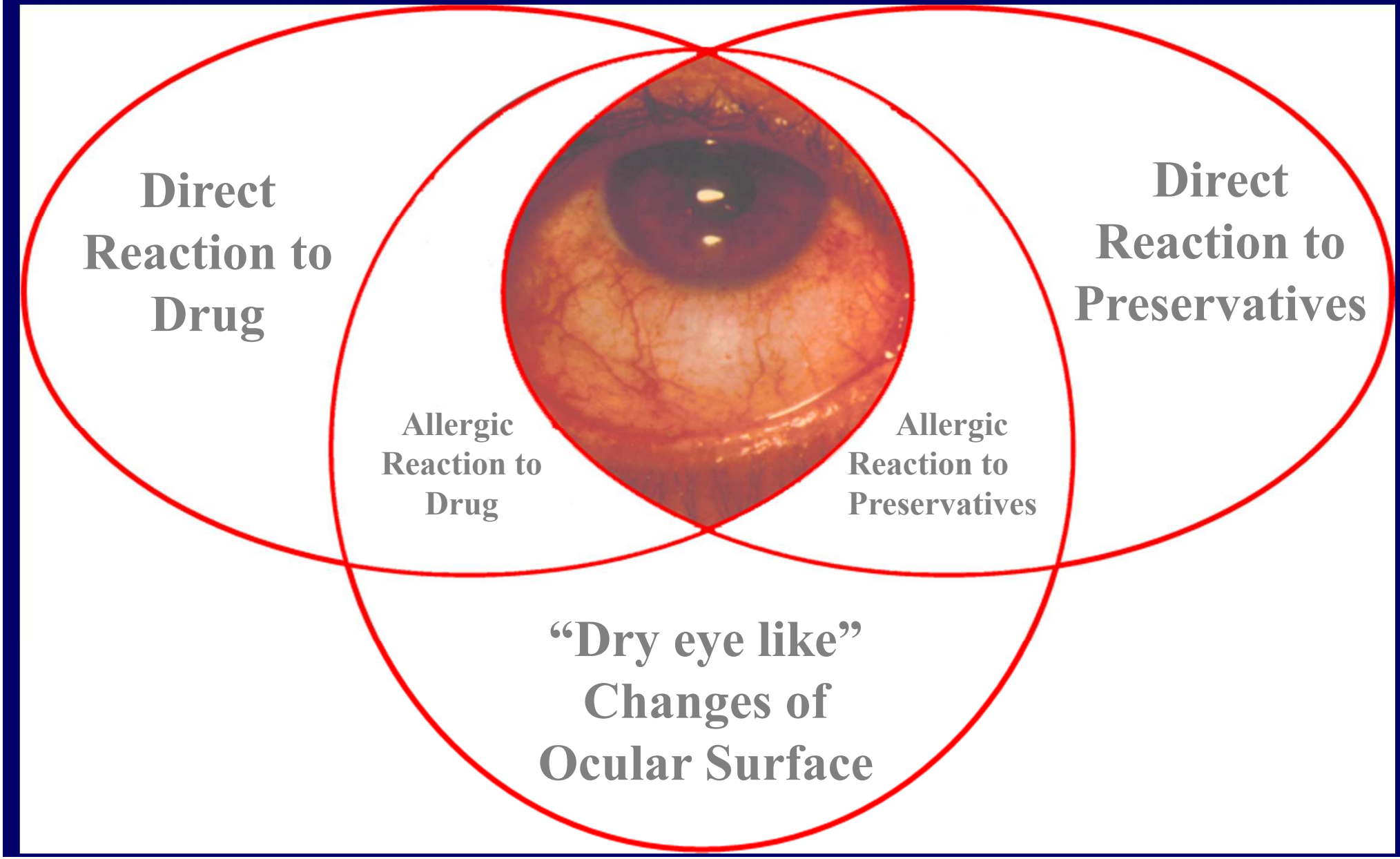


Dry eye



Mediators in tears are the the main actors of dry eye-related inflammation

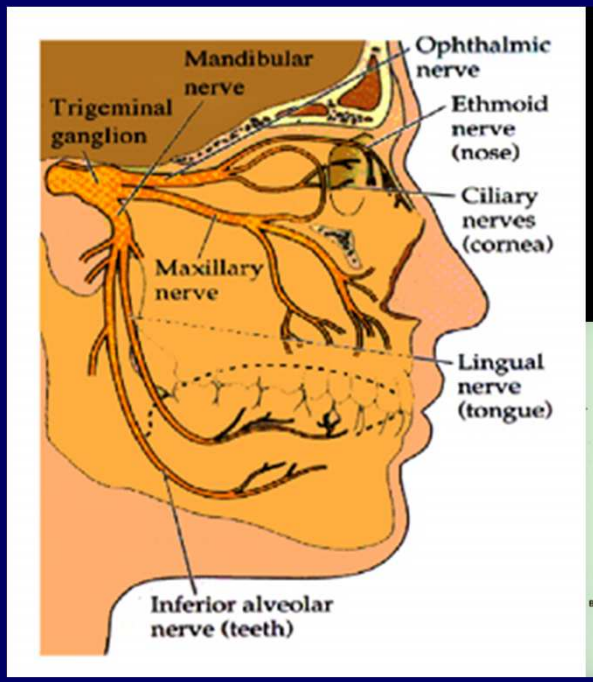
Ocular surface Toxicity

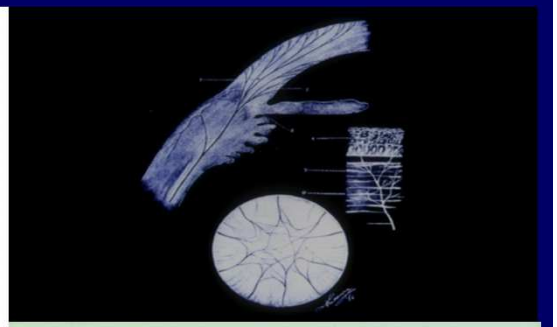


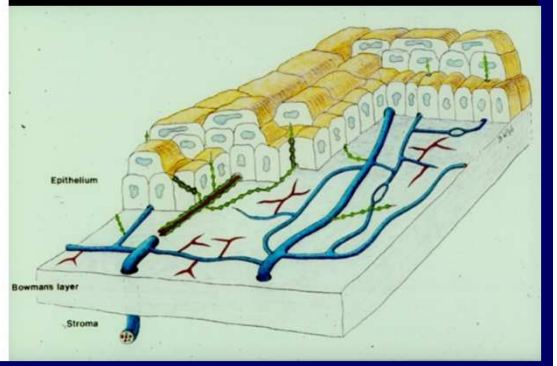
Inflammation → Pain

CORNEAL INNERVATION

- Corneal sensory innervation is the richest of the human body (100+ times more than than the tooth pulp)







- The cornea is an avascular tissue depending on sensory nerves not only for mechanical sensitivity but also for its trophic support

- Three types of sensory nerves: mechanic (20%), chemical (70%), cold fibers (10%).

Mechanical sensitivity testing is NOT sufficient!

Corneal sensitivity testing

- **Mechanical stimulation of the corneal epithelium with a cotton thread:**

  - Complete anhesthesia: no feel, no blink

  - Hypoesthesia: feeling without blinking

  - Normoesthesia: blink at every touch

- **In the last 50 years corneal sensitivity testing was based only on mechanical stimuli.**

  Improvement of cotton-thread test ➔ Cochet-Bonnet esthesiometer (0-6mm nylon thread)

Belmonte esthesiometer

- **Nociceptive corneal sensation evoked by:**
  - **Mechanical stimulus (air)**
  - **Chemical stimulus (CO_2)**
  - **Thermal stimuli (hot / cold air)**

- **Possibility to investigate all the different corneal nerve fibers**

- **Possibility to understand roles of any kind of corneal hypo/hyperesthesia on the patogenesis of ocular surface disease, adjusted to peculiar characteristics of each patients**

STIMOLO MECCANICO

ARIA

FALSO ESTIM.

90 ml/min

STIMOLO MECCÁNICO ON/OFF

REGISTRARE STÍMOLO MECCANICO

TEMPO STIMOLO | 1.5 Sc. | TEMPERATURA D´USCITA | 20º

STIMOLO CHIMICO

CO2

FALSO ESTIM.

