



Diyabetik Sıçanlarda Vitre İçine Uygulanan Mezenkimal Kök Hücrelerin Etkisi

Eren Çerman¹, Ülkü Arıĝ², Muhsin Eraslan¹, Fatih Mahmut Bulut², Özlem Şahin¹, Selvinaz Özkara³, Fügen Vardar Aker³, Erdal Karaöz⁴, Tunç Akkoç²

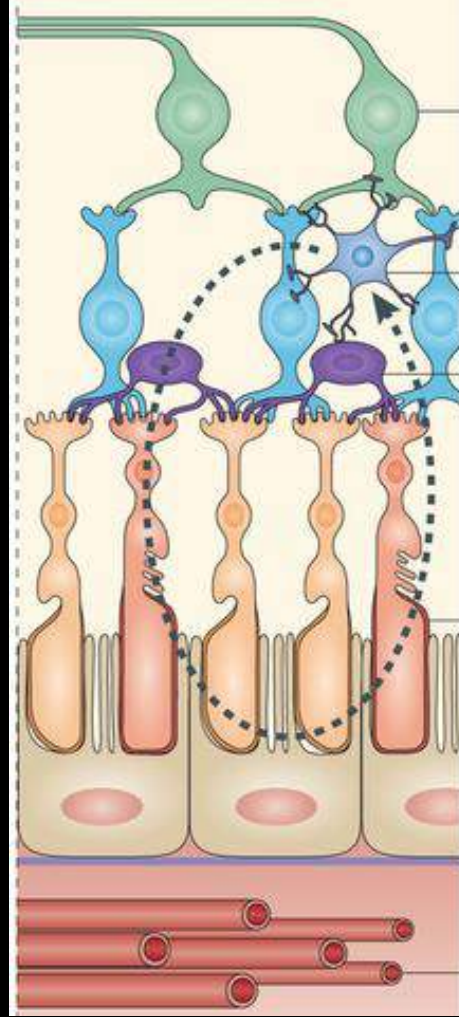
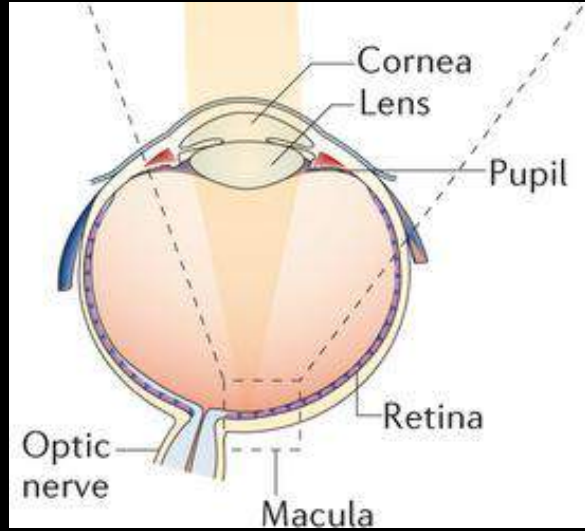
¹Marmara Üniversitesi Göz Hastalıkları Anabilim Dalı

²Marmara Üniversitesi, Çocuk Sağlığı ve Hastalıkları Ana Bilim Dalı Pediatrik Allerji İmmunoloji Bölümü, İstanbul

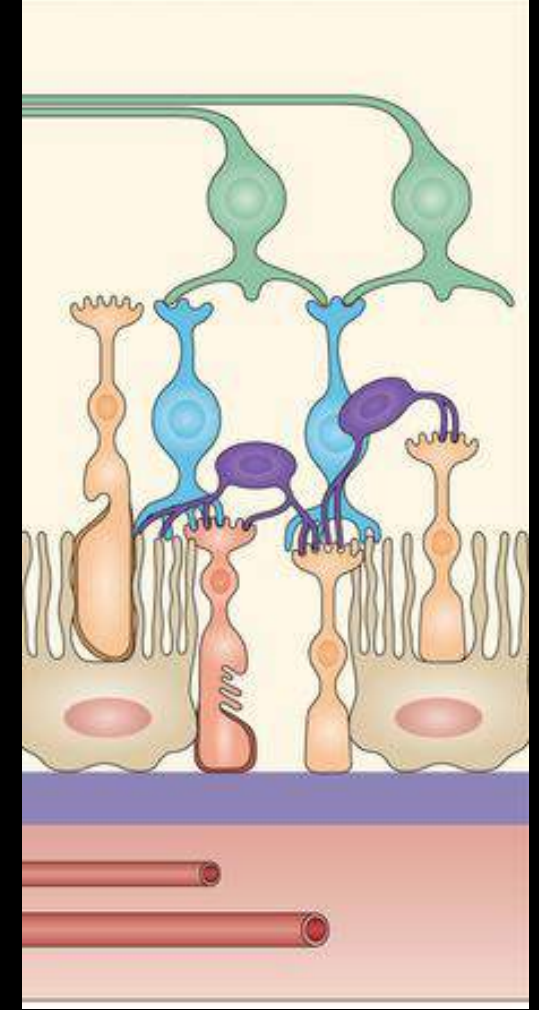
³Haydarpaşa Numune Eğitim ve Araştırma Hastanesi, Patoloji Ana Bilim Dalı, İstanbul

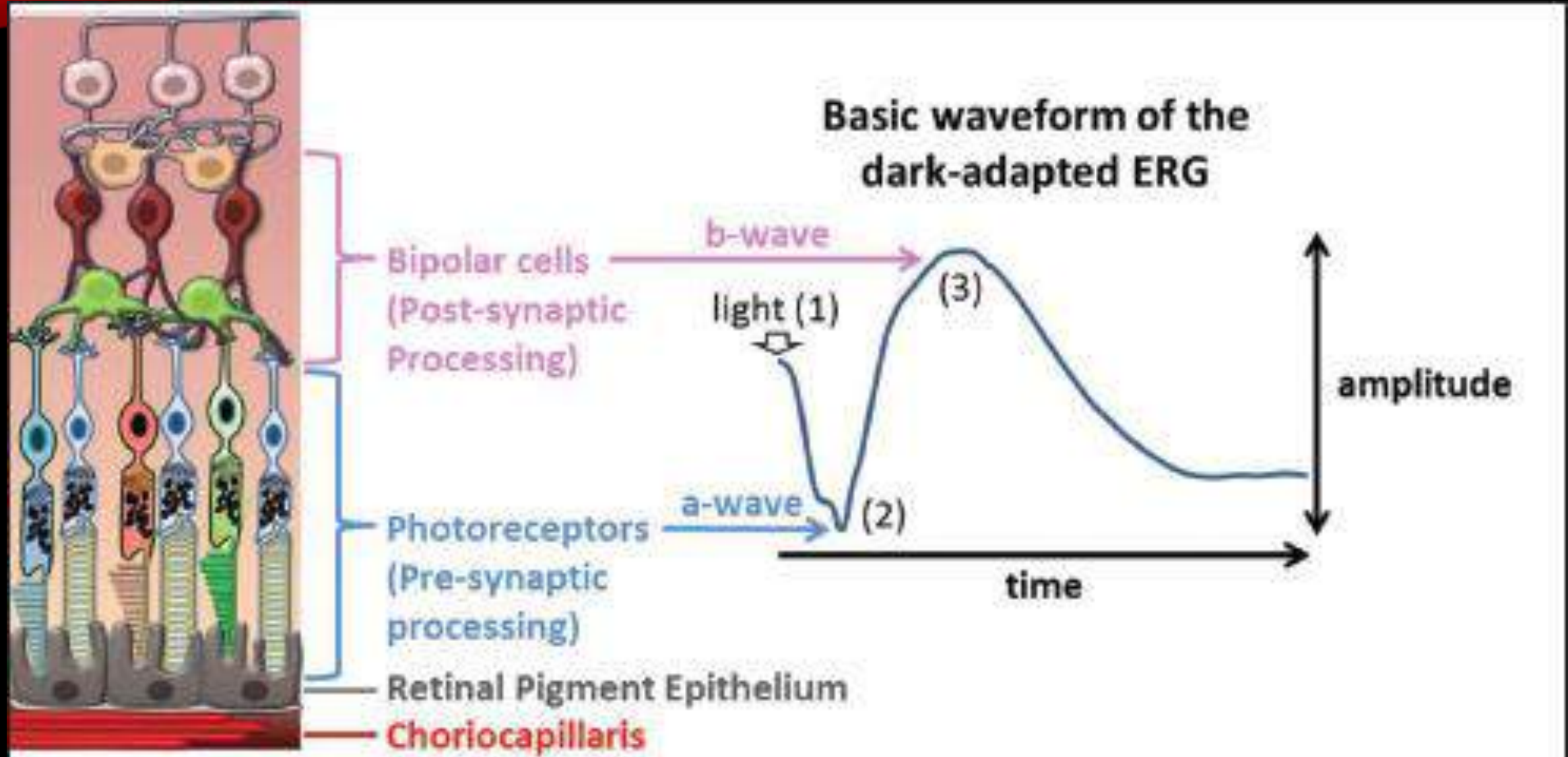
⁴Kocaeli Üniversitesi, Kök Hücre ve Gen Tedavileri Araştırma ve Uygulama Merkezi, Kocaeli

Çalışma içerisinde adı geçen herhangi bir ticari firmayla finansal bağlantım yoktur.

