Department of Ophthalmology and Visual Science
Chiba University Graduate School of Medicine

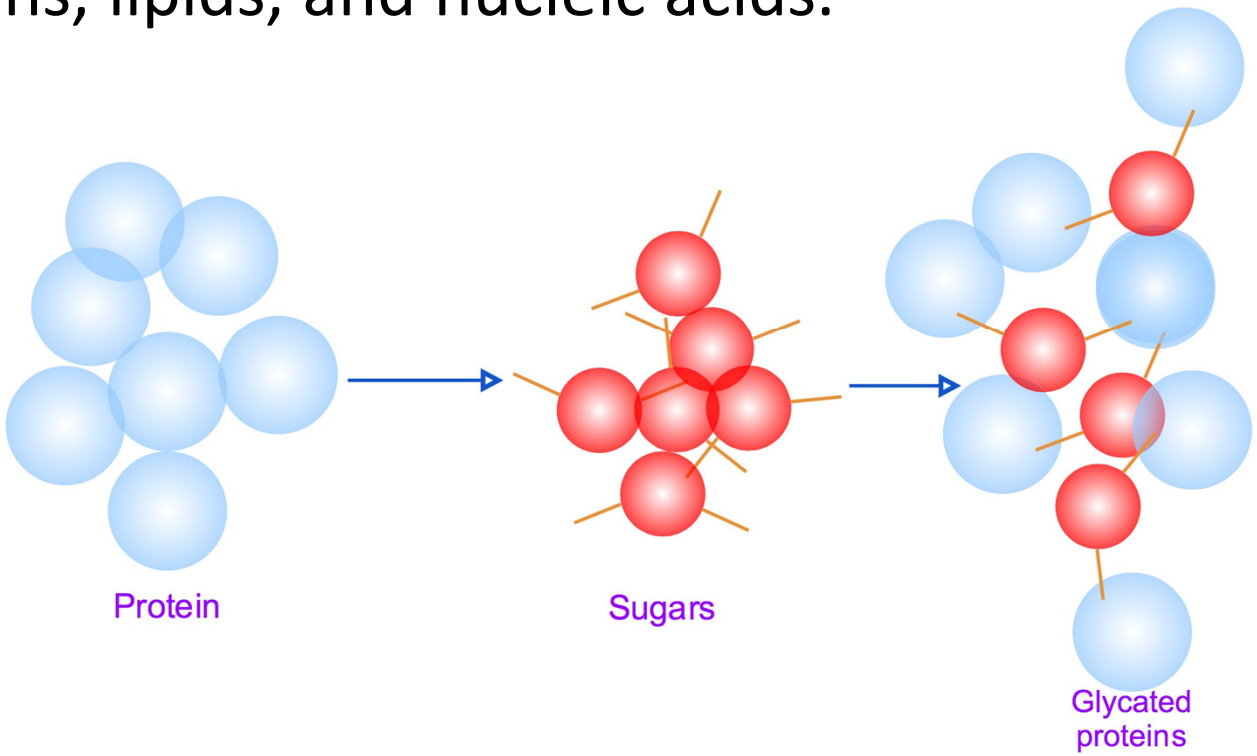
NF-kB and SP1 expression in AGEs exposed retina and influence of different neurotrophic factors on it