40 %

STIMOLO CHIMICO ON/OFF

REGISTRARE STIMOLO CHIMICO

TEMPO STIMOLO | 1.5 Sc. | TEMPERATURA D´USCITA | 20º

FLUSSO CO2 | FLUSSO ARIA

STIMULO TERMICO FREDDO

ARIA | TEMPERATURA STIMOLO

FALSO ESTIM. | 90 ml/min | 20.2 º C

STIMOLO TÉRMICO ON/OFF

REGISTRARE STÍMOLO TERMICO

TEMPO STIMOLO | 1.5 Sc. | TEMPERATURA AMBIENTE | 20º

VALORI NORMALI ESTESIOMETRIA DELLA CORNEA

Età	SOGLIE MECCÁNICO (ml/min) Occhi Scuri		SOGLIE CHIMICO (%CO2) Occhi Chiari	
25	60,8	51,1	29,6	17,1
30	67,3	57,6	32,1	19,6
35	73,8	64,1	34,6	22,1
40	80,3	70,6	37,1	24,6
45	86,8	77,1	39,6	27,1
50	93,3	83,6	42,1	29,6
55	99,8	90,1	44,6	32,1
60	106,3	96,6	47,1	34,6
65	112,8	103,1	49,6	37,1
70	119,3	109,6	52,1	39,6
75	125,8	116,1	54,6	42,1
80	132,3	122,6	57,1	44,6

Neurogenic inflammation

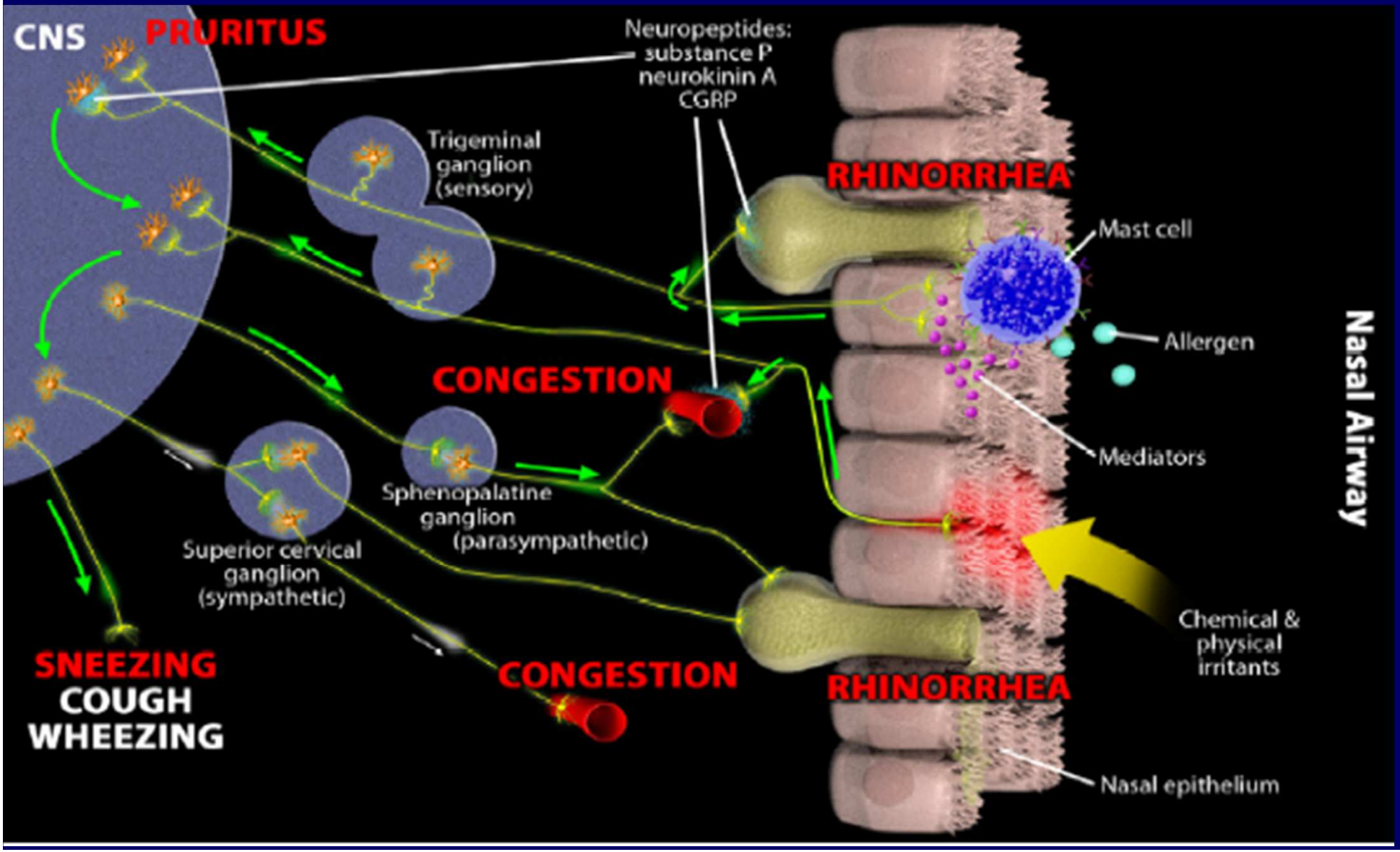
- Inflammation that results from the release of substances from primary sensory nerve terminals.

- These neuromediators act on target cells and exert their biological activity on MC and immune cells to sustain inflammation (Richardson 2002).

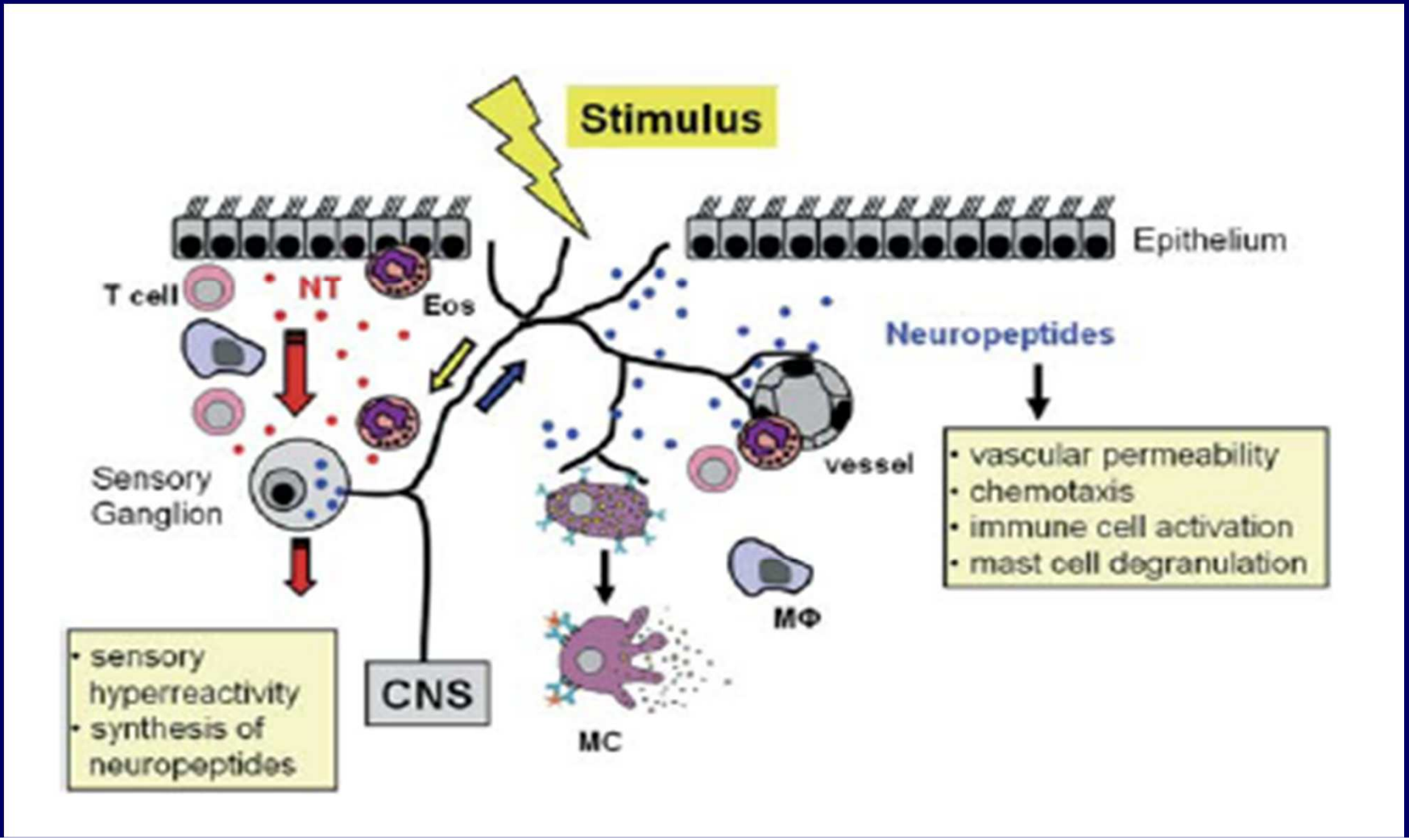
The role of the nervous system in rhinitis