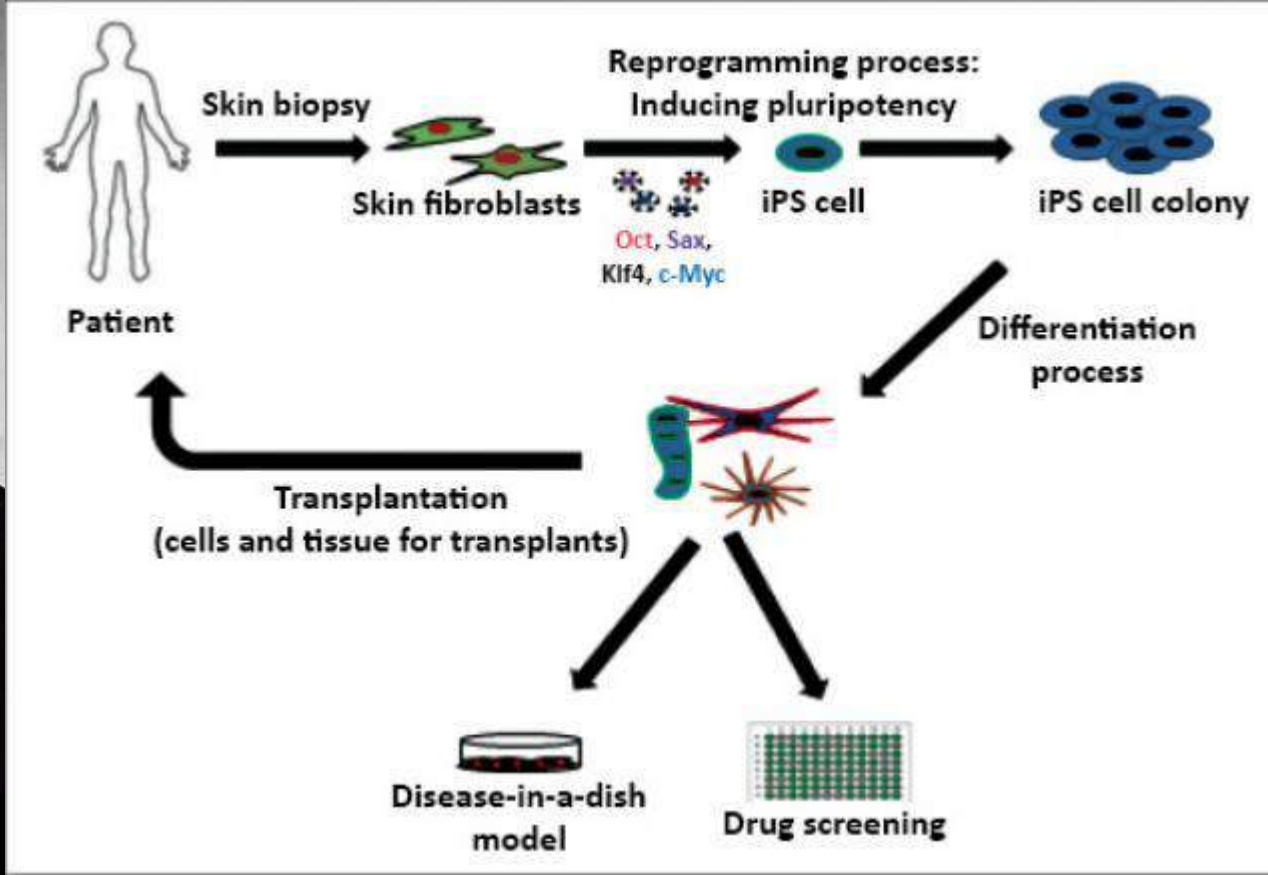


Diyabetik Retinopati
Yaşabağlı Maküla Dejeneransı
Stargardt hastalığı
Retinitis Pigmentoza





Giriş



Nobel ödülünü aldıktan sonra retina rejenerasyonu üzerinde çalışacaklarını açıkladı.

6 kişi üzerinde klinik çalışmaya başlandı. Sonuçları henüz açıklanmadı.

Shinya Yamanaka

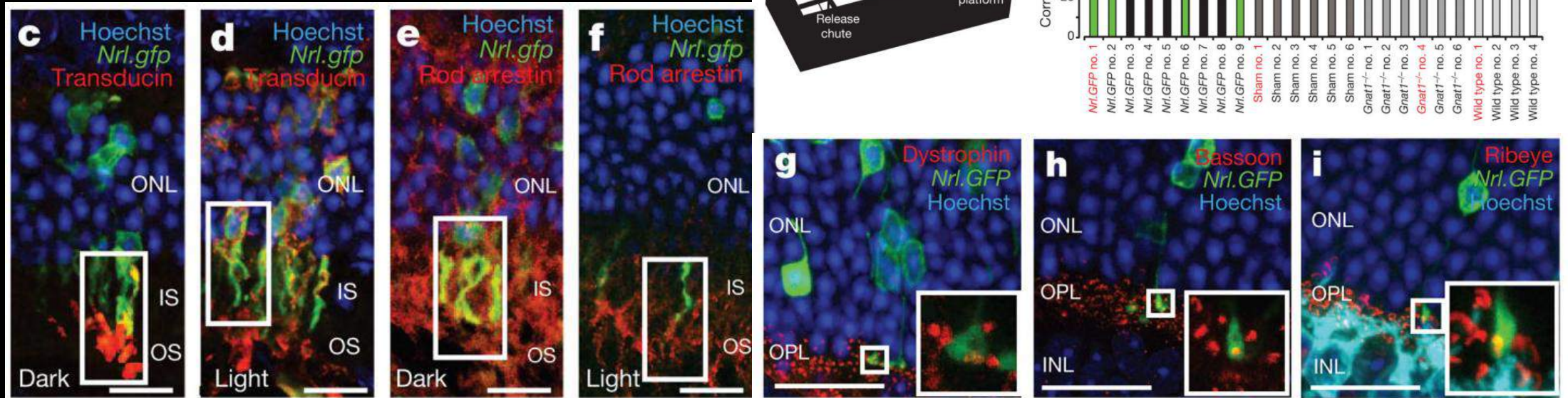
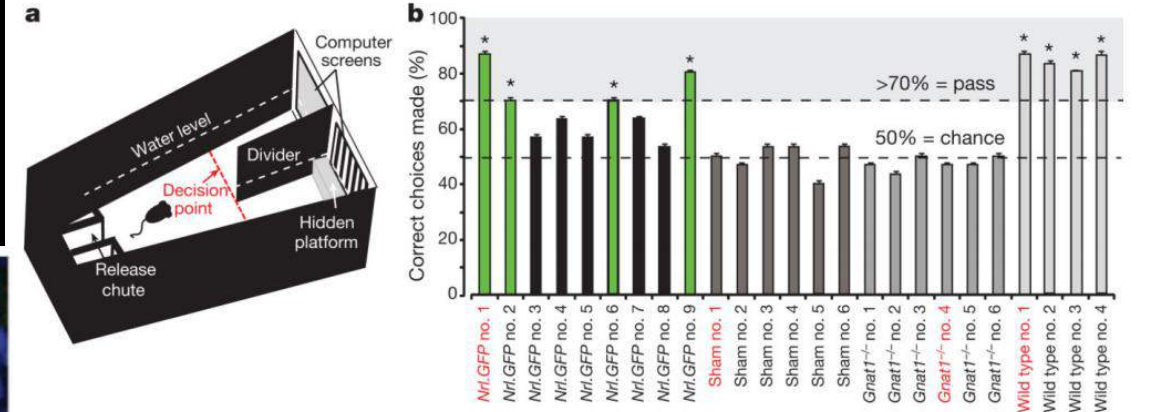
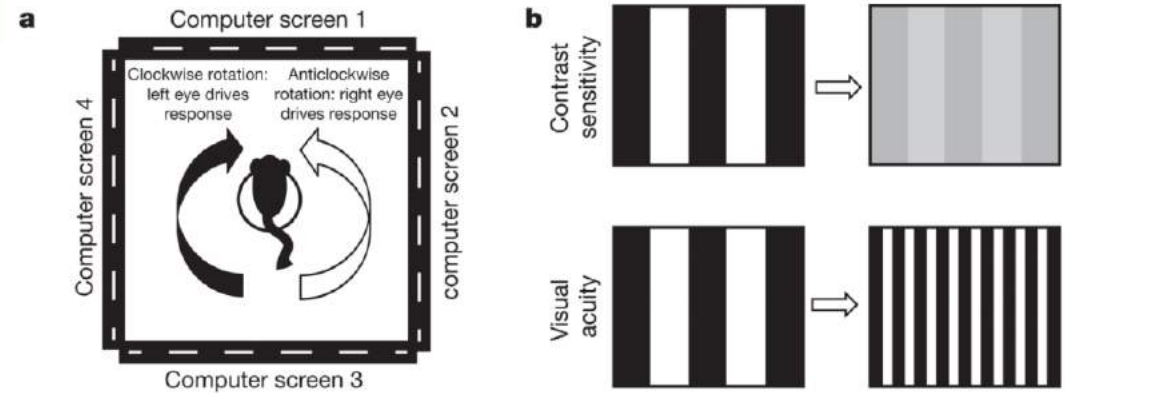
2012 yılında Nobel Tıp ödülünü almıştır.

Published in final edited form as:

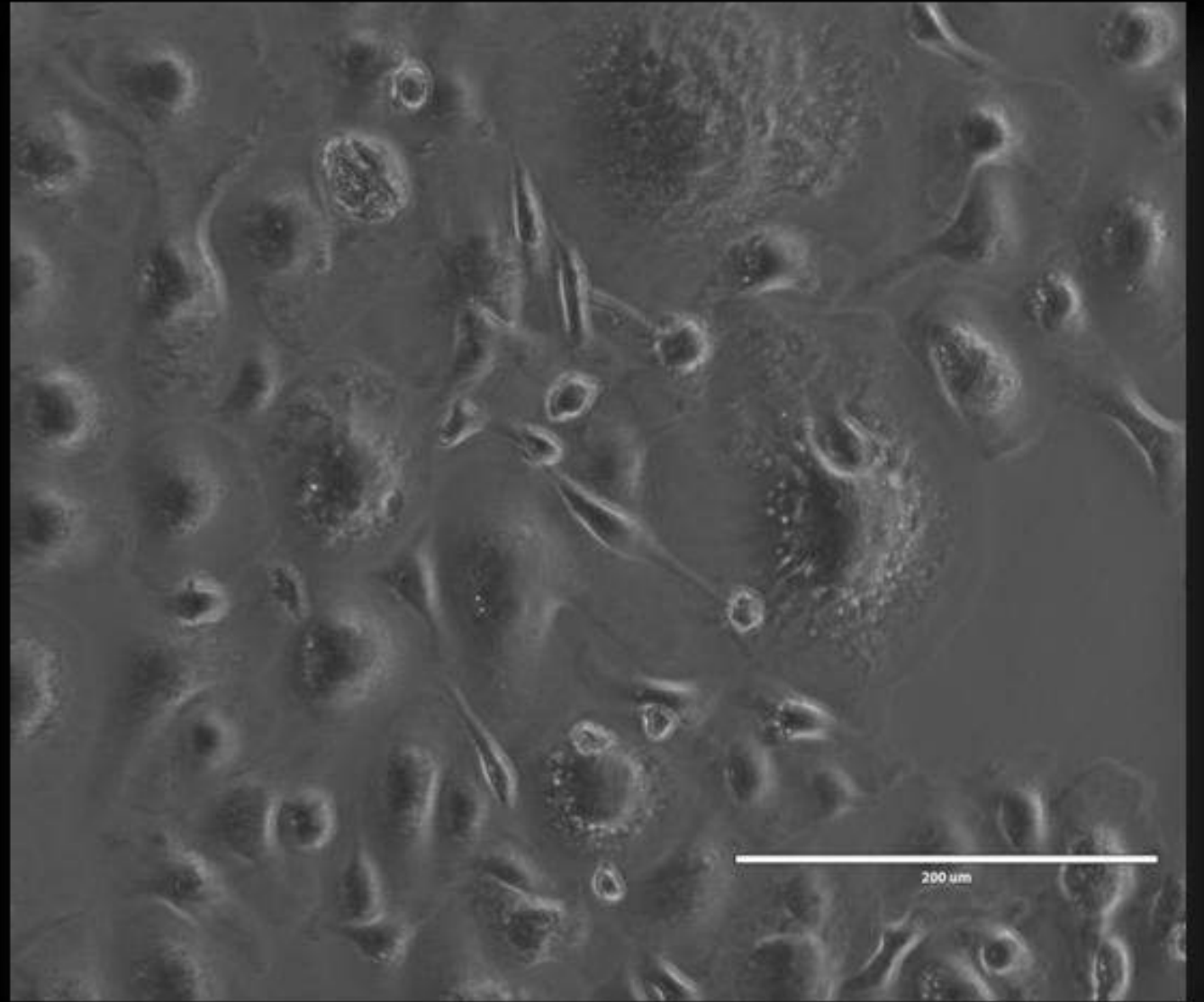
Nature. 2012 May 3; 485(7396): 99–103. doi:10.1038/nature10997.