Guzel Bikbova, Toshiyuki Oshitari, Shuichi Yamamoto

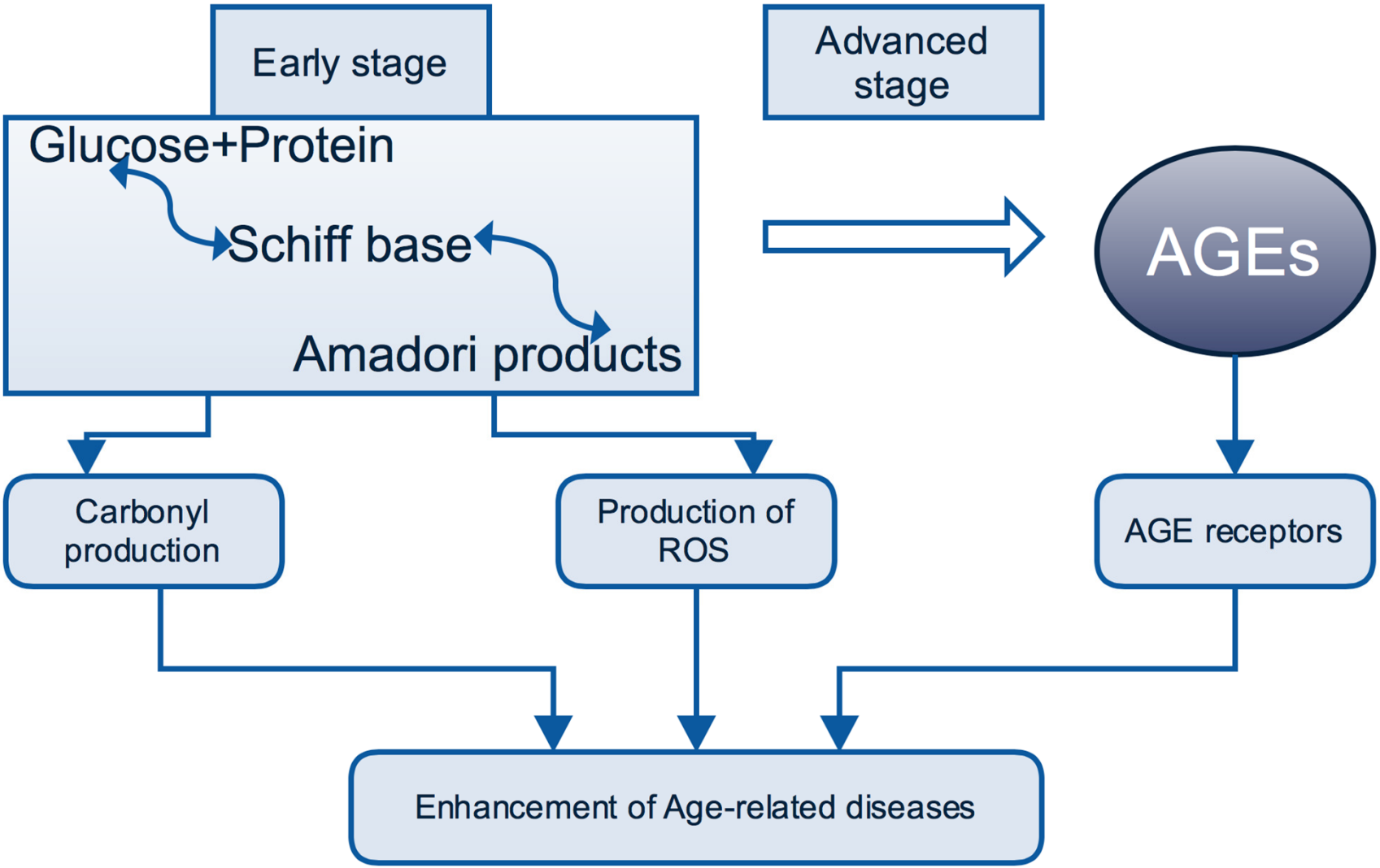
Valencia 2015

INTRODUCTION

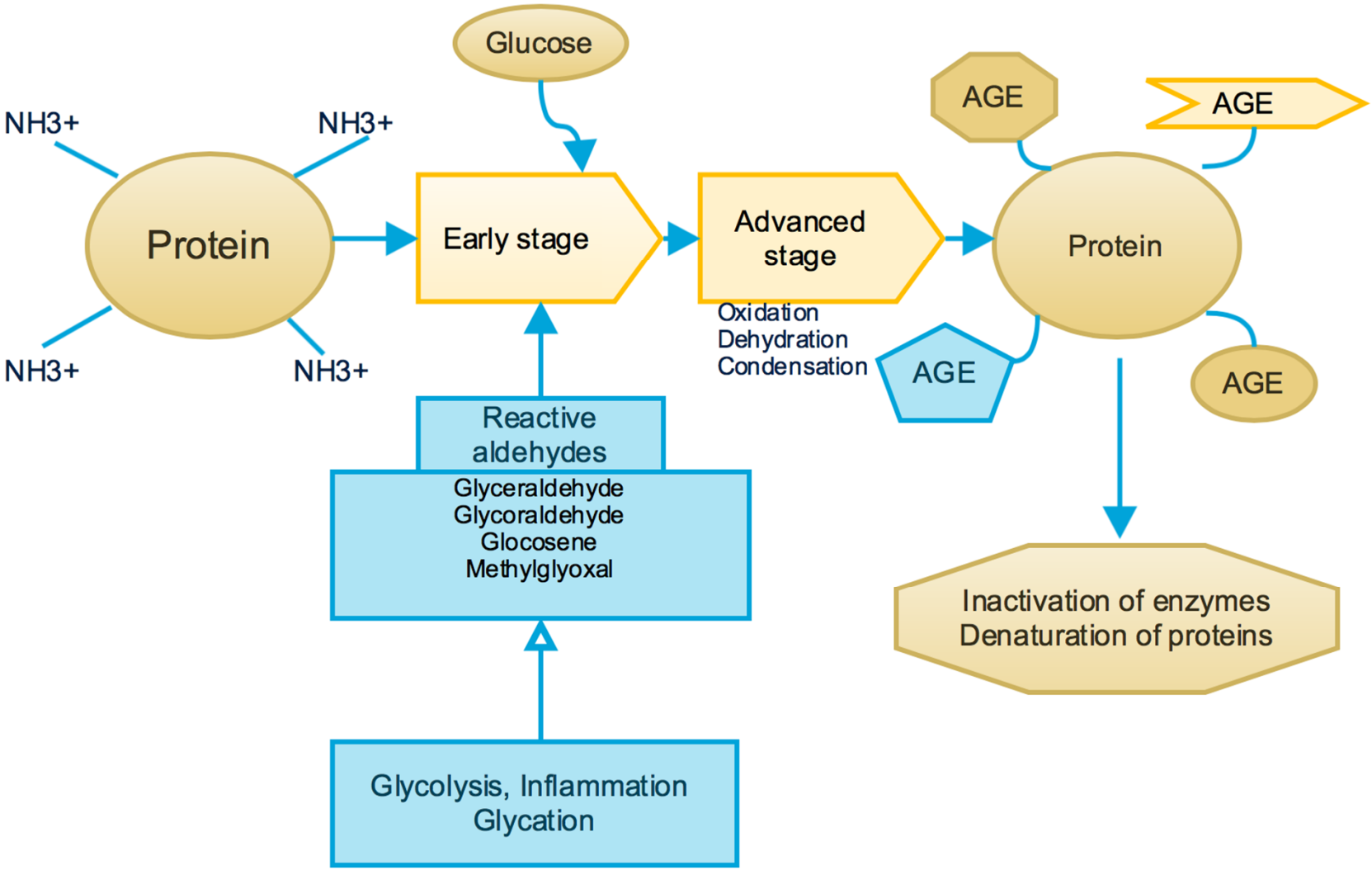
Advanced glycation end products - a heterogeneous group of molecules formed from the nonenzymatic reaction of reducing sugars with free amino groups of proteins, lipids, and nucleic acids.



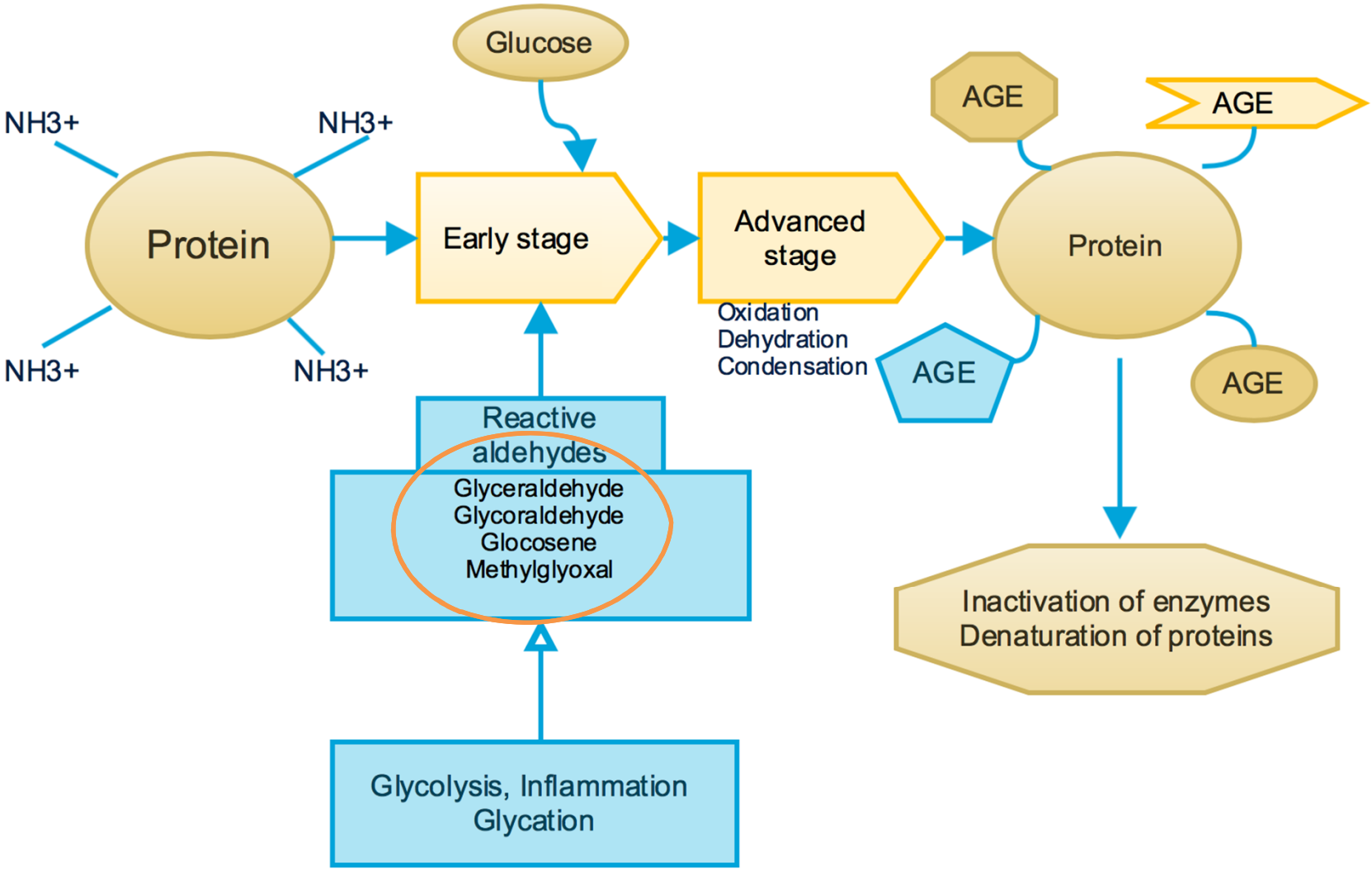
MAILLARD REACTION



Generation of intermediate aldehydes and protein degeneration involved in AGE formation.



Generation of intermediate aldehydes and protein degeneration involved in AGE formation.



RECEPTORS FOR AGEs

- Currently identified cell surface receptors which recognize AGEs include
  - RAGE;
  - macrophage scavenger receptor class A (SR-A) (Suzuki, et al,1997);
  - SR-B (SR-B1 and CD36);
  - lectin-like oxidized low density lipoprotein receptor-1 (LOX-1) (Jano et al, 2002) ;
  - galectin-3 complex (Vlassara et al, 1995);
  - fasciclin , epidermal growth factor (EGF)-like, laminin-type EGF-like, and link domain-containing scavenger receptors-1 and -2 (FEEL-1 and -2);
  - megalin (Saito et al,2003);
  - toll-like receptor 4 (TLR4).