Seema Sarin, MD,[a] Bradley Undem, PhD,[a,b] Alvin Sanico, MD,[a,b,d] and Alkis Togias, MD[a,b,c] *Baltimore, Md*

J ALLERGY CLIN IMMUNOL
NOVEMBER 2006



Not only allergens but any kind of noxious stimulus can induce neuropeptides' release from corneal nerve endings



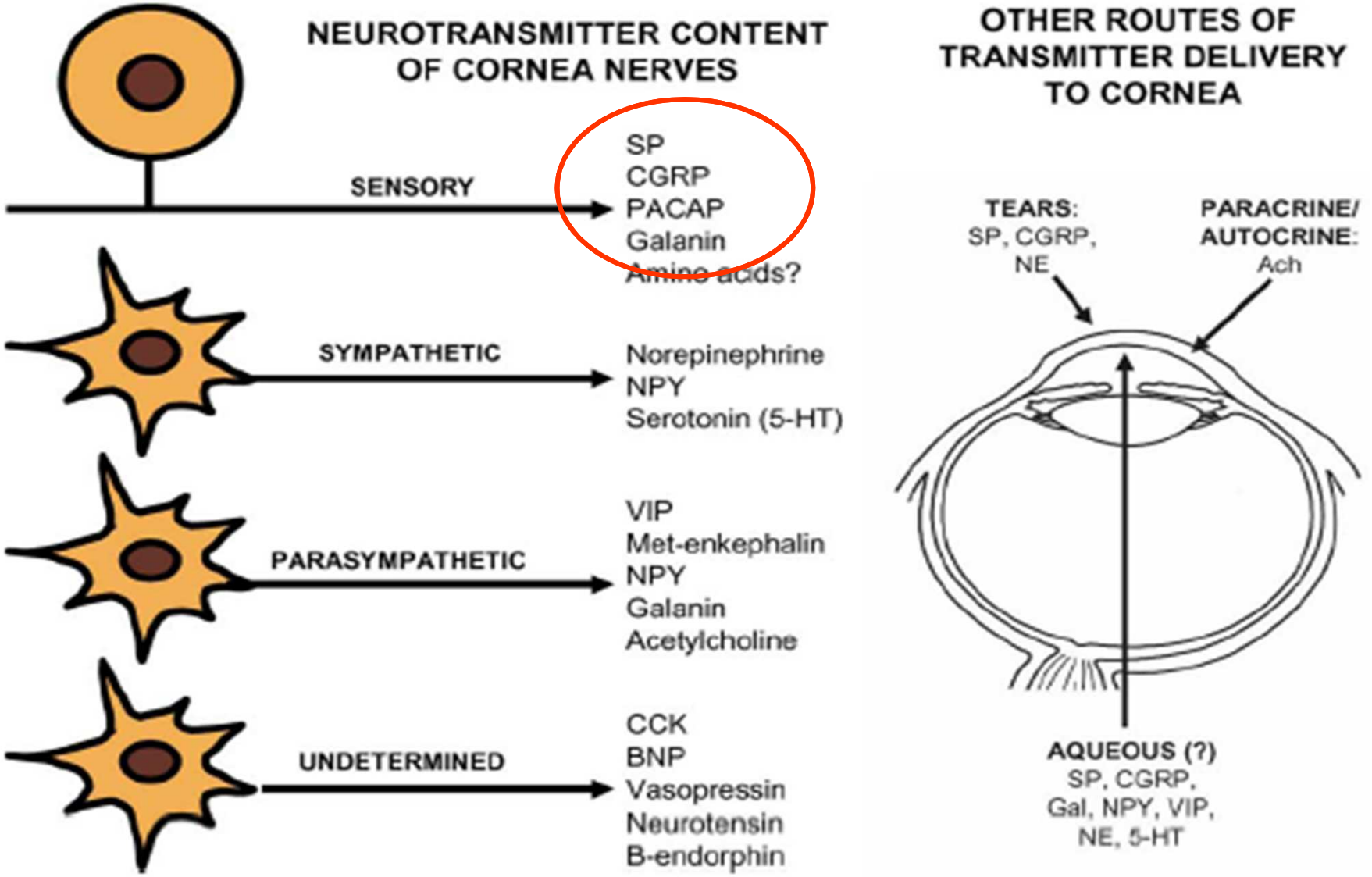


Fig. 7. Neurochemistry of the corneal innervation and the pathways by which nerve transmitter substances reach the cornea.

The neuropeptides substance P (SP) and Calcitonine gene-related peptide (CGRP) are considered to be the major mediators of neurogenic inflammation and pain.

Bornes 2001, Groneberg 2004

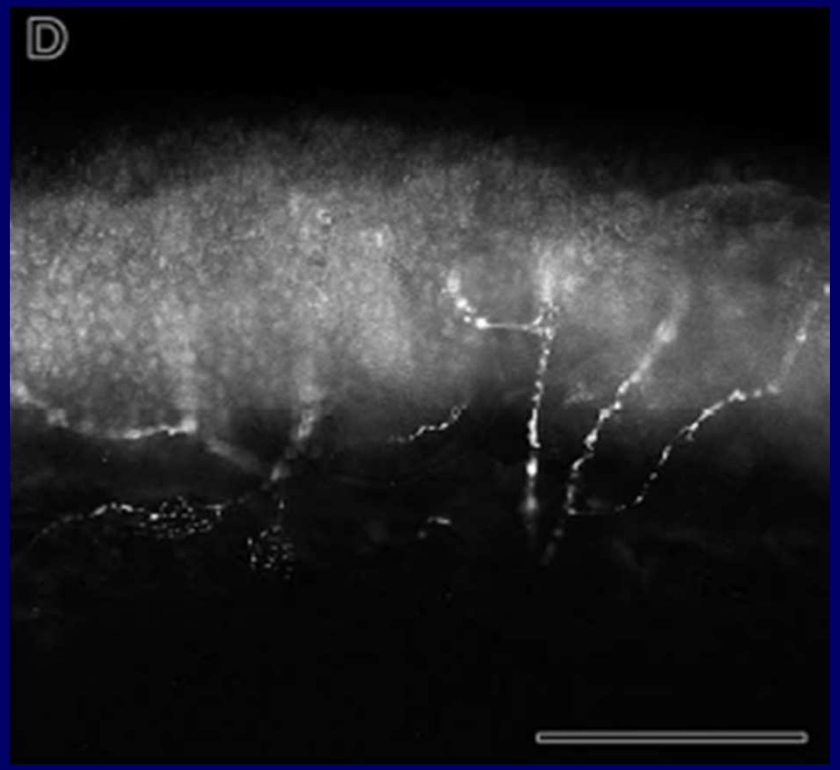
SUBSTANCE P (SP)

Substance P induces pain, vasodilation,
increase in vascular permeability,
stimulation of mast cell, B-T lymphocytes
chemoattactant for Eos (Lambiase et al 1998, 2013)

Substance P is produced by eosinophils
monocytes, macrophages, lymphocytes
and dendritic cells (Lai 1998)

SUBSTANCE P (SP)

- Is present in the cornea in physiologically relevant concentrations
- Its is a 11 amino acids peptide generally associated with intense, persistent, or chronic pain.