Restoration of vision after transplantation of photoreceptors

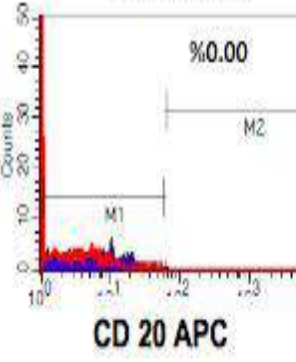
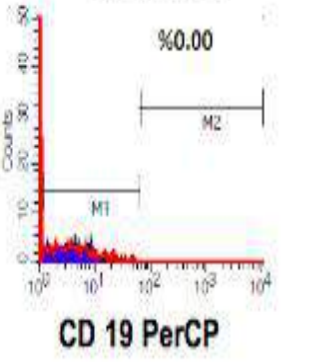
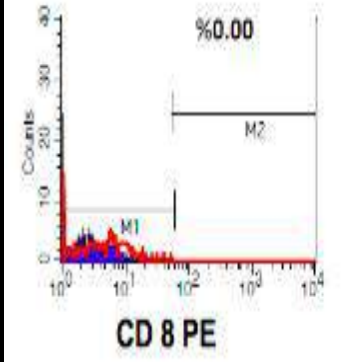
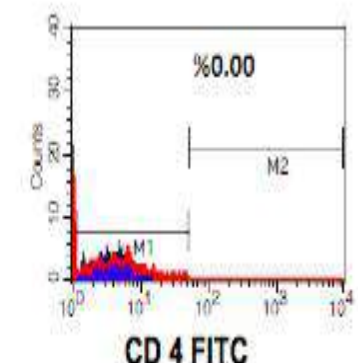
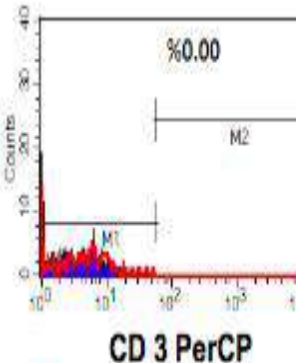
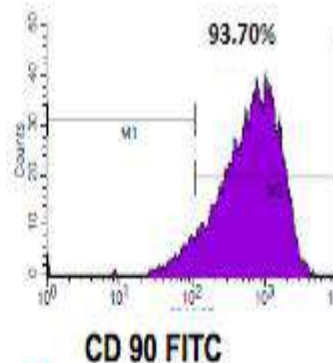
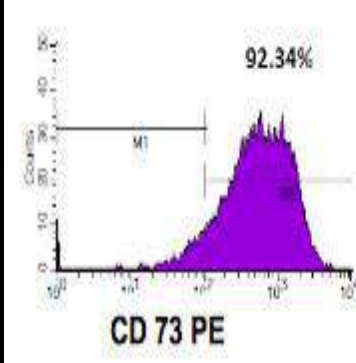
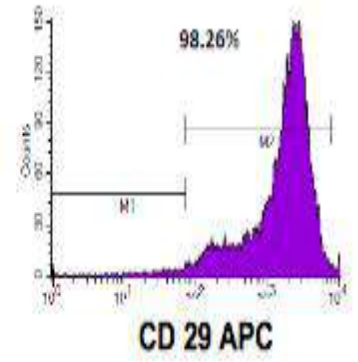
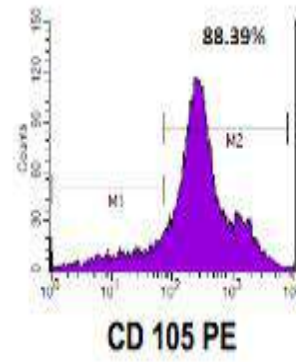
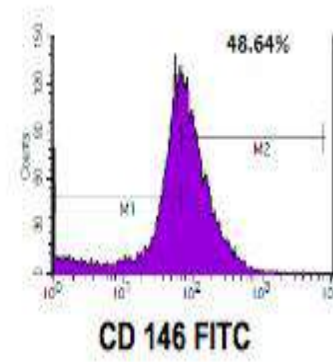
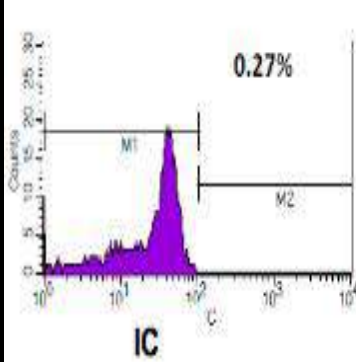
R. A. Pearson¹, A. C. Barber¹, M. Rizzi¹, C. Hippert¹, T. Xue³, E. L. West¹, Y. Duran¹, A. J. Smith¹, J. Z. Chuang⁴, S. A. Azam¹, U. F. O. Luhmann¹, A. Benucci², C. H. Sung⁴, J. W. Bainbridge¹, M. Carandini², K.-W. Yau³, J. C. Sowden⁵, and R. R. Ali^{1,6}



Sıçan Kemik İliđi Kaynaklı MKH'lerin
İnvert Mikroskop Görüntüsü, Pasaj 3
(x20 Büyütme)



Kök Hücrelerin akım sitometrisi ile karakterizasyonu



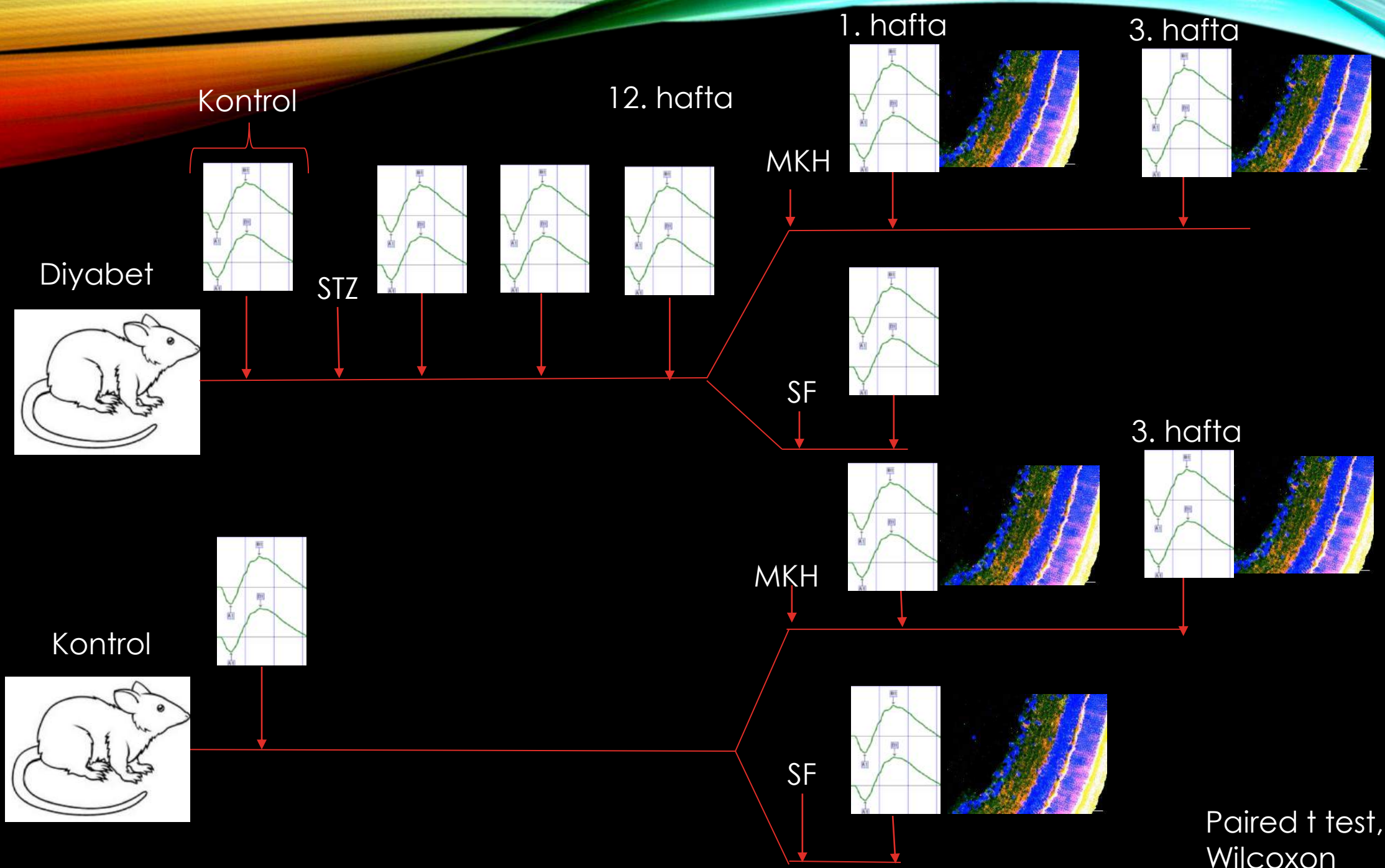
Deney Hayvanları ve kullanılan ilaçlar

Erkek, >300 gr Rattus norvegicus albino Wistar
12 saat gündüz gece adaptasyonunda