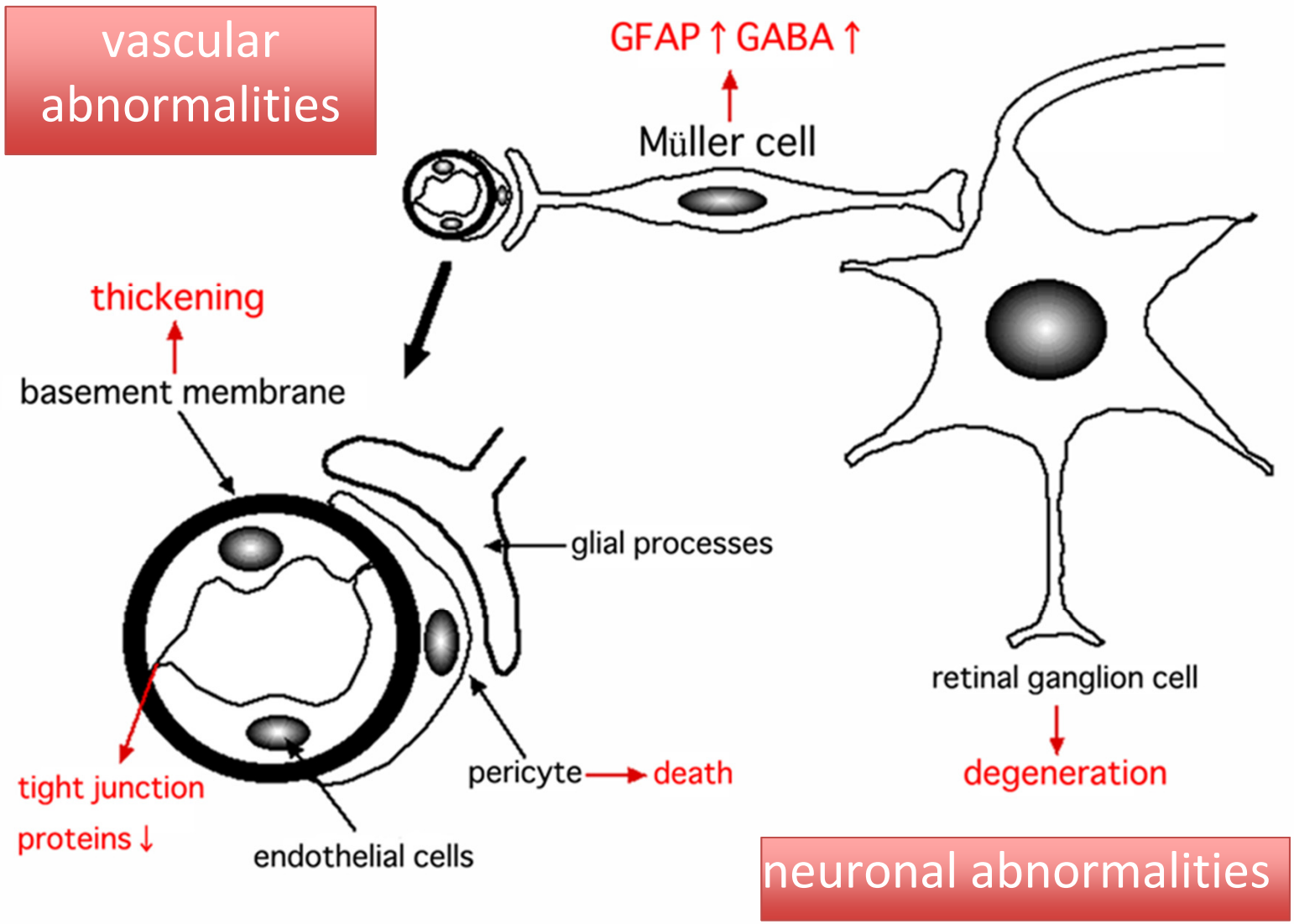
INTRODUCTION

- Tanaka et al. 2000, found that AGEs can activate the *RAGE* gene through NFkB and SP1, causing enhanced AGE-RAGE interactions in human vascular endothelial cells.
- This activation can exacerbate diabetic microvasculopathy.

INTRODUCTION

- A typical intracellular signaling pathway of RAGE involves formation of intracellular oxidation stress and activation of transcription factor NFκB.
- Specificity protein 1 (SP1) is a transcription factor that either activates or represses transcription in response to physiological and pathological stimuli. It regulates the expression of a large number of genes involved in a variety of processes such as cell growth, apoptosis, differentiation, and immune responses.

Pathogenesis of the early phase DR

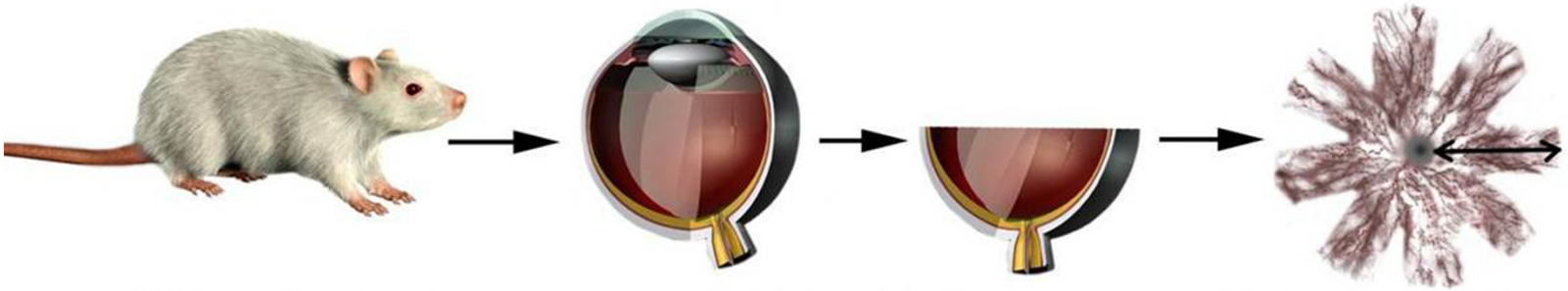


Oshitari et al. Vasc Health Risk Manag 2008

PURPOSE

- To determined the neuroprotective and regenerative effects of four neurotrophic factors, namely, NT-4, HGF, GDNF, and TUDCA in high dose of AGE exposed retinas.
- To examined whether the expressions of NF-kB and SP1 were correlated with the neuroprotective and regenerative effects of different neurotrophic factors in high dose of AGEs exposed rat retinas.

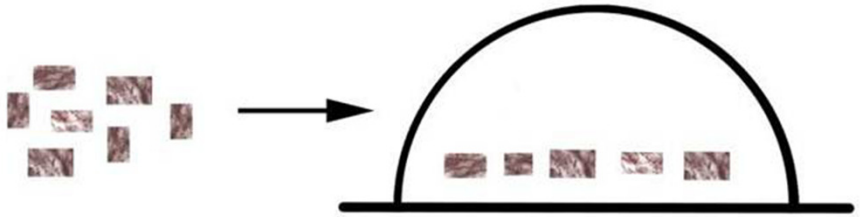
3D CULTURE



kill the animals under CO_2 inhalation

isolation of retina

retinal explants (0.4 mm square) where prepeared 1.5 mm from the disc

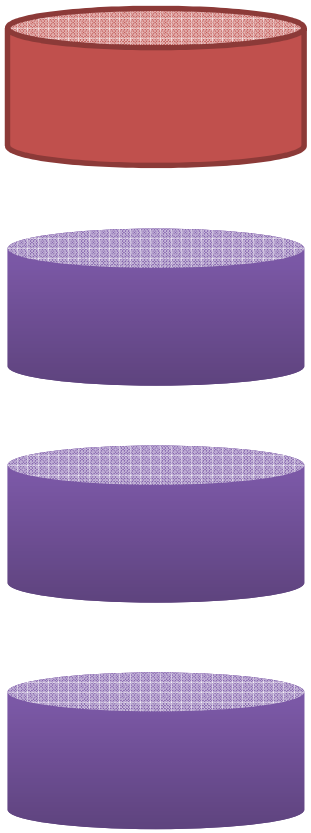


fix in collagen gel and culture in serum free media

serum free media

minimum essential medium
glucose 2.7mg/ml
insulin 5 µ g/ml
bovine serum albumin 792 µ g/ml
Na_2SeO_3 5.2mg/ml
$NaHCO_3$ 3.7mg/ml
HEPES 3.6mg/ml