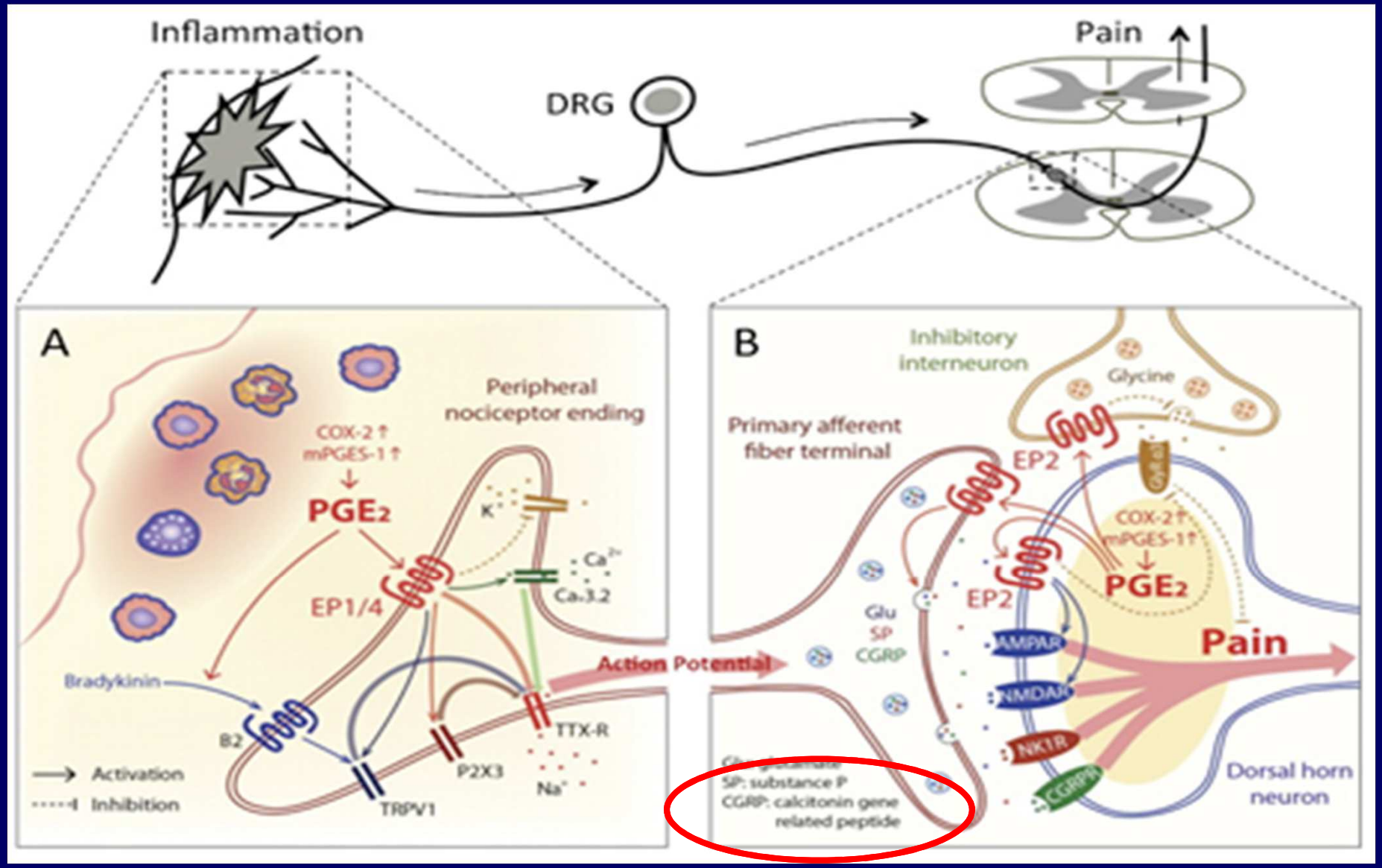
Substance P (SP) positive nerve fibers

L.J. Müller et al. / Experimental Eye Research 76 (2003) 521–542

Substance P and Pain

- Nociceptors in the damaged area initiate a sensation of pain.
- These receptors are stimulated after damage due to a release of chemicals to which they are sensitive. In the cornea, these receptors are primarily chemical sensors, but they also respond to mechanical and thermal stimulation.
- After stimulation, they send receptor potentials, which in turn trigger afferent **action potentials**.

Inflammation ➔ Pain



Investigative Ophthalmology & Visual Science, September 1997, Vol. 38, No. 10

Increased Plasma Levels of Substance P in Vernal Keratoconjunctivitis

Alessandro Lambiase,† Stefano Bonini,†‡ Alessandra Micera,* Paola Tirassa,* Laura Magrini,§ Sergio Bonini,§ and Luigi Aloe**

0.001; Table 1). Moreover, NGF levels were dramatically increased in the plasma of VKC patients (11,037 ± 10,641 pg/ml; median, 130 pg/ml; $P < 0.001$) compared with levels in the plasma of control subjects (47.5 ± 8.5 pg/ml; median, 42.5 pg/ml).

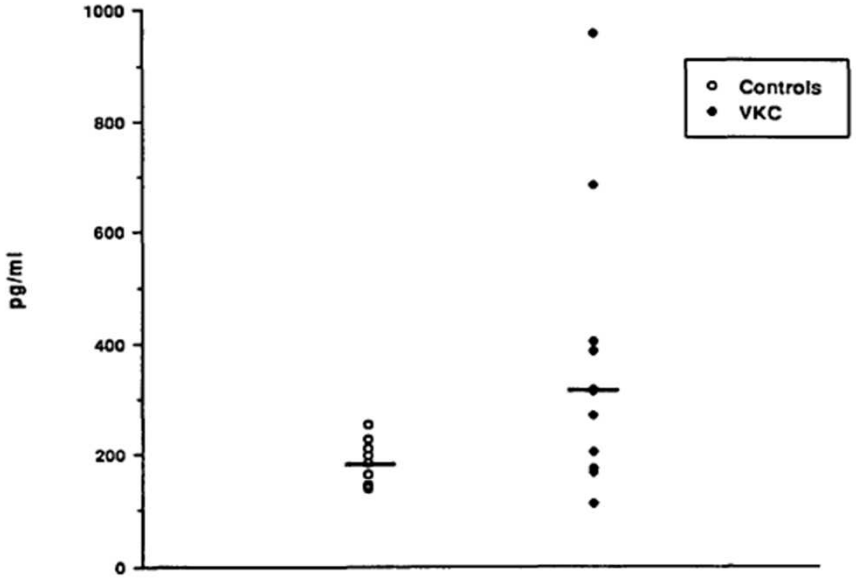


FIGURE 1. Substance P (SP) plasma concentration in vernal keratoconjunctivitis (VKC) patients and control subjects. The horizontal bars represent the medians of the values. Readings in the two groups differ significantly ($P < 0.001$).

TABLE 1. Characteristics of Patients With Vernal Keratoconjunctivitis

Patient Number	*Age (yr), Sex*	*Previous and Associated Atopic Diseases*	*Substance P (pg/ml)*	*NGF (pg/ml)*	*Total IgE (kU/l)*	*RAST Positive**	*ECP (μg/l)*	*Circulating Eosinophils (× μl)*	*Biopsy*
1	15, M	—	956	117,430	142	—	23.5	220	No
2	23, F	Asthma, rhinitis, eczema	684	2363	133	Par. off., Dph.	21.8	200	Yes
3	16, M	Rhinitis	404	121	39	Gram., Par. off.	47.5	540	Yes
4	7, M	—	387	77	44	—	53.9	600	Yes
5	21, F	Rhinitis	318	130	37	—	8	100	Yes
6	4, F	—	314	114	15	—	ND	ND	No
7	8, M	—	270	701	13	Gram., Par. off.	32.4	290	Yes
8	8, M	Eczema	203	10	407	Gram., Par. off., Dph.	108.6	800	Yes
9	32, F	—	174	167	196	Dph.	ND	ND	Yes
10	10, F	—	165	274	30	Par. off., Dph.	ND	ND	Yes
11	7, M	Asthma	110	20	224	Gram., Par. off., Dph.	67.3	300	No

ECP = cosinophil cationic protein; NGF = nerve growth factor; ND = not done.