Anestezi için → Periton içine 100 mg/kg ketamin hidroklorid
10 mg/kg xylazine

Diyabet için → Periton içine 60mg/kg streptozotosin (STZ)

20 µL solüsyonda 200×10^3 Mezenkimal Kök hücre içeren solüsyon vitre içine enjekte edildi



Paired t test,
Wilcoxon

ERG

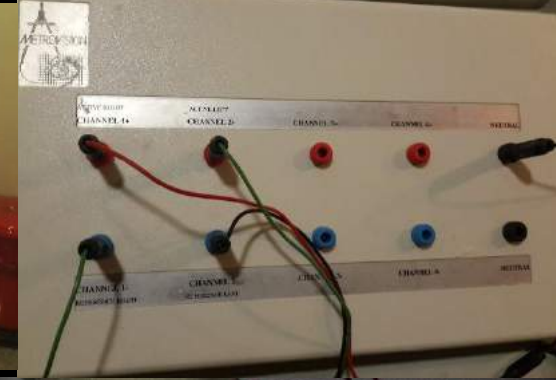
12 saat adaptasyon sonrası skotopik ERG

MetroVison ERG cihazı kullanıldı

Flaş ERG

Gümüş elektrodlar

Viscotears, Mydfrin, Tropicamid



Işık gücü ve ERG

$$-25\text{db} = 0.01 \text{ cd.s.m}^{-2}$$

$$-10 \text{ db} = 0.1 \text{ cd.s.m}^{-2}$$

$$-5 \text{ db} = 0.3 \text{ cd.s.m}^{-2}$$

$$\text{RC} = 3 \text{ cd.s.m}^{-2}$$

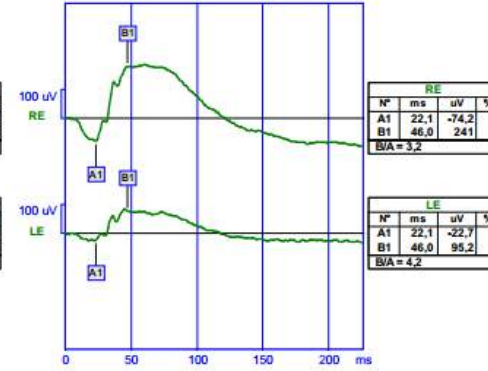
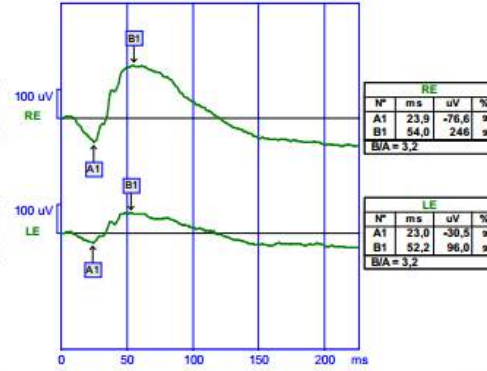
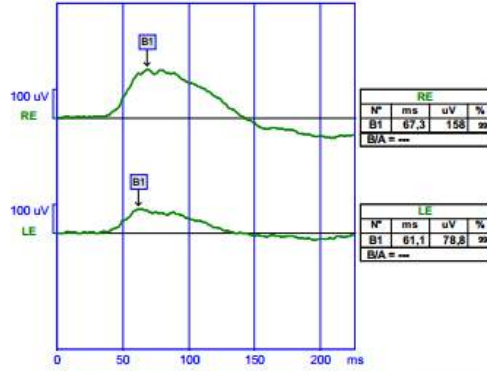
$$\text{OP} = 3 \text{ cd.s.m}^{-2}$$

patient's ID : k11
file number :
birth date : // // // // // *

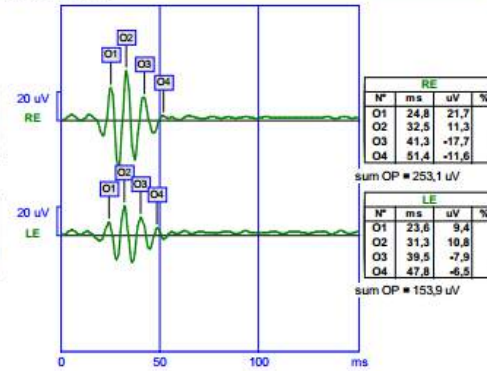
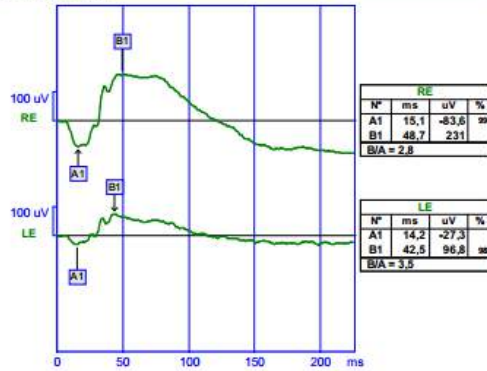
Rx :
exam. date : 31/01/2013 15:58
exam :

VISUAL ELECTROPHYSIOLOGY EXAM

ERG rod: white flash (-25dB) 1mn 20s Val= 8 Rej= 0 ERG combined: (-10dB) 2mn 51s Val= 8 Rej= 0 ERG combined: (-5dB) 3mn 49s Val= 8 Rej= 0
RE stimulated RE stimulated RE stimulated



ERG combined: rod+cone 5mn 0s Val= 8 Rej= 0 ERG oscillatory potentials RE stimulated 6mn 27s Val= 8 Rej= 0



T.C. SAĞLIK BAKANLIĞI
MARMARA ÜNİVERSİTESİ PENDİK
EĞİTİM VE ARAŞTIRMA HASTANESİ

Vision Monitor
Mon2010J

Metrovision
4 rue des platanes
59640 Pétrenchies
France
tel +33 3 20 17 19 50
http://www.metrovision.com