Experimental Design



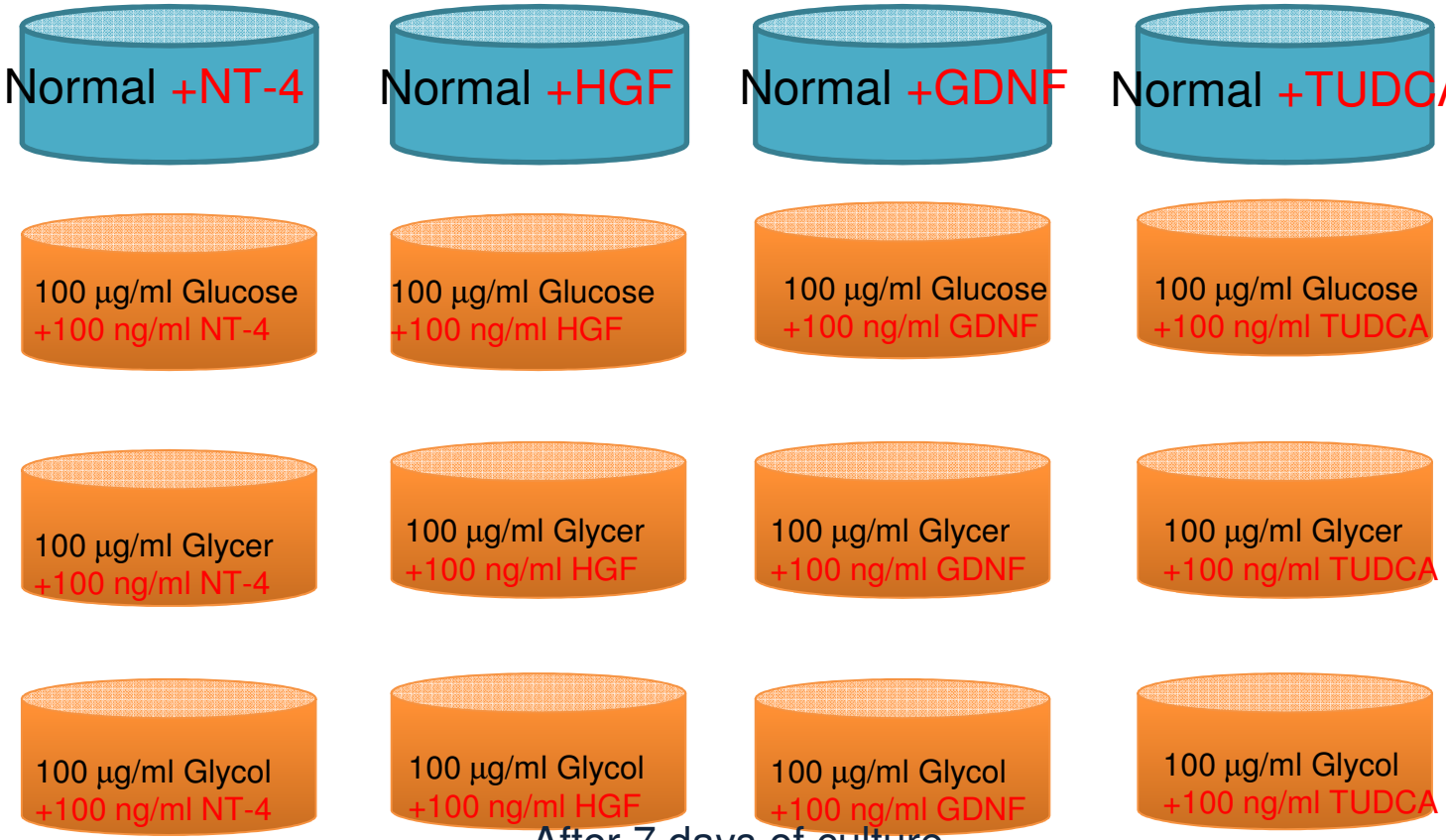
Normal

100 μg/ml Glucose-AGE-BSA

100 μg/ml Glycolaldehyde-AGE-BSA

100 μg/ml Glyceraldehyde-AGE-BSA

Experimental Design



After 7 days of culture

↓

Count numbers of neurites

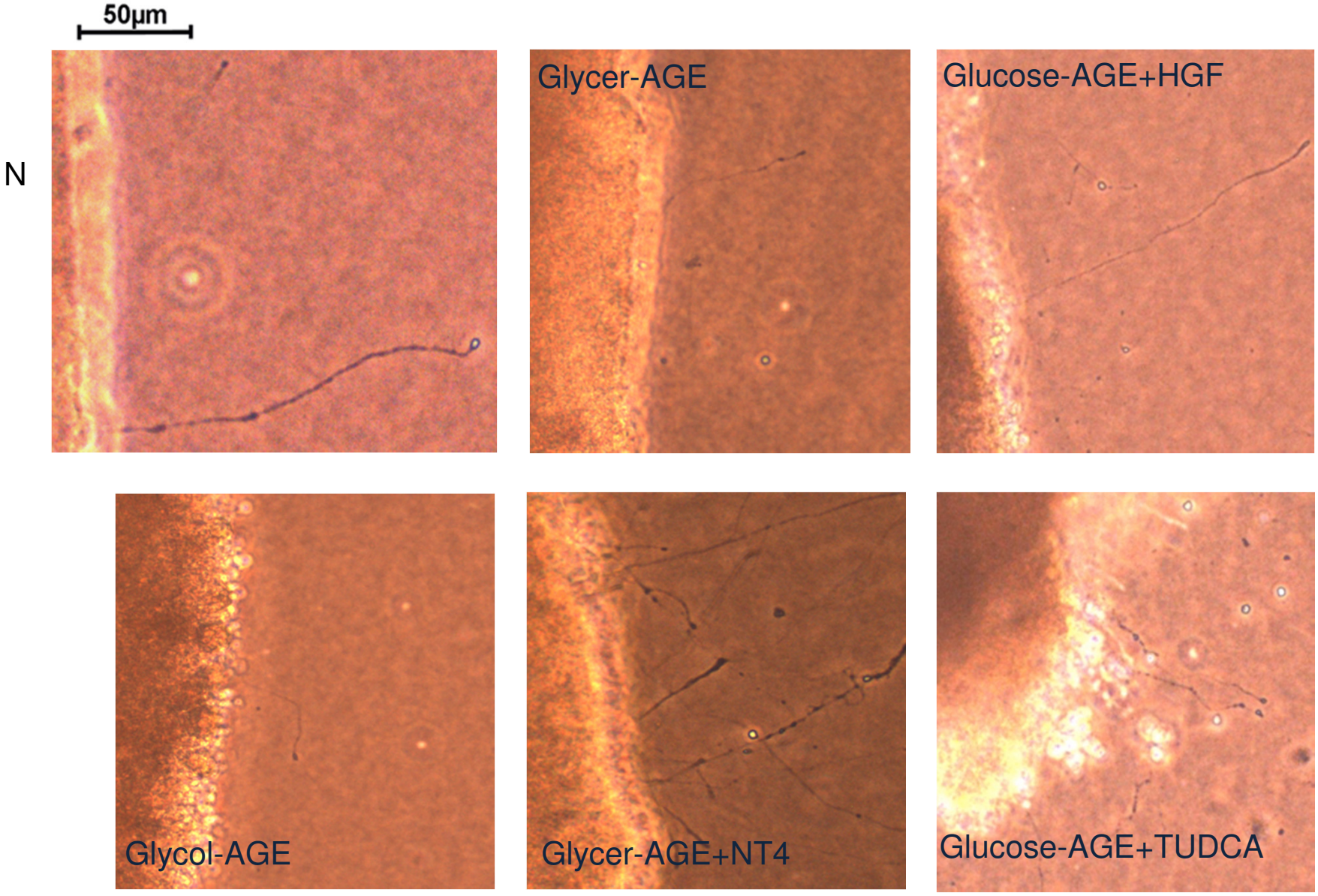
↓

Fix explants/prepare cryosections

↓

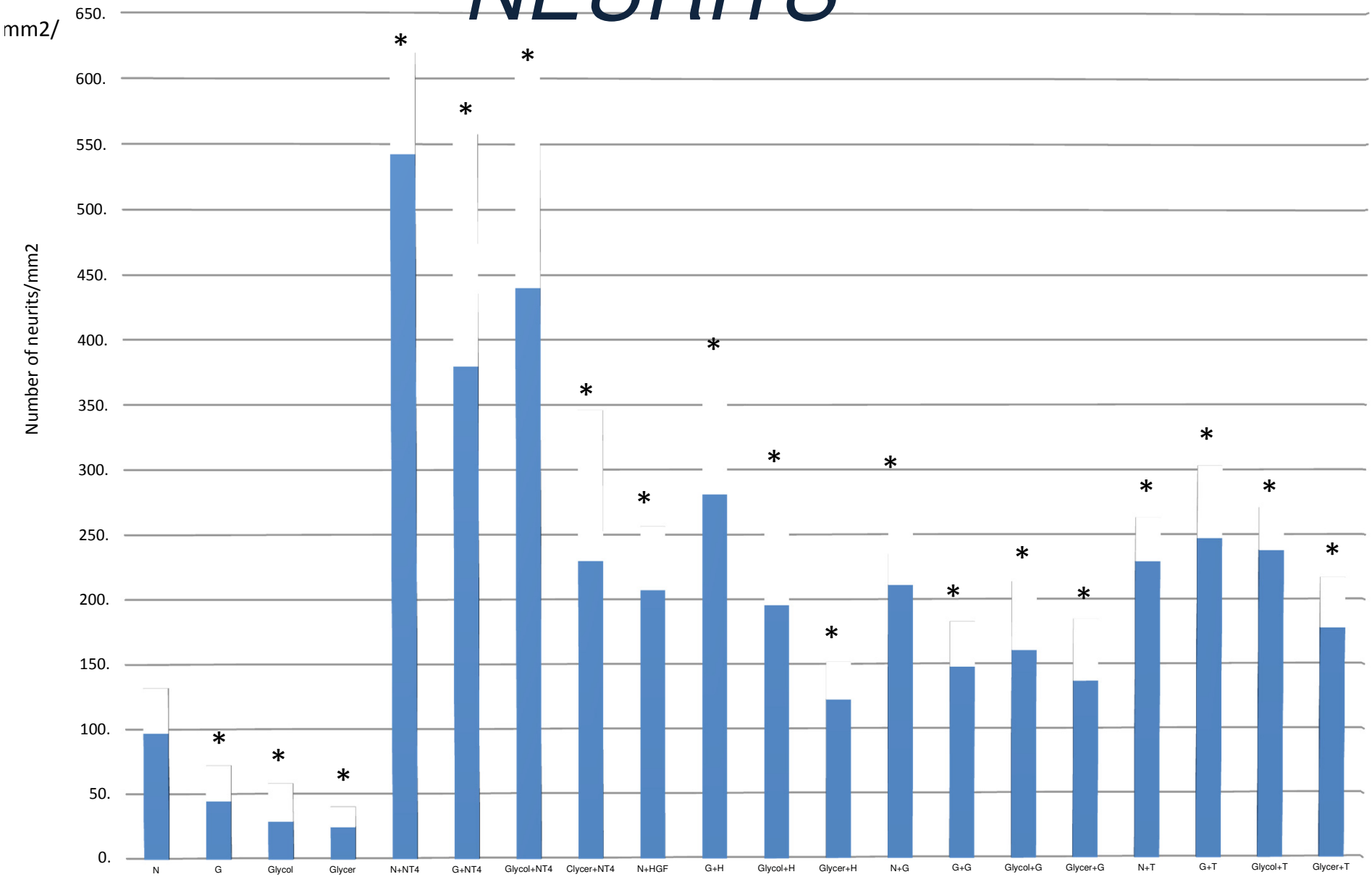
Perform TUNEL staining
SP1, NF-kB immunostaining