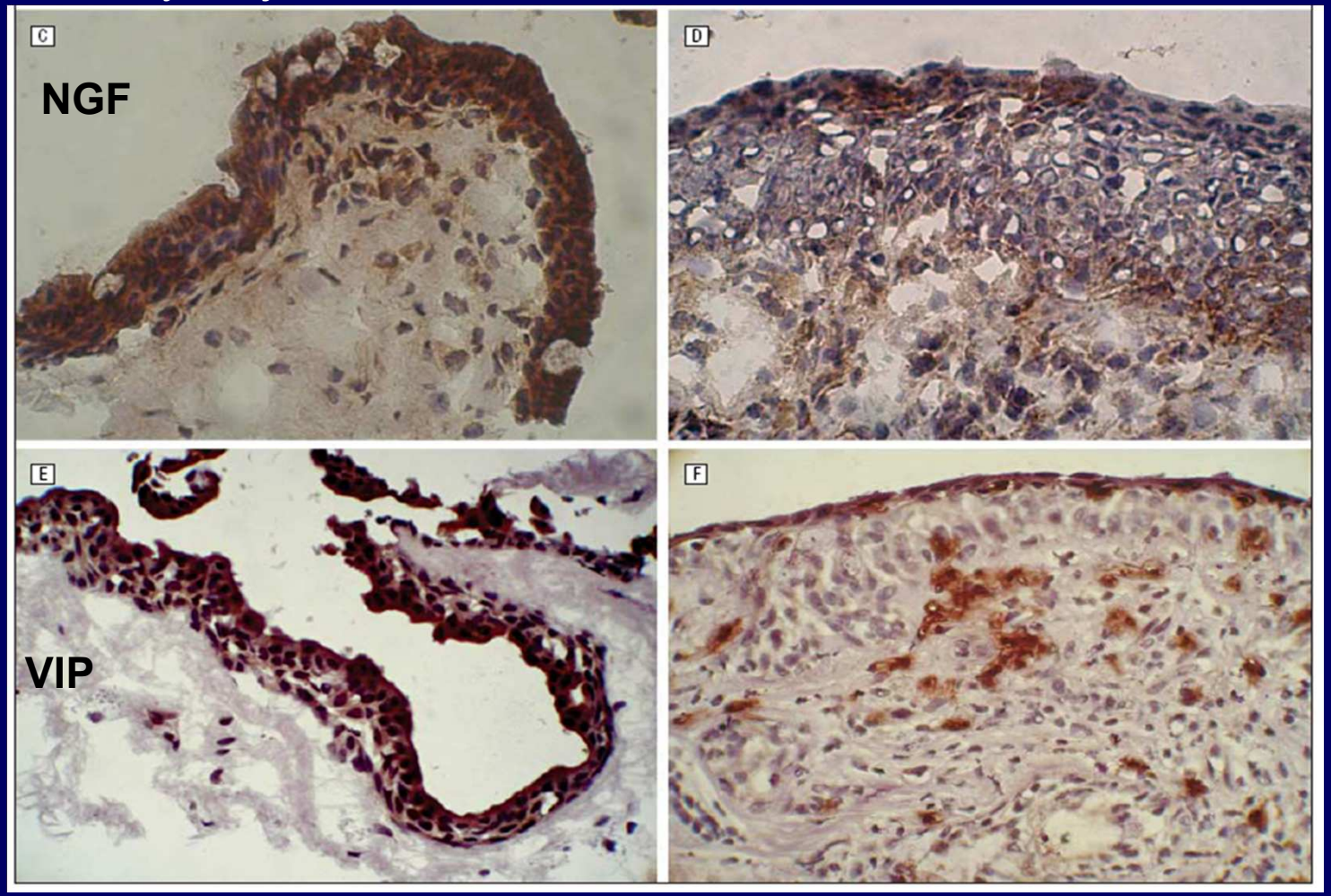
Altered Expression of Neurotransmitter Receptors and Neuromediators in Vernal Keratoconjunctivitis

Laura Motterle, MD; Yolanda Diebold, PhD; Amalia Enríquez de Salamanca, PhD; Victoria Saez, BS; Carmen Garcia-Vazquez, BS; Michael E. Stern, PhD; Margarita Calonge, MD; Andrea Leonardi, MD