serial n654

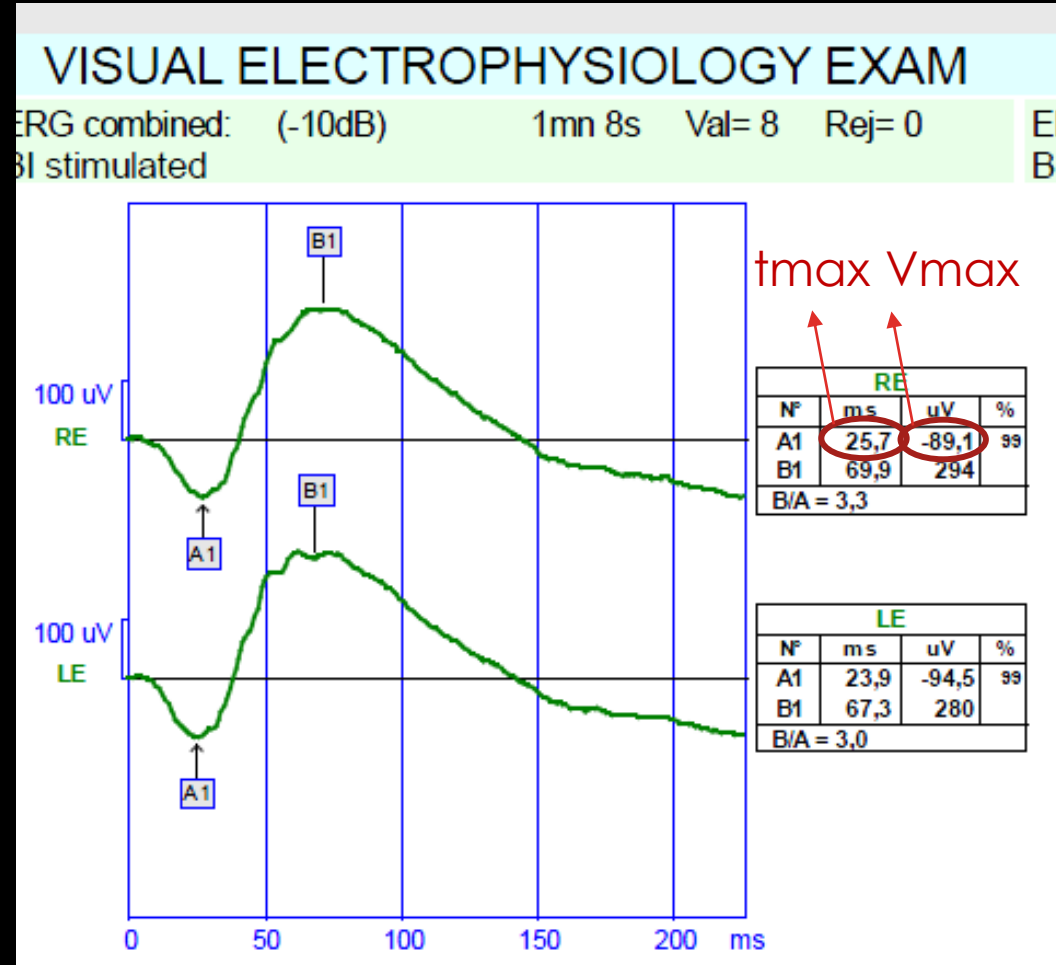
© 2011 Metrovision

a dalgasının Tahmini

Gaussian dağılım fomülü

$$V(A) = Vmax \exp\left[-\frac{(t-tmax)^2}{2\sigma^2}\right]$$

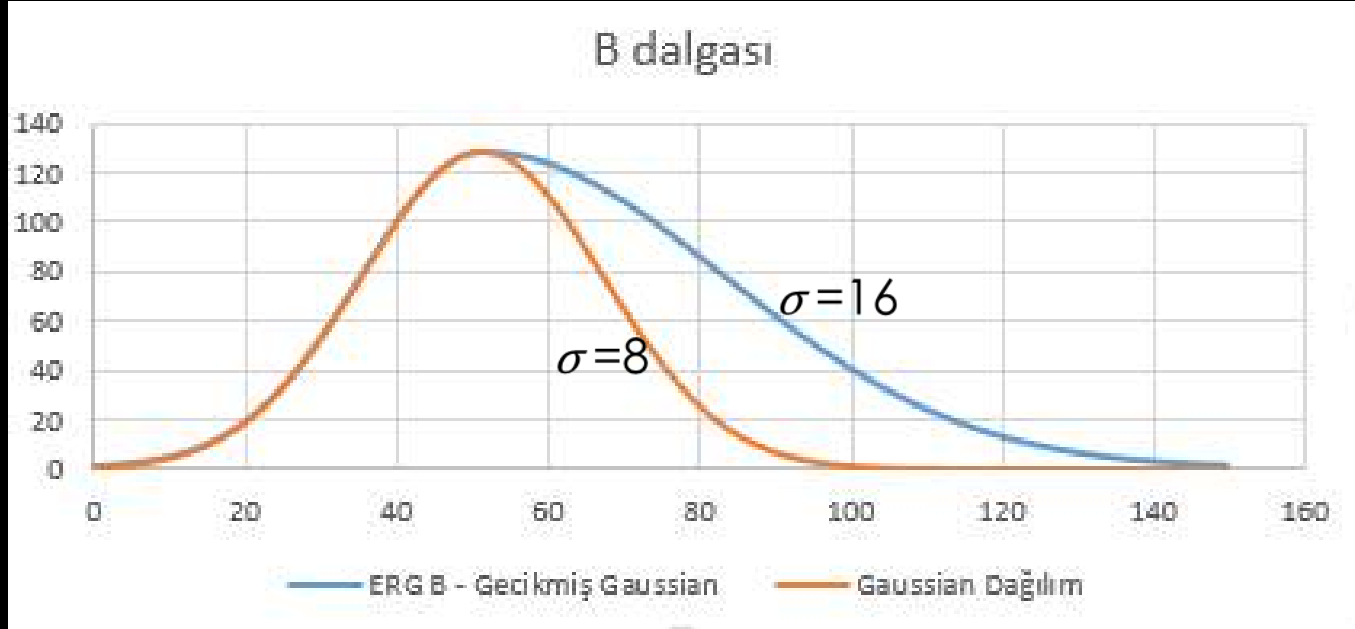
`=ŞAJ$12*(EXP((-1)*(((P2-ŞAI$12)^2)/(2*(ŞAI$15^2))))))`



b Dalgasının Tahmini

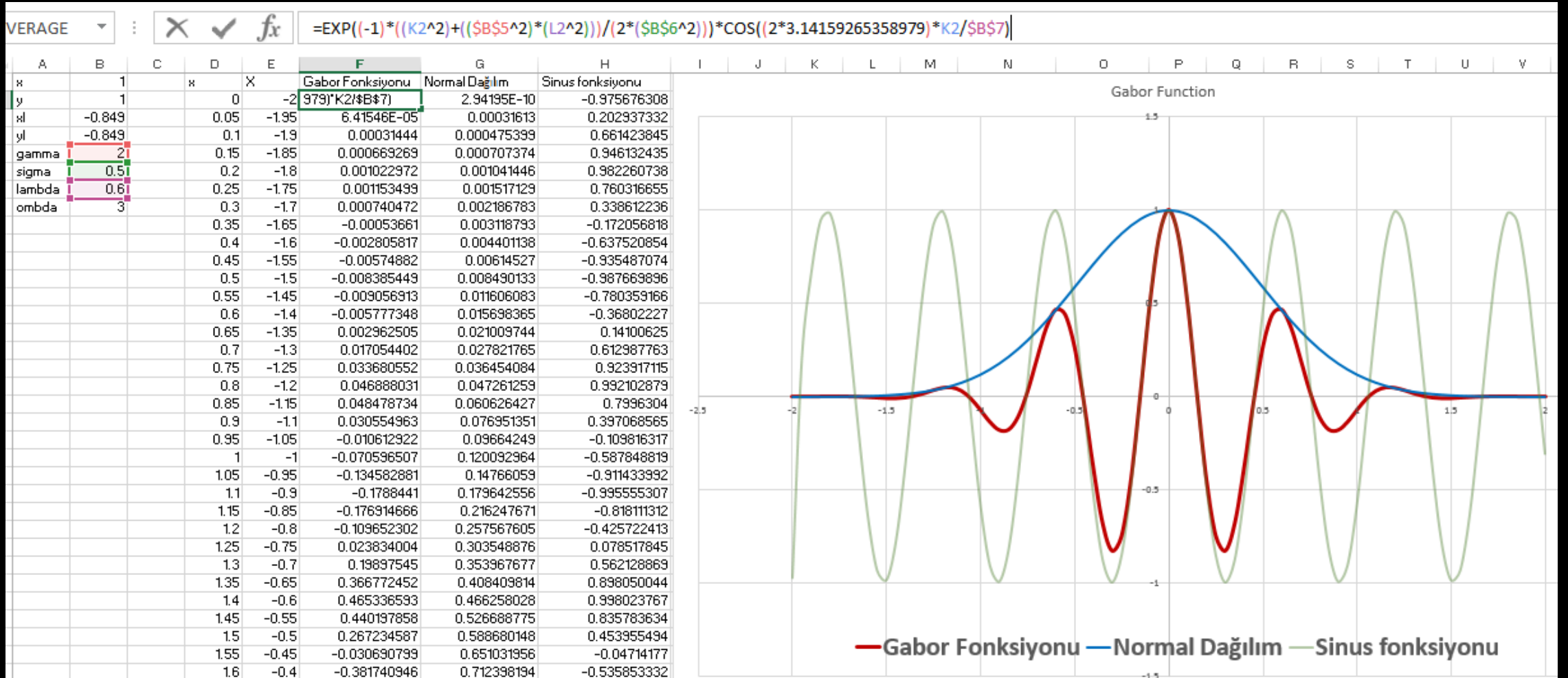
Gecikmiş Gaussian dağılım fomülü

$$V(A) = Vmax \exp\left[-\frac{(t-tmax)^2}{2\sigma^2}\right]$$

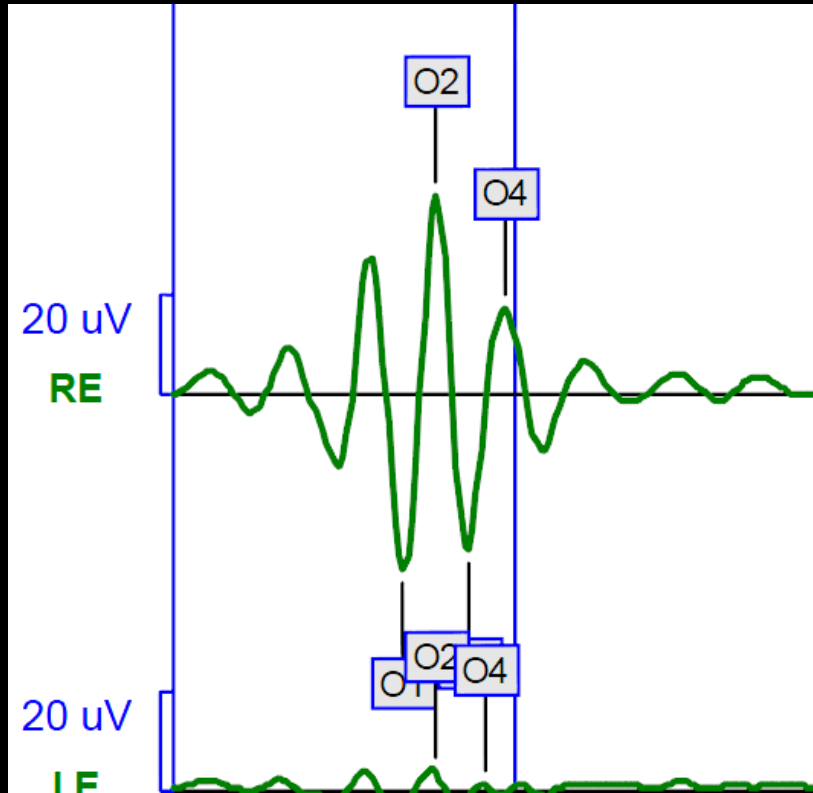


Osillatuar Potansiyel : Gabor Fonksiyonu

$$g(x, y; \lambda, \theta, \psi, \sigma, \gamma) = \exp\left(-\frac{x'^2 + \gamma^2 y'^2}{2\sigma^2}\right) \cos\left(2\pi\frac{x'}{\lambda} + \psi\right)$$



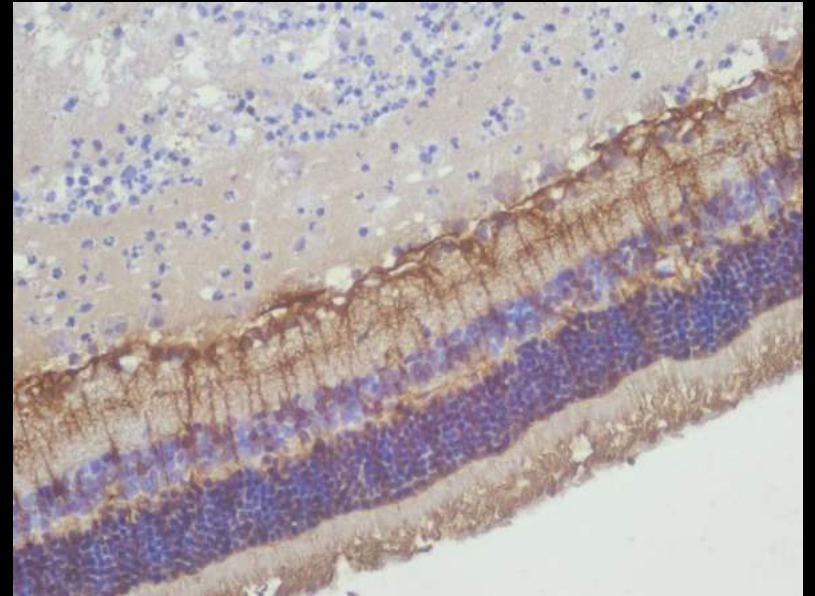
Osillatuar Potansiyel : Gabor Fonksiyonu



Immünohistokimya

- Vimentin (Müller)
- Glial Fibriller Asidik Protein (GFAP) (Müller)
- Rodopsin
- Dapi

Yapılan boyamada diyabet ile kontrol grubu arařtırmacılar kör olarak alıřıldı ve boyanma istatistiksel olarak arařtırıldı.