REGENERATED NEURITS



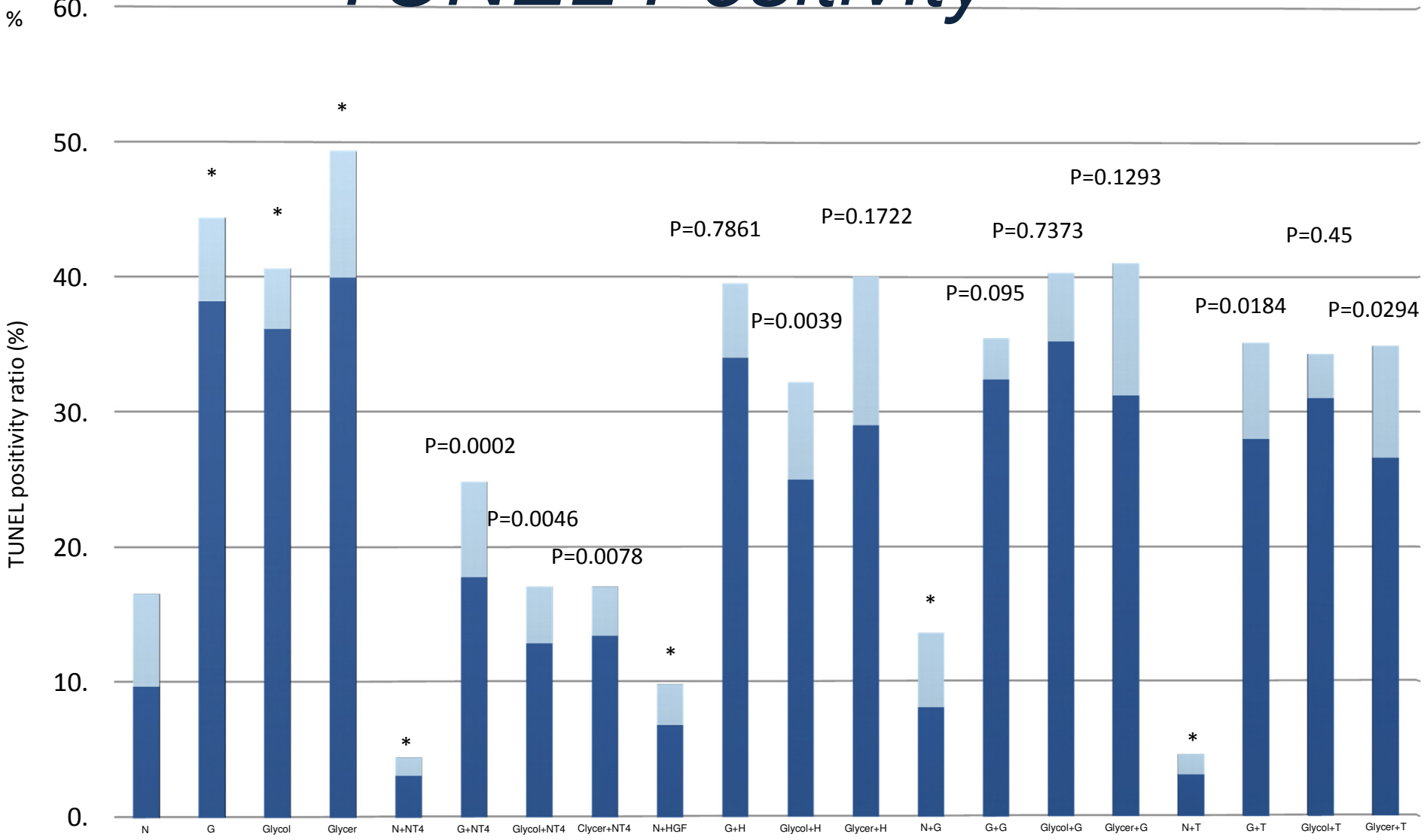
*- P<0.0001

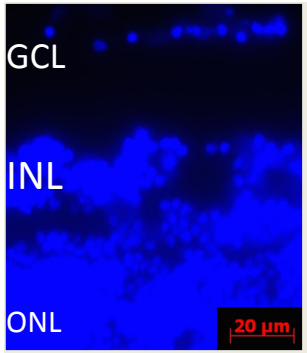
NEURITS



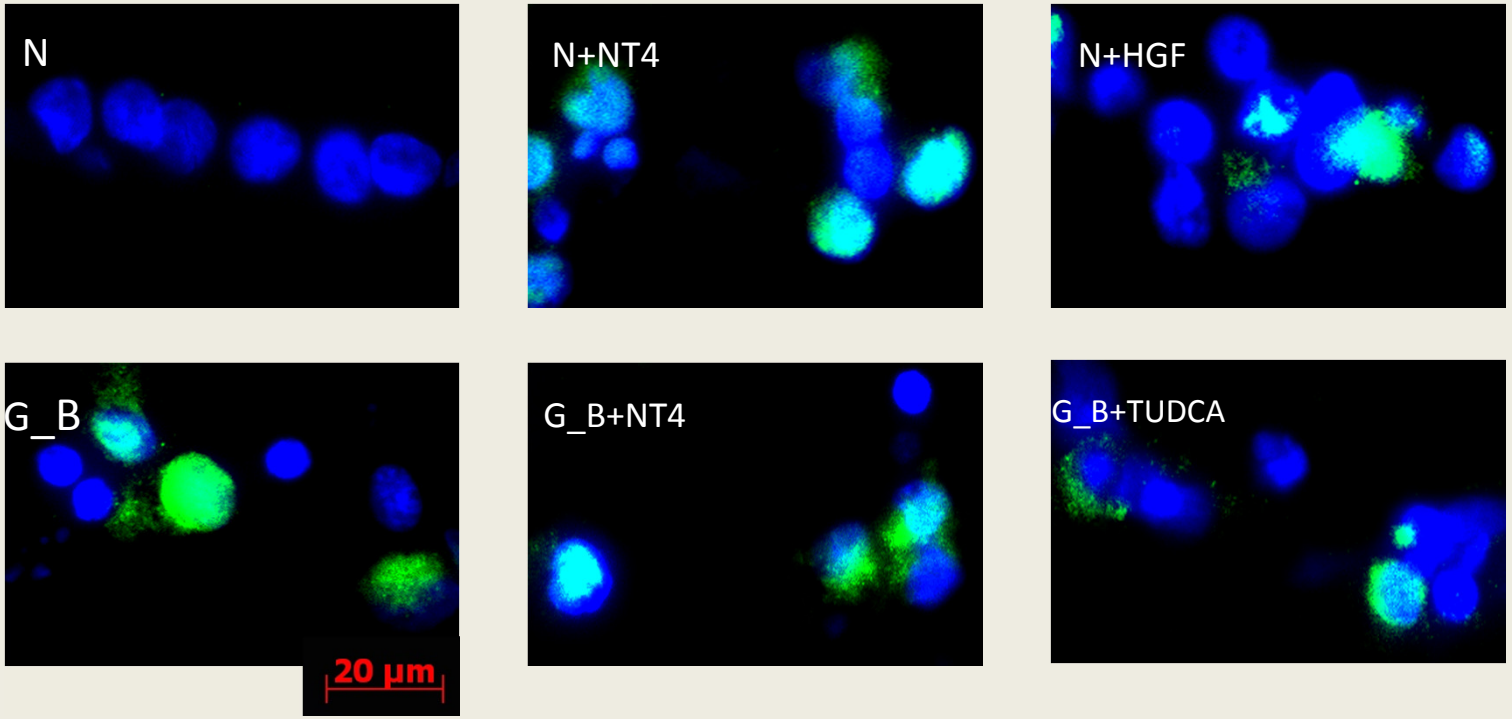
*- P<0.0001

TUNEL Positivity



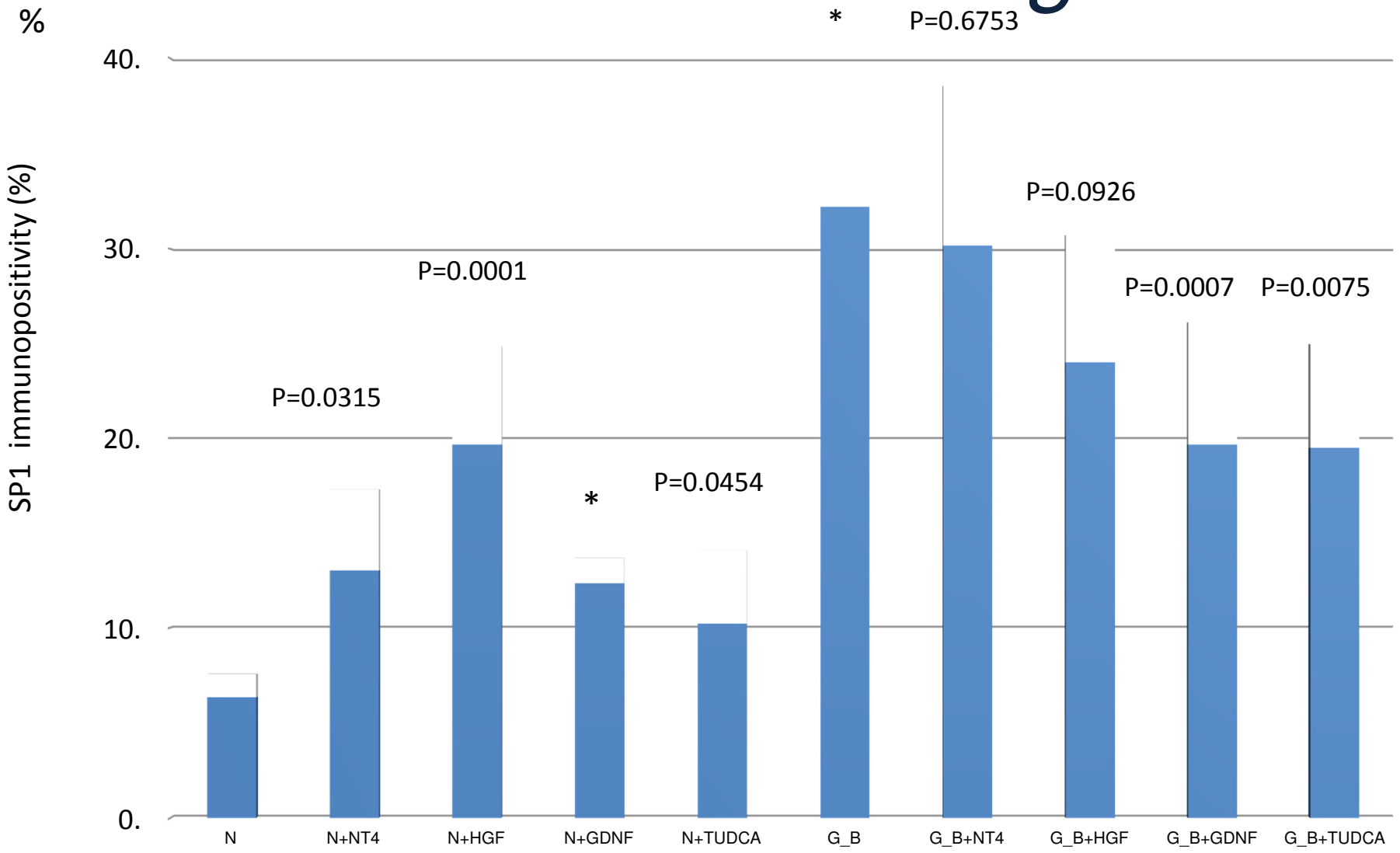


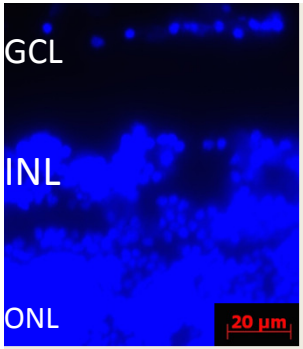
SP1 immunostaining



*- P<0.0001

SP1 immunostaining



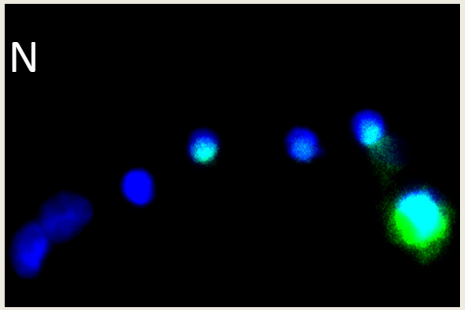


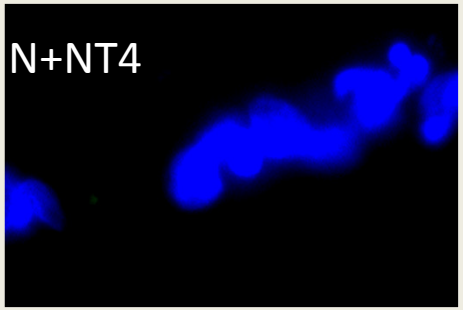
NF-kB immunostaining

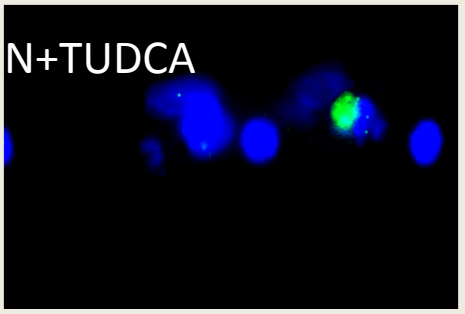
B

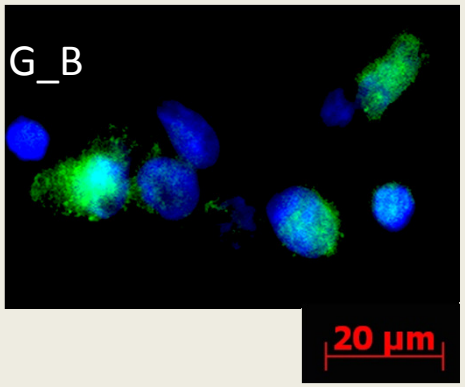
C

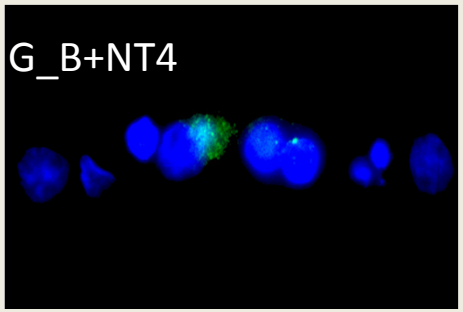
A

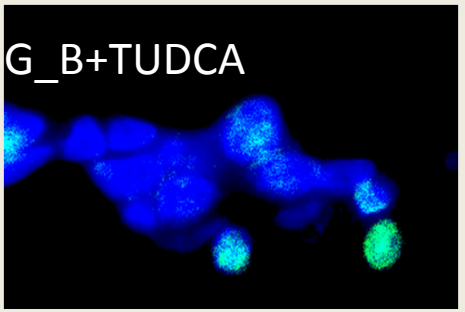






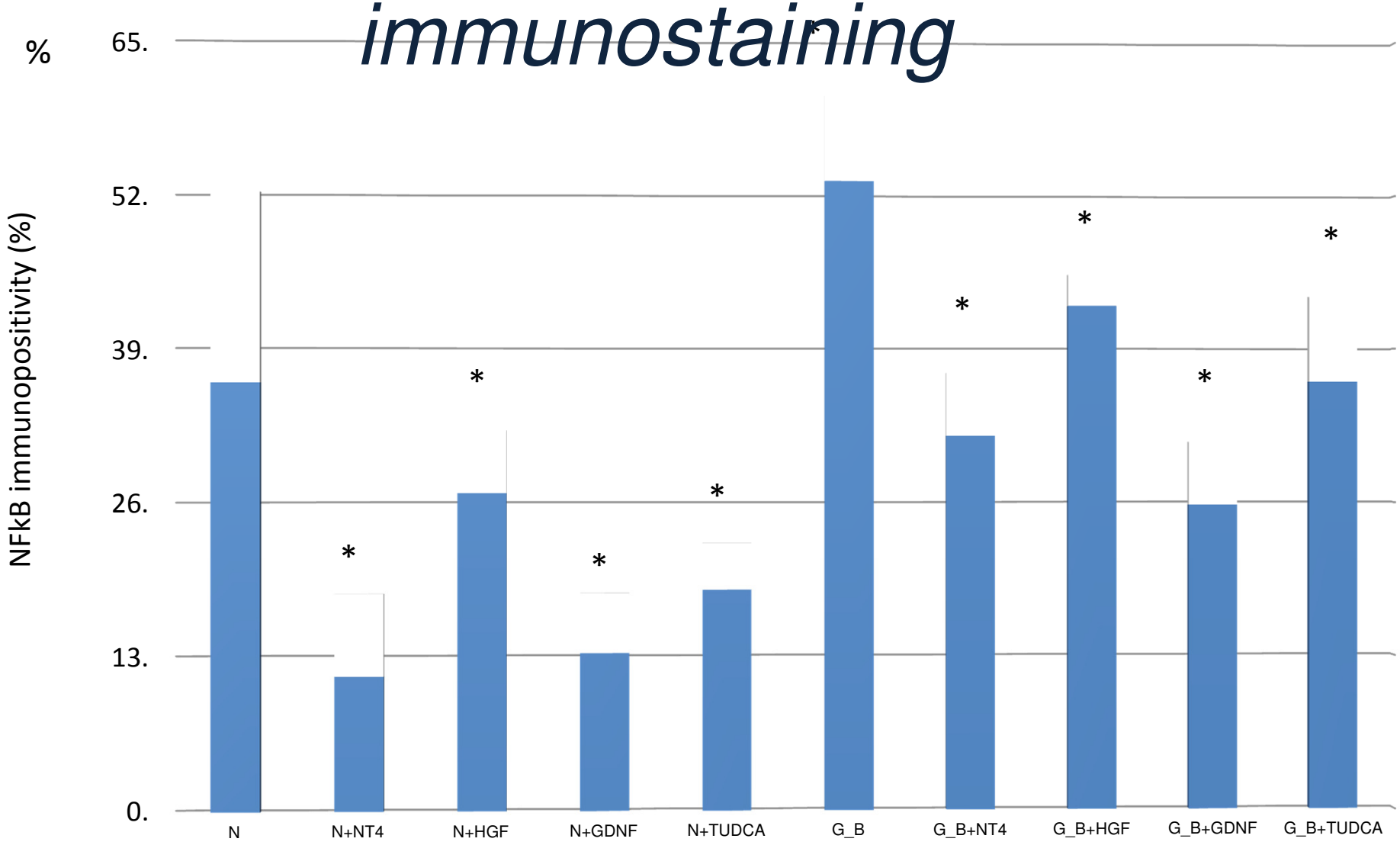


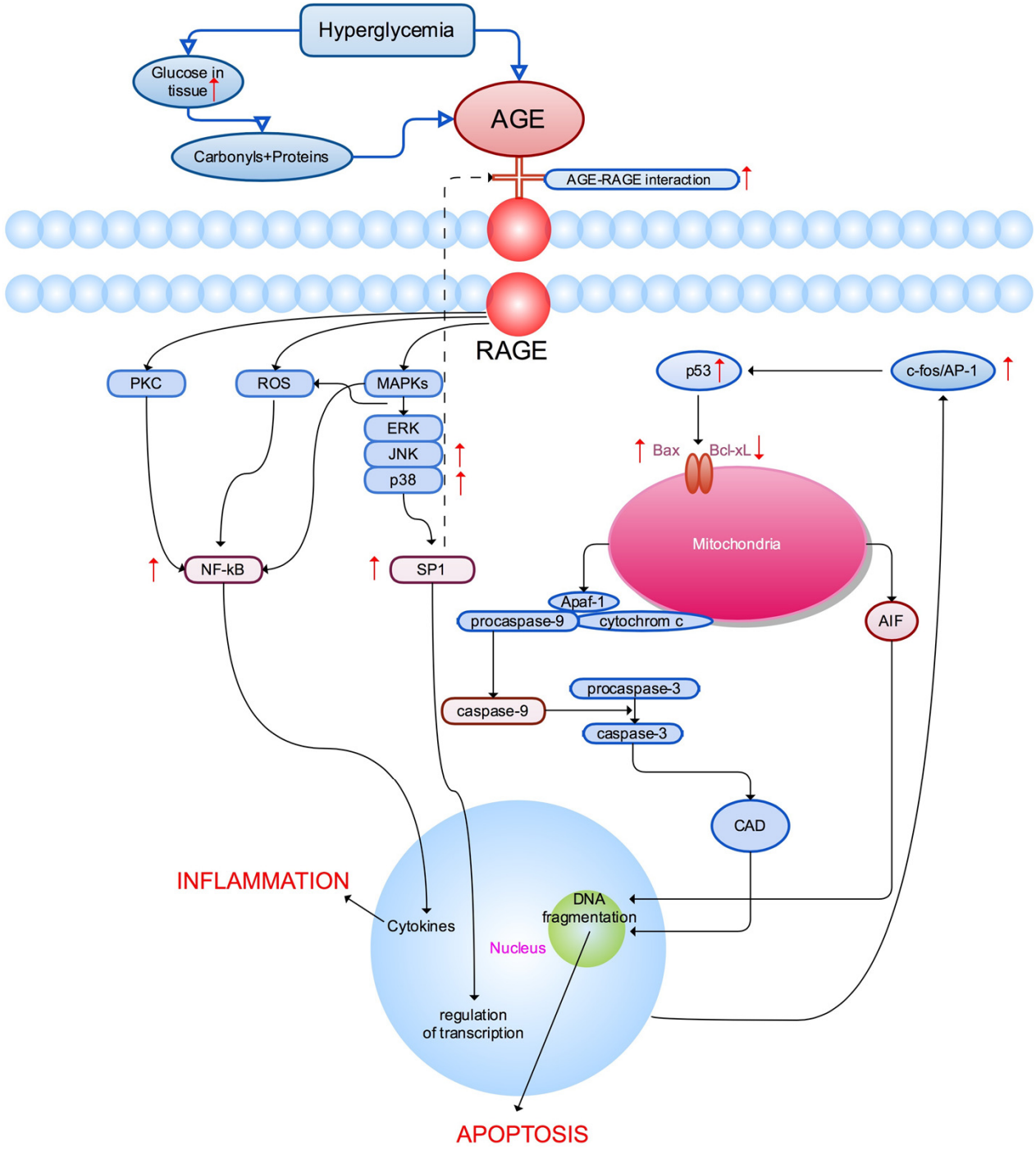


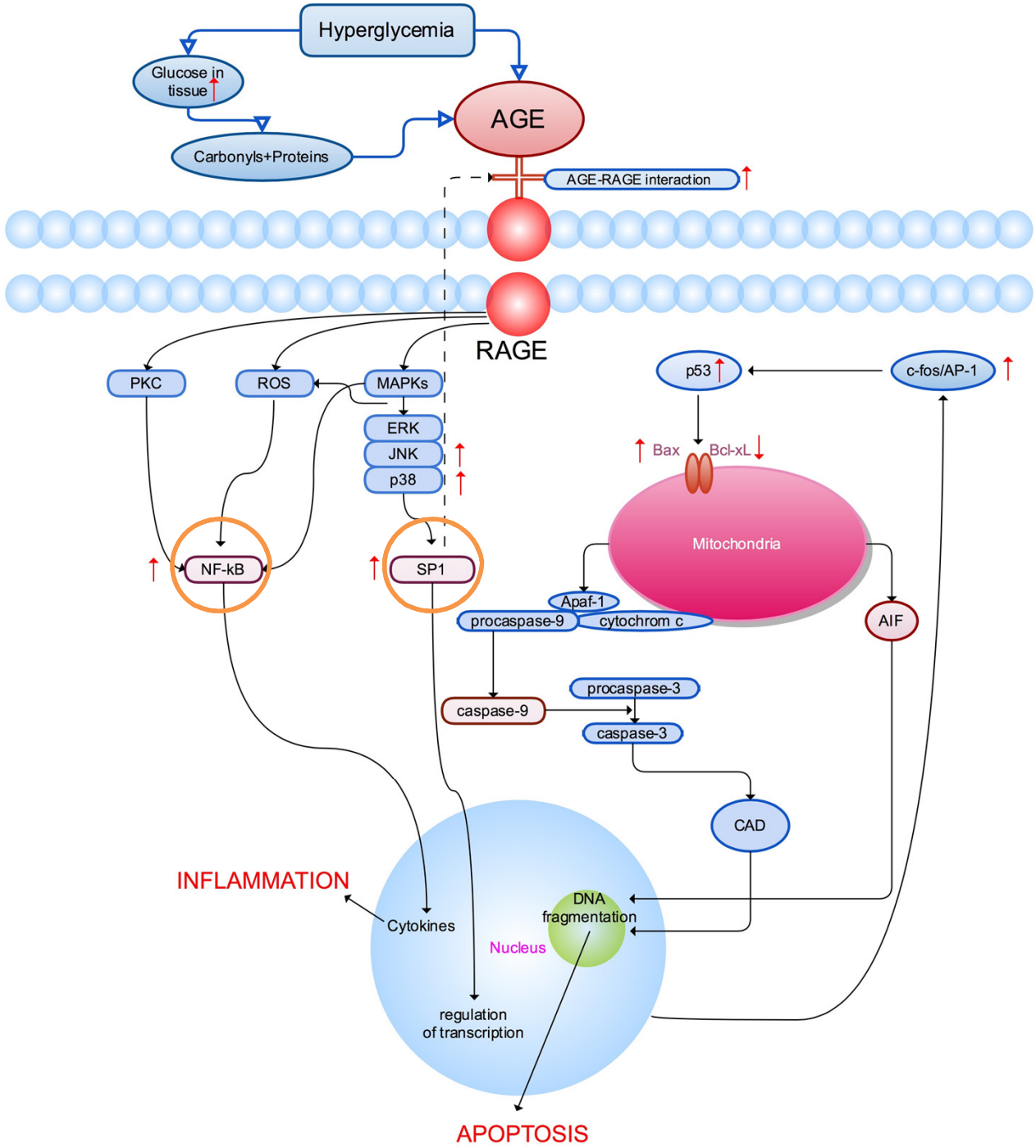