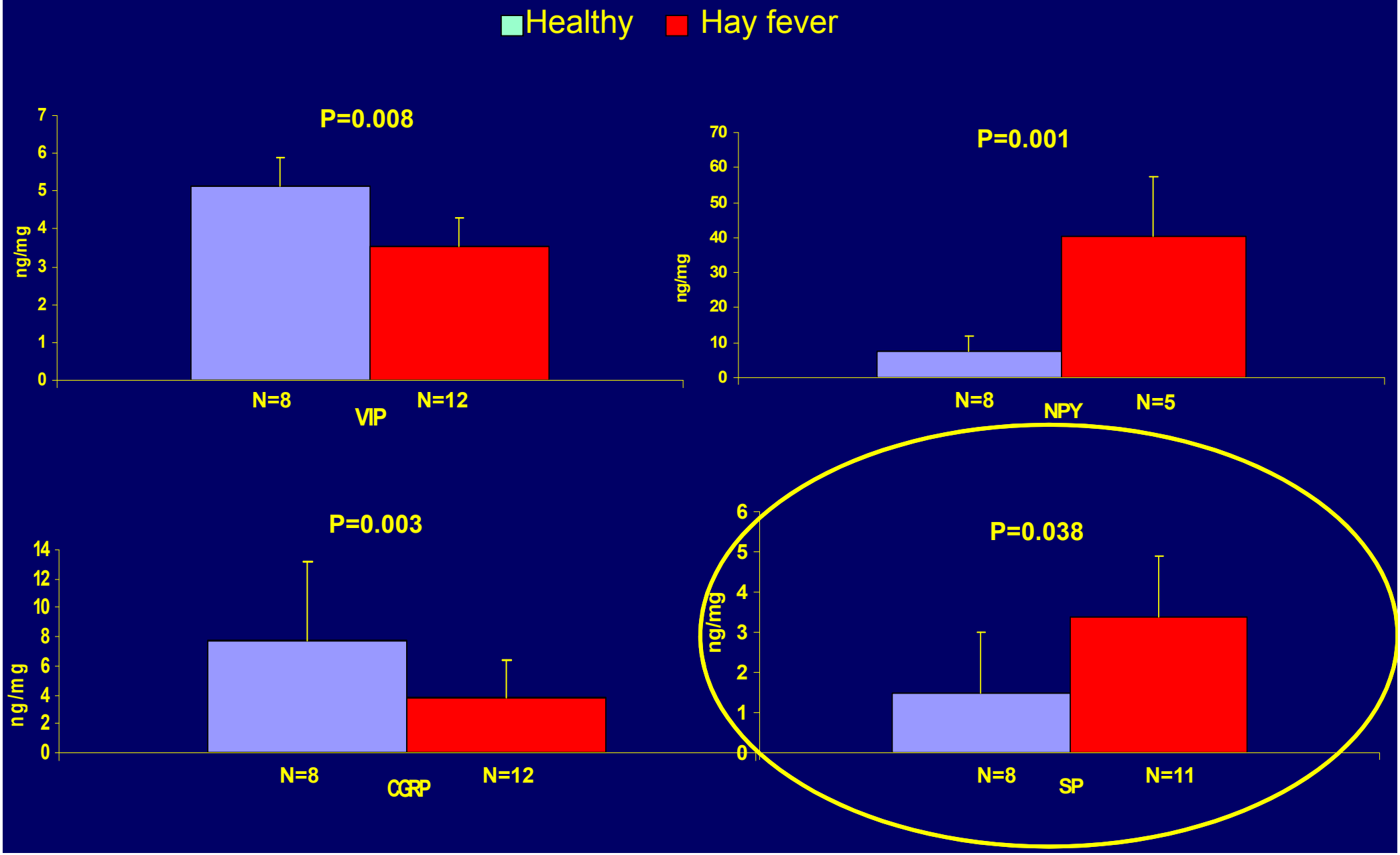
Arch Ophthalmol. 2006;124:462-468

Healthy conjunctiva

VKC conjunctiva



Neuropeptides levels in tears of patients with hay fever



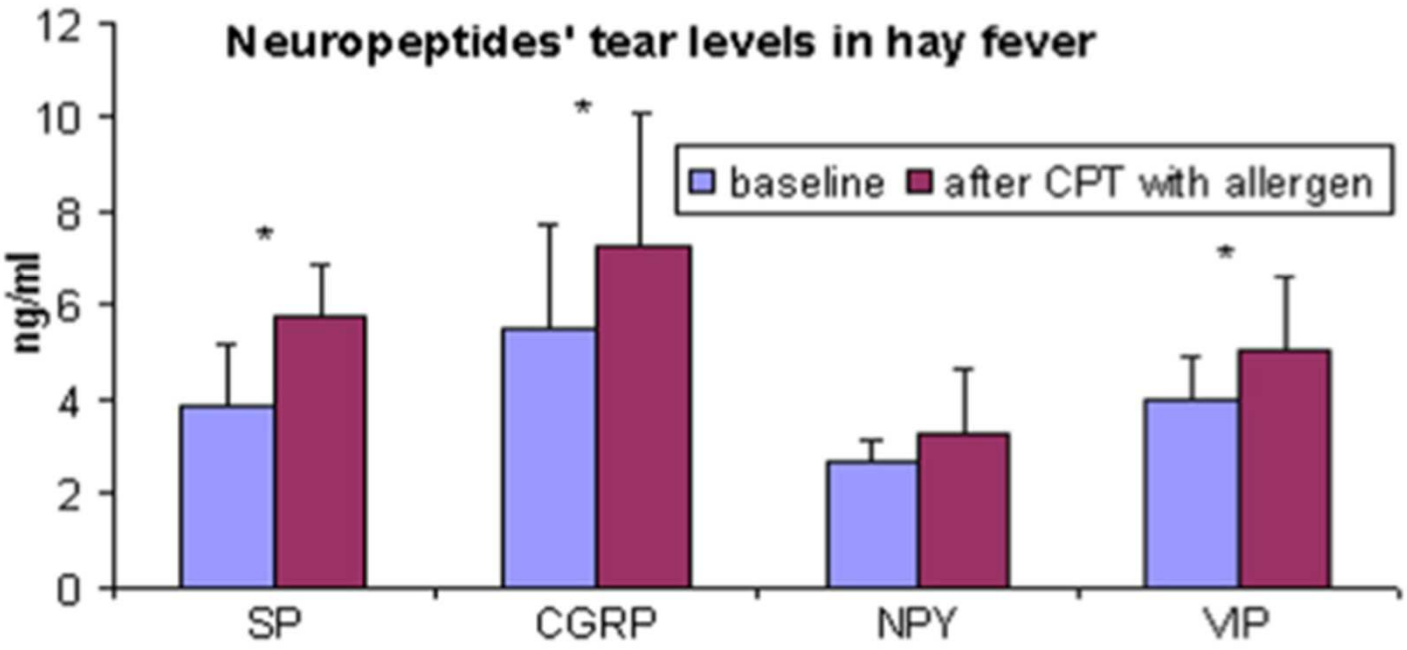


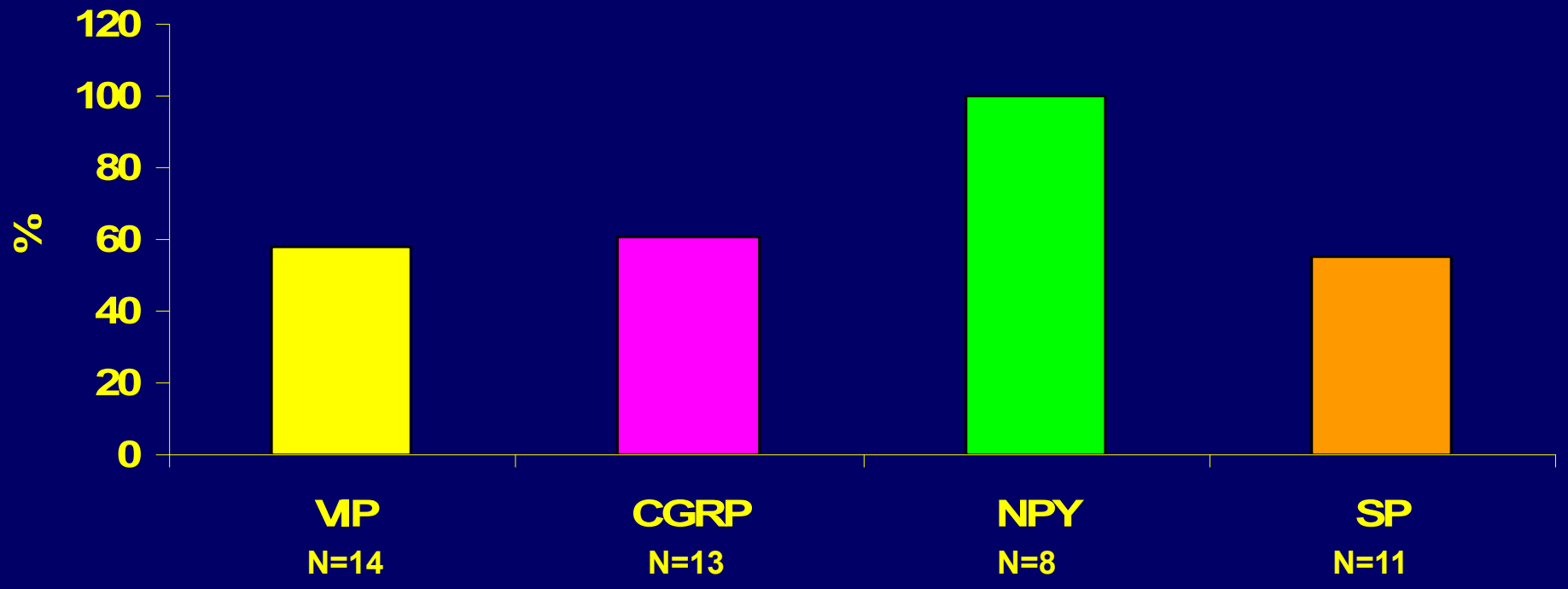
Figure 2. In allergic patients SP, CGRP, and VIP but not NPY tear levels significantly increase after a positive CPT.

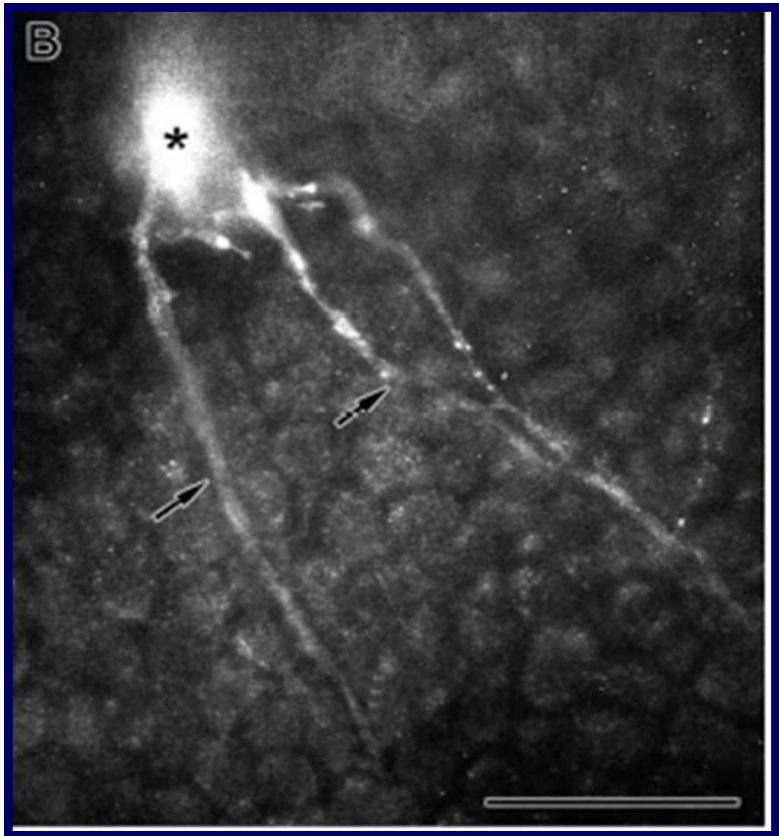
Table 1 Treatment with preservative-free cromolyn sodium 4%-chlorpheniramine maleate 0.2% eye drops inhibited the local release of substance P, calcitonine gene-related peptide, neuropeptide Y, and vasoactive intestinal peptide after conjunctival provocation test

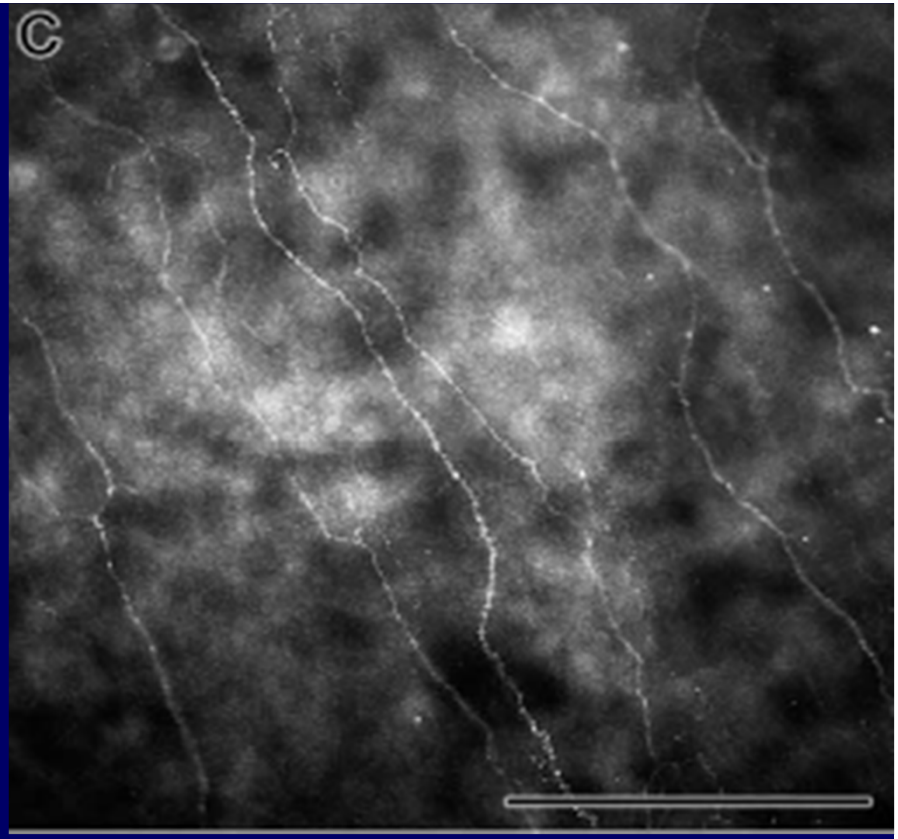
	Baseline (visit 1)			After treatment with cromolyn sodium (visit 2)		
Neuropeptides	Before CPT	After CPT	*P* value	Before CPT	After CPT	*P* value
Substance P (ng/ml)	3.2 ± 2	5.1 ± 2.3	0.03	3.2 ± 2.3	3.7 ± 1.4	NSS
CGRP (ng/ml)	3.9 ± 1.5	6.2 ± 2.4	0.04	5 ± 1.5	4.9 ± 2.5	NSS
NPY (ng/ml)	2.8 ± 0.4	3.7 ± 1.5	NSS	3.2 ± 1.2	4 ± 1	NSS
VIP (ng/ml)	3.6 ± 0.6	5.2 ± 1.7	0.03	3.7 ± 0.6	4.2 ± 0.8	NSS

CGRP, calcitonine gene-related peptide; CPT, conjunctival provocation test; NPY, neuropeptide Y; NSS, non statistically significant; VIP, vasoactive intestinal peptide.

Percentage of patients showing an increase of Neuropeptides levels in tears after specific CPT



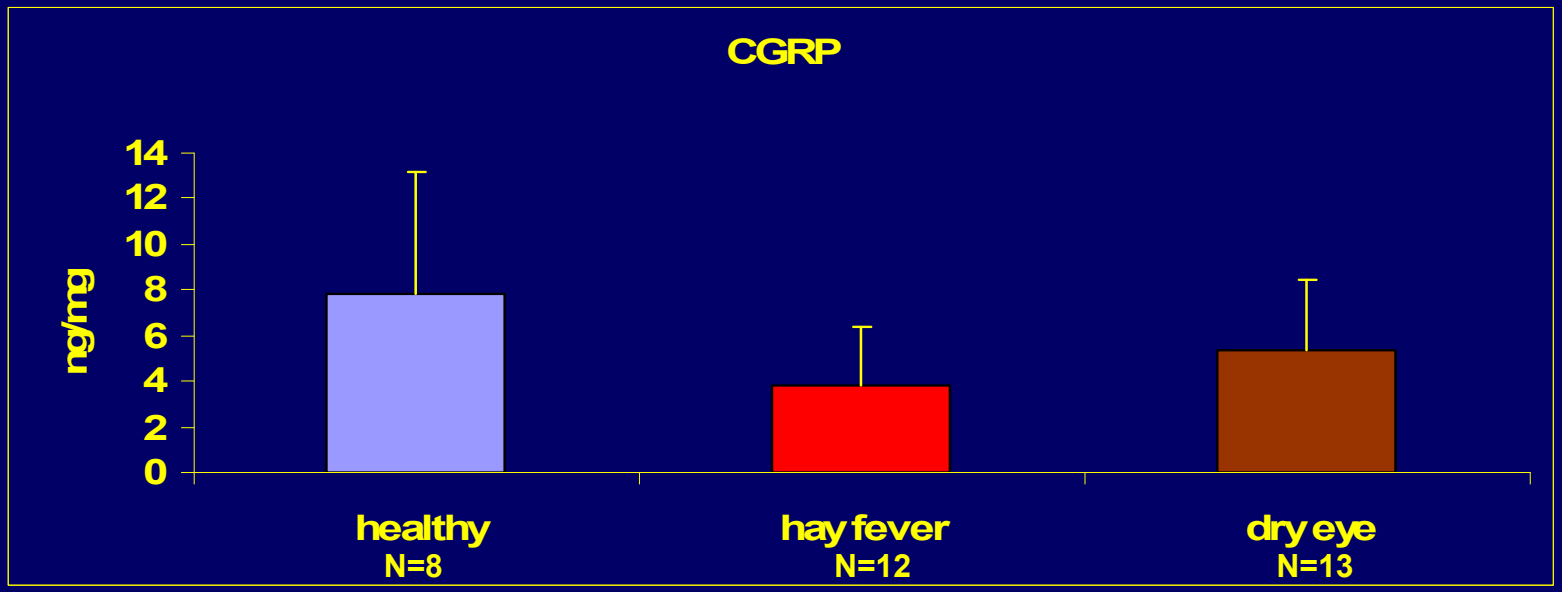


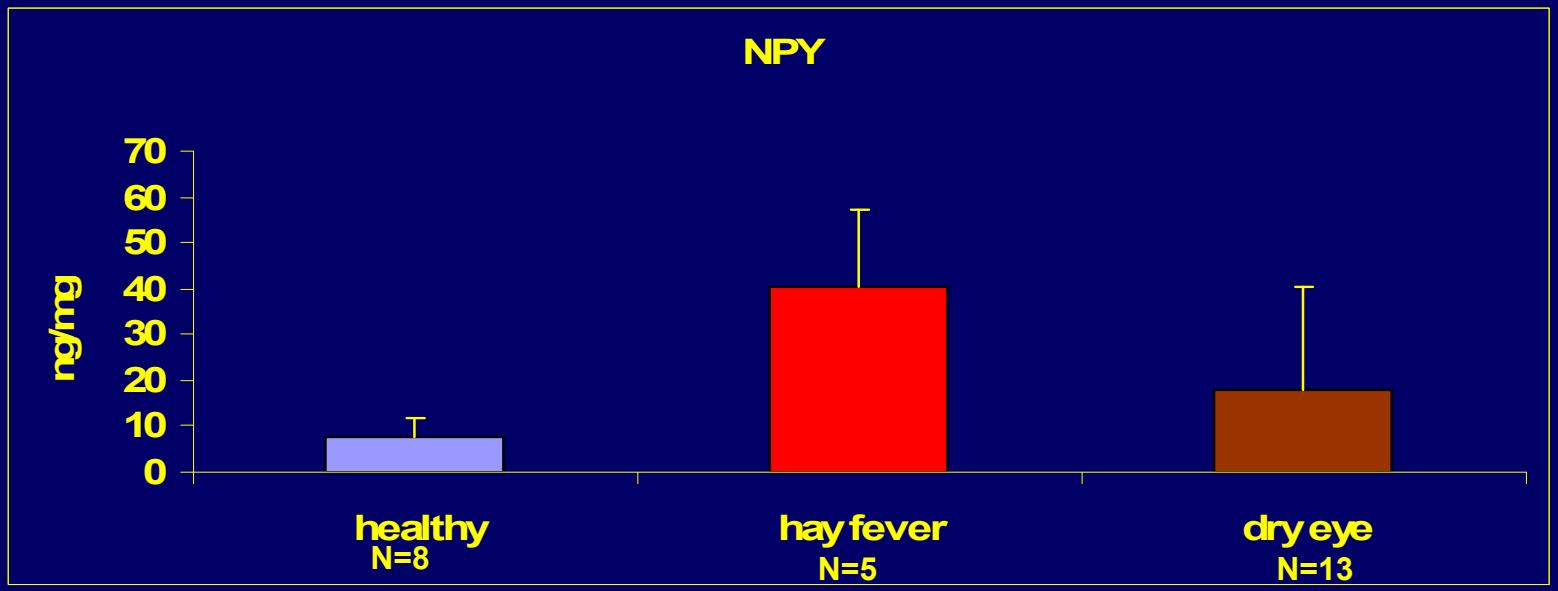


CGRP positive nerve fibers in the subbasal plexus

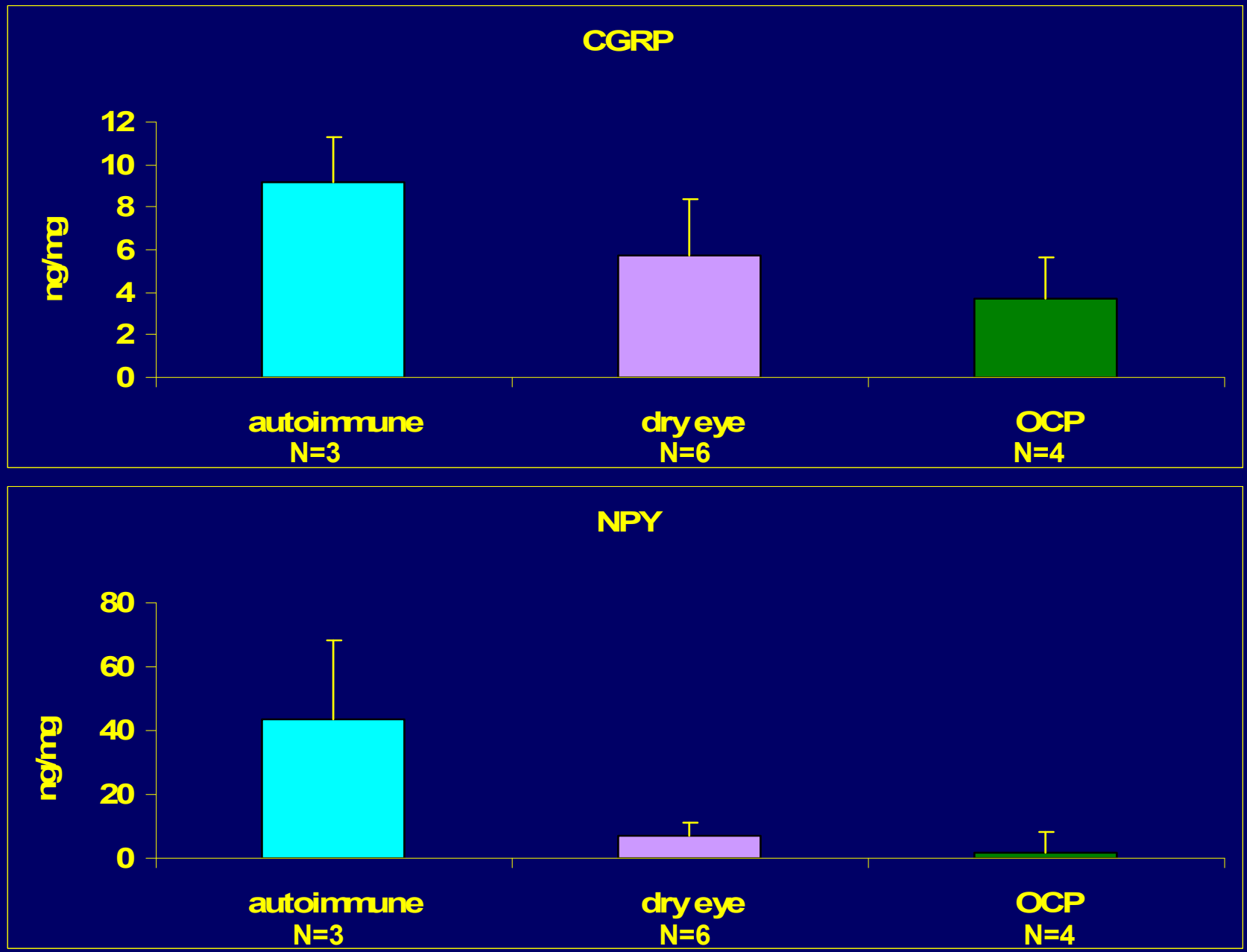
L.J. Müller et al. / Experimental Eye Research 76 (2003) 521–542

Tear CGRP and NPY levels in patients with hay fever and dry eye





Tear CGRP and NPY levels in patients with dry eye



Conclusions

➔ Sensory neuropeptides are the main actors of neurogenic pain during ocular surface inflammation.

➔ Possible strategy for pain management at the (inflammed) ocular surface?

- Depletion of neuropeptides: topically applied capsaicin has been successfully used to reduced periocular post-herpetic pain induced by substance P released by damaged sensory nerve terminals.
- However, capsaicin may reduce corneal sensitivity ➔ reduce inflammation to recude of pain…

Wide spectrum of anti-inflammatory drugs available

- Steroids: for chronic use loteprednol preferable (better IOP control)

- Ciclosporin-a: FDA-approved, not yet approved by EMA

- Tacrolimus: already used for uveitis, in clinical trials for dry eye

- NSAIDs (better if preservative free to reduce toxicity in chronic use)

To avoid ocular surface toxicity ➔ systemic antibiotics available

Eur J Clin Pharmacol (2012) 68:479–503
DOI 10.1007/s00228-011-1161-x

REVIEW ARTICLE

Macrolides: from in vitro anti-inflammatory and immunomodulatory properties to clinical practice

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Brief communication

Azithromycin decreases MMP-9 expression in the airways of lung transplant recipients ☆

Stijn E. Verleden [a], Jennifer Vandooren [b], Robin Vos [a], Stijn Willems [a], Lieven J. Dupont [a]

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Molecular and Cellular Pharmacology

Azithromycin fails to reduce inflammation in cystic fibrosis airway epithelial cells

Vinciane Saint-Criq [a,b], Manon Ruffin [a,b], Carine Rebeyrol [a,b], Loïc Guillot [a,b], Jacky Jacquot [a,b], Annick Clement [a,b,c], Olivier Tabary [a,b,*]

Macrolides Reduce Levels of: IL-6, IL-8, TNF-alfa