Immü Floresans

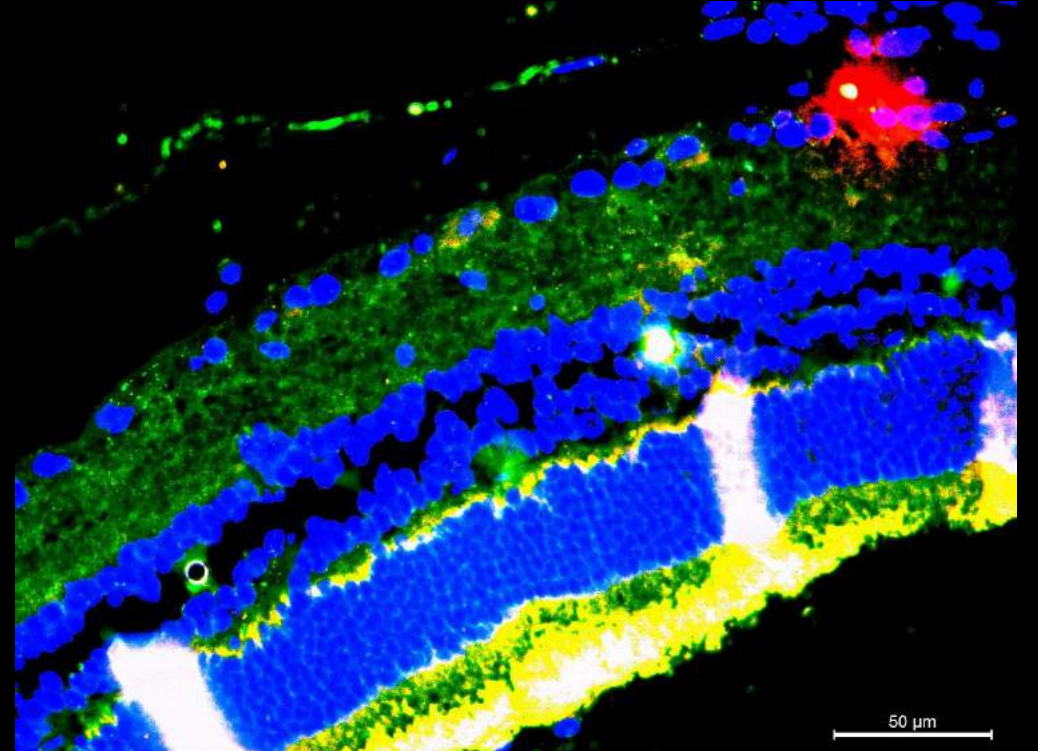
GFP ile MKH'ler.

Vimentin ve GFAP ile Müller hücreleri

Rodopsin ile fotoreseptörler

Dapi ile hücre çekirdekleri işaretlendi.

İki sekonder antikor kullanılarak GFP ekspresyonu gösteren hücrelerin hangi retina hücresine dönüşmüş olabileceği araştırıldı.



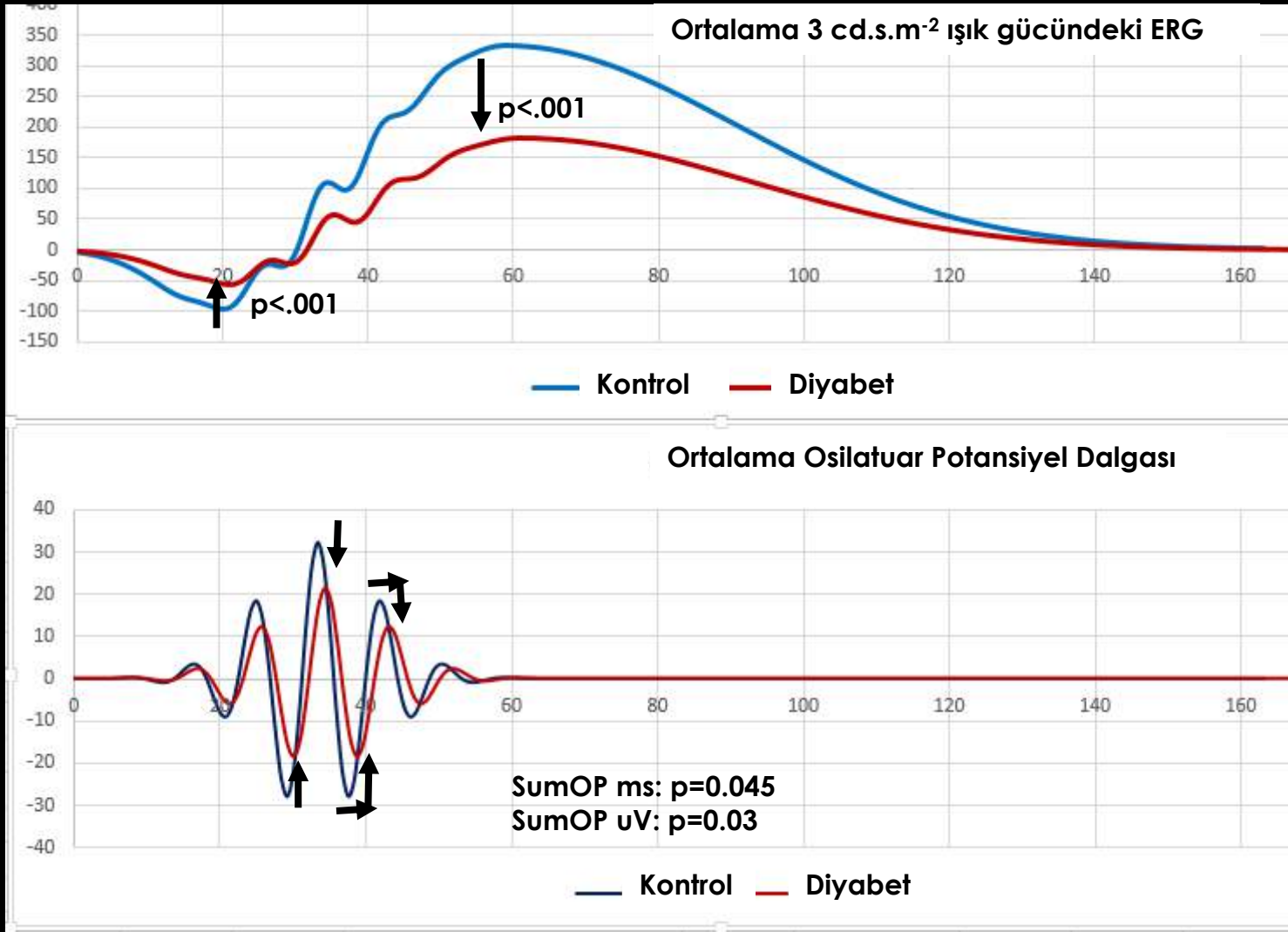
SONUÇLAR VE TARTIŞMA

68 Wistar Albino ratın 148 ERG sonucu incelendi.
24 ratın 48 gözüne ait ERG
27 adet 12 haftalık diyabetik ratın 54 ERG

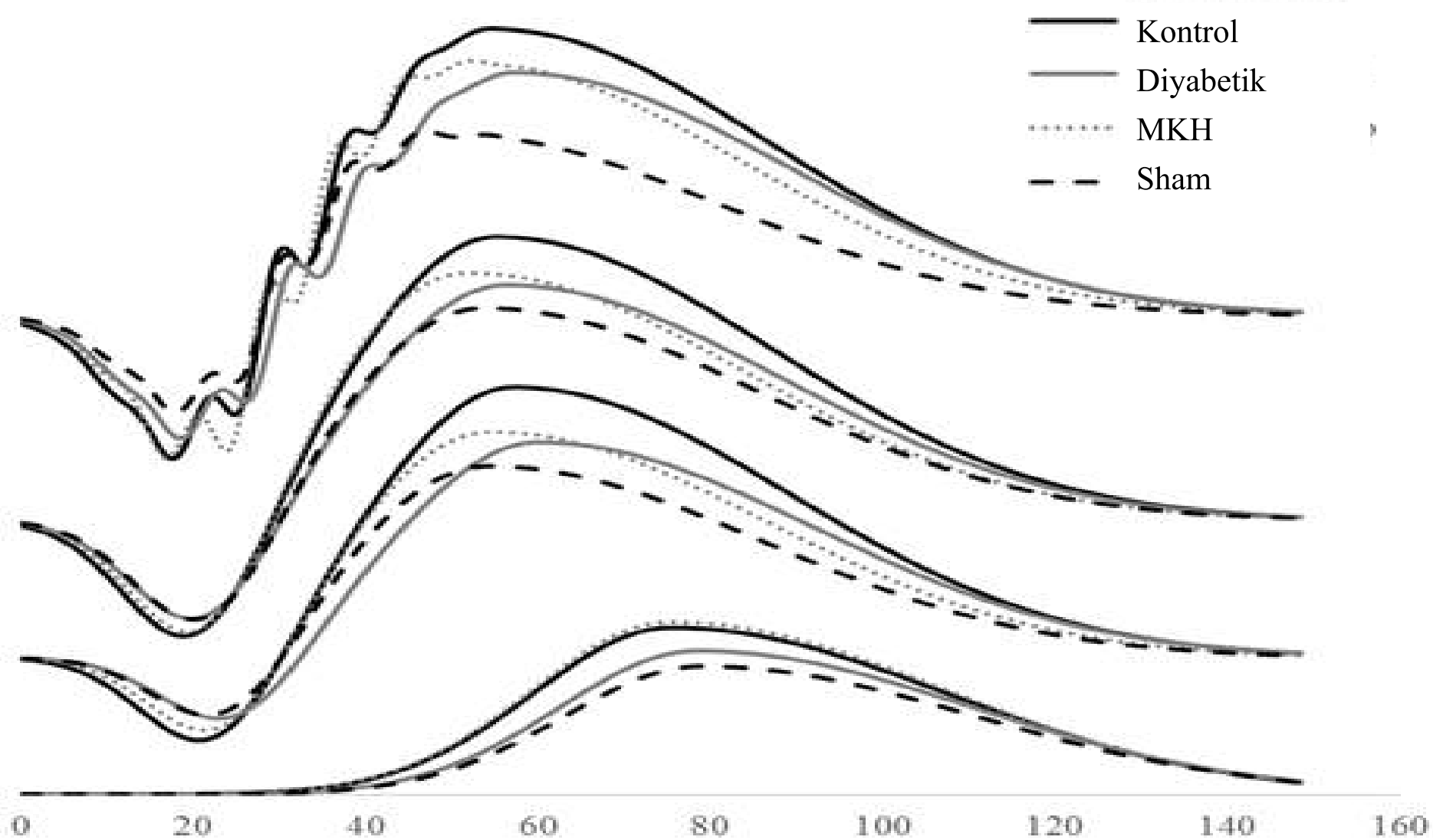
Tedaviden sonra

1. haftada 7 ratın 14 gözüne ait ERG
2. Haftada 13 ratın 26 gözüne ait ERG
3. Haftada 5 ratın 10 gözüne ait ERG

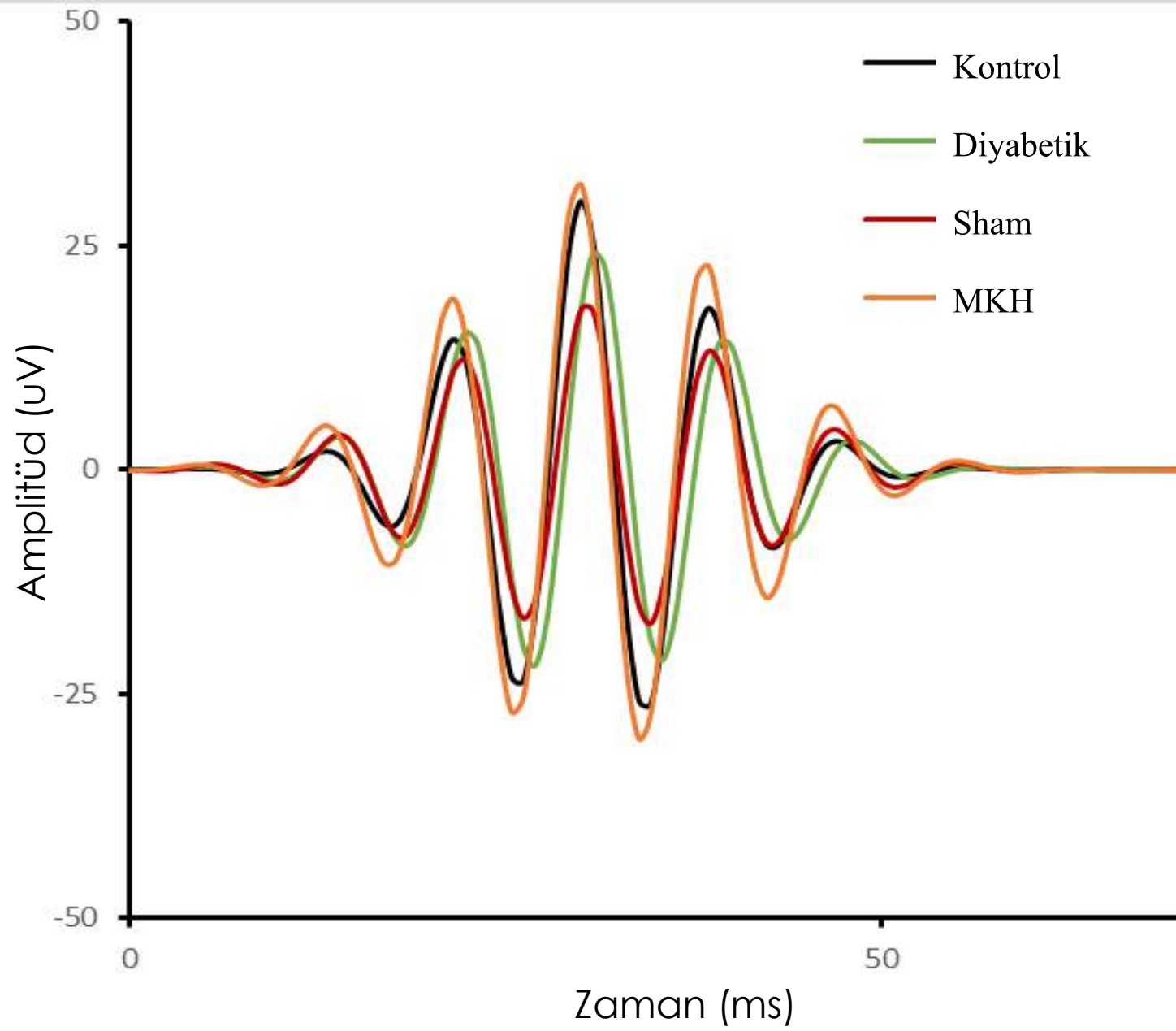
Kontrol ERG - Diyabetik ERG



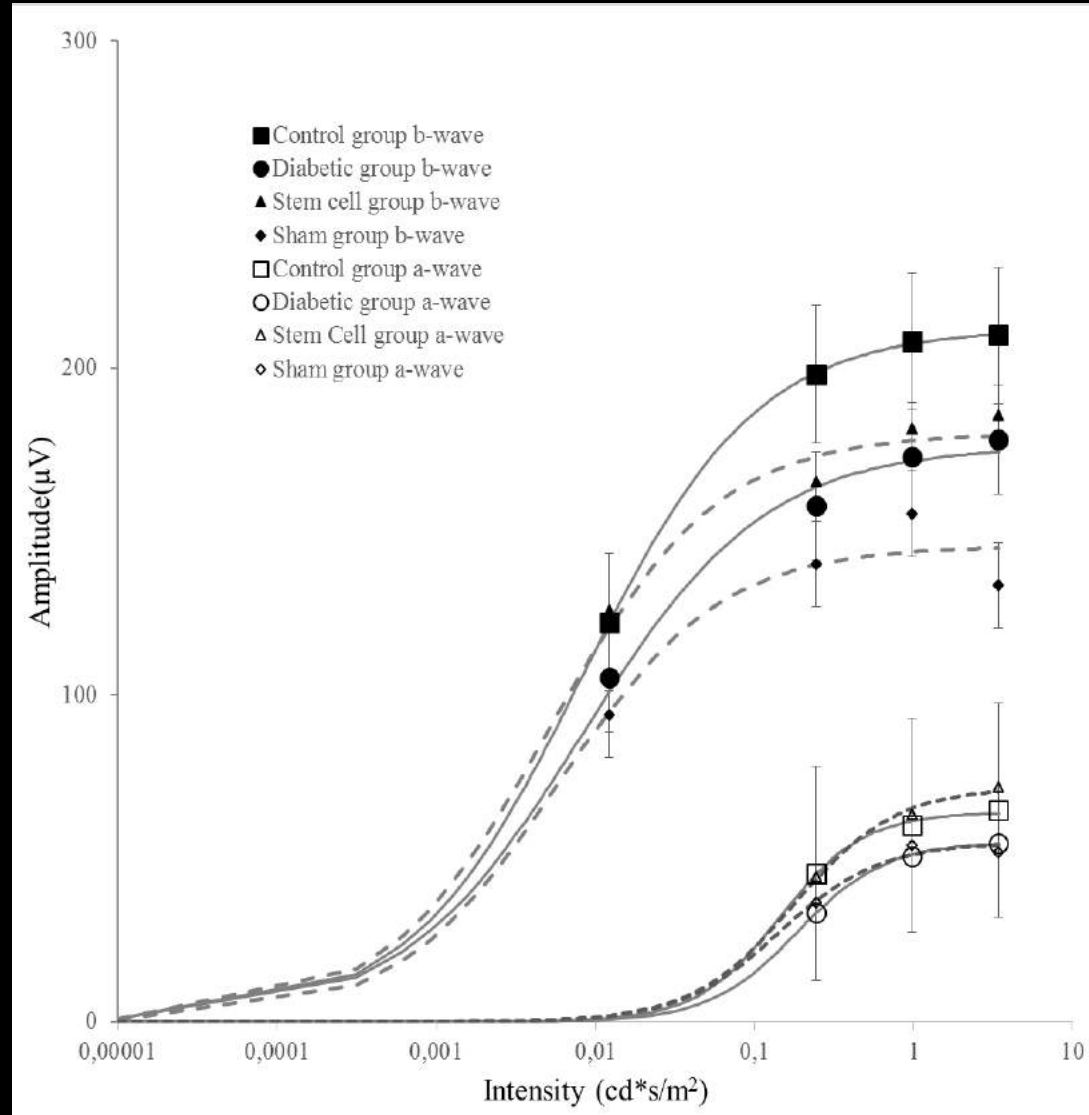
paired T test



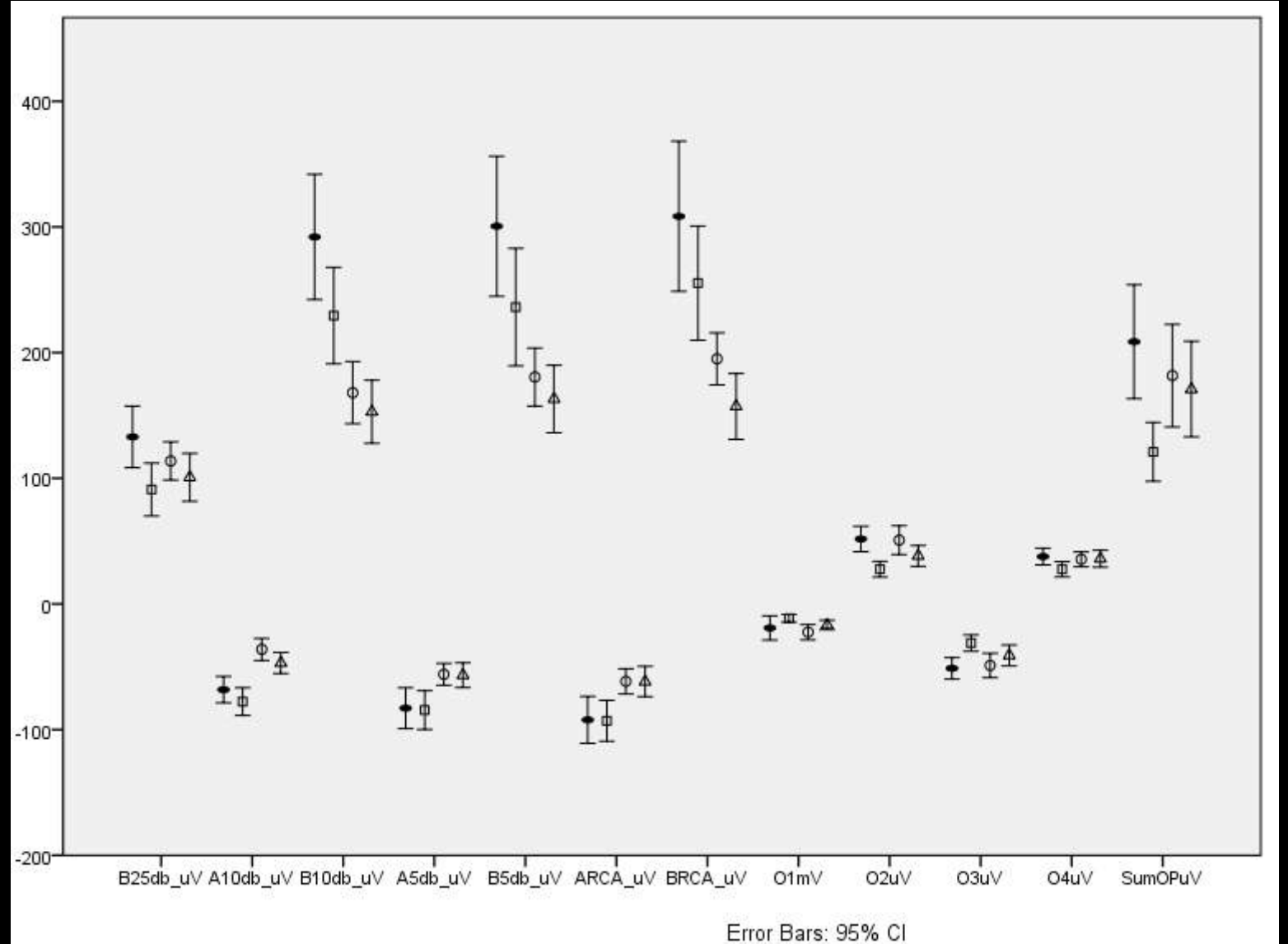
Zaman (ms)

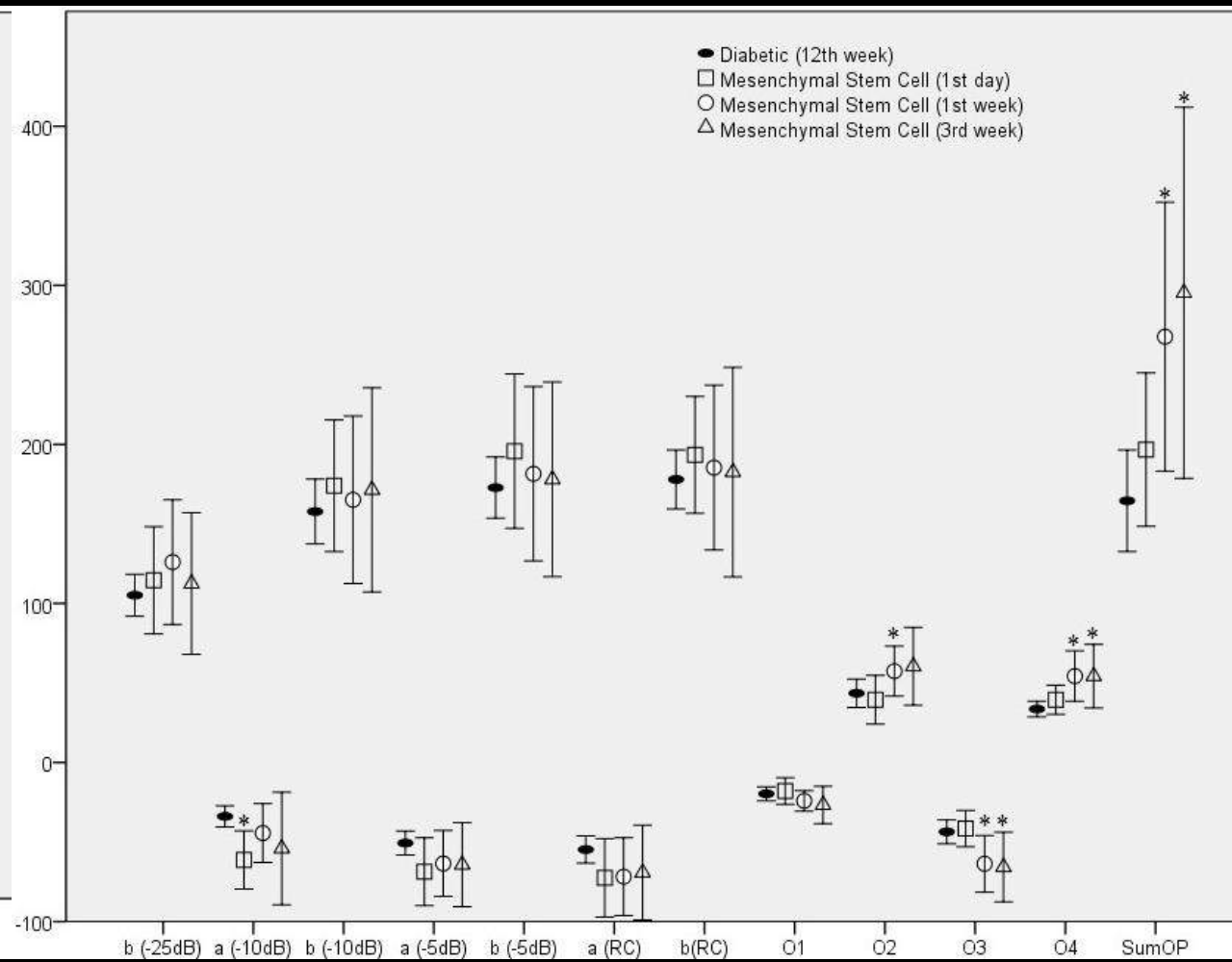
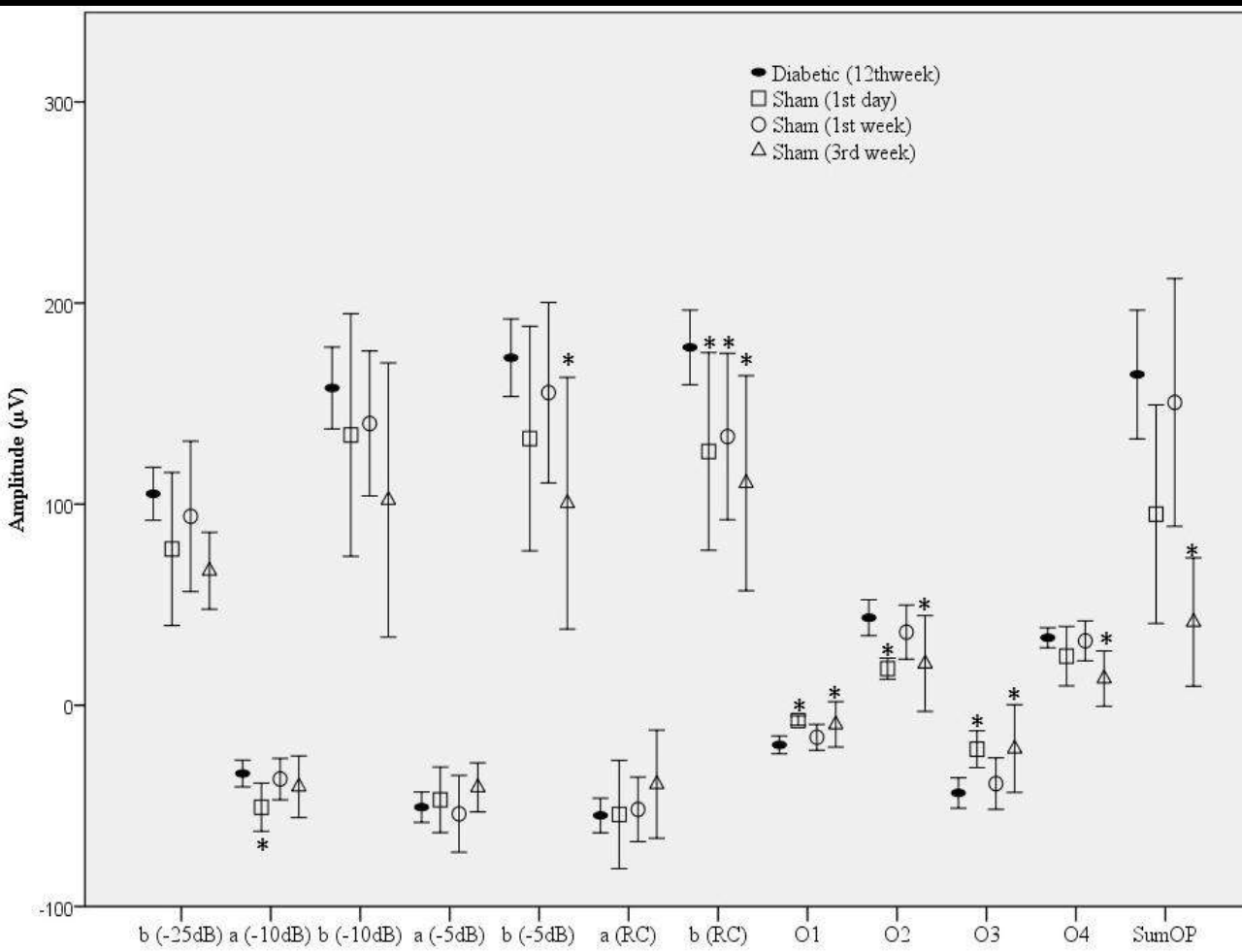


Ortalama ışık yoğunluğu- cevap diyagramı