NF-kB immunostaining

* - P< 0.05




Hyperglycemia
Glucose in tissue
Carbonyls+Proteins
AGE
AGE-RAGE interaction
RAGE
PKC
ROS
MAPKs
ERK
JNK
p38
NF-kB
SP1
p53
c-fos/AP-1
Bax
Bcl-xL
Mitochondria
Apaf-1
procaspase-9
cytochrom c
AIF
procaspase-3
caspase-9
caspase-3
CAD
INFLAMMATION
Cytokines
DNA fragmentation
Nucleus
regulation of transcription
APOPTOSIS


Hyperglycemia
Glucose in tissue
Carbonyls+Proteins
AGE
AGE-RAGE interaction
RAGE
PKC
ROS
MAPKs
ERK
JNK
p38
NF-kB
SP1
p53
c-fos/AP-1
Bax
Bcl-xL
Mitochondria
Apaf-1
procaspase-9
cytochrom c
AIF
procaspase-3
caspase-9
caspase-3
CAD
INFLAMMATION
Cytokines
DNA fragmentation
Nucleus
regulation of transcription
APOPTOSIS

DISCUSSION

All examined neurotrophic factors decreased the number of NF-κ B immunopositive cells in glucose-AGE-BSA exposed retina,but NT-4 had the highest significant effect.

However, the number of SP1 immunopositive cells was increased by the addition of neurotrophic factors in serum-free media and more significantly in the HGF and NT-4 groups.

Thus after the addition of neurotrophic factors to glucose-AGE-BSA media, the level of SP1 transcription factor remained high in the NT-4 and HGF group. However, there was a slight decrease in the GNDF and TUDCA groups

DISCUSSION

It is possible that the increased expression of SP1 may result from an enhanced phosphorylation of SP1, because the SP1 promoter contains several SP1-binding elements and is positively regulated by its own gene product, SP1 protein.

Suppression of NF-κ B expression in retinal neurons by several neurotrophic factors can result in neuroprotection, and reducing inflammation and oxidative stress suggests therapeutic potential of neuroprotective therapy in various ocular pathologies associated with AGEs accumulation.

CONCLUSIONS

Neurite regeneration inhibition by high dose AGEs is correlated with increased expression of NF-κB and SP1.

NT-4 enhances neurite regeneration in AGEs exposed retinas more than other neurotrophic factors such as HGF, GDNF, and TUDCA. This effect of NT-4 is correlated with NF-κ B suppression.

SP1 overexpression may be related to neuronal regeneration in neurotrophic factors incubated retinas.

CHIBA UNIVERSITY

Thank you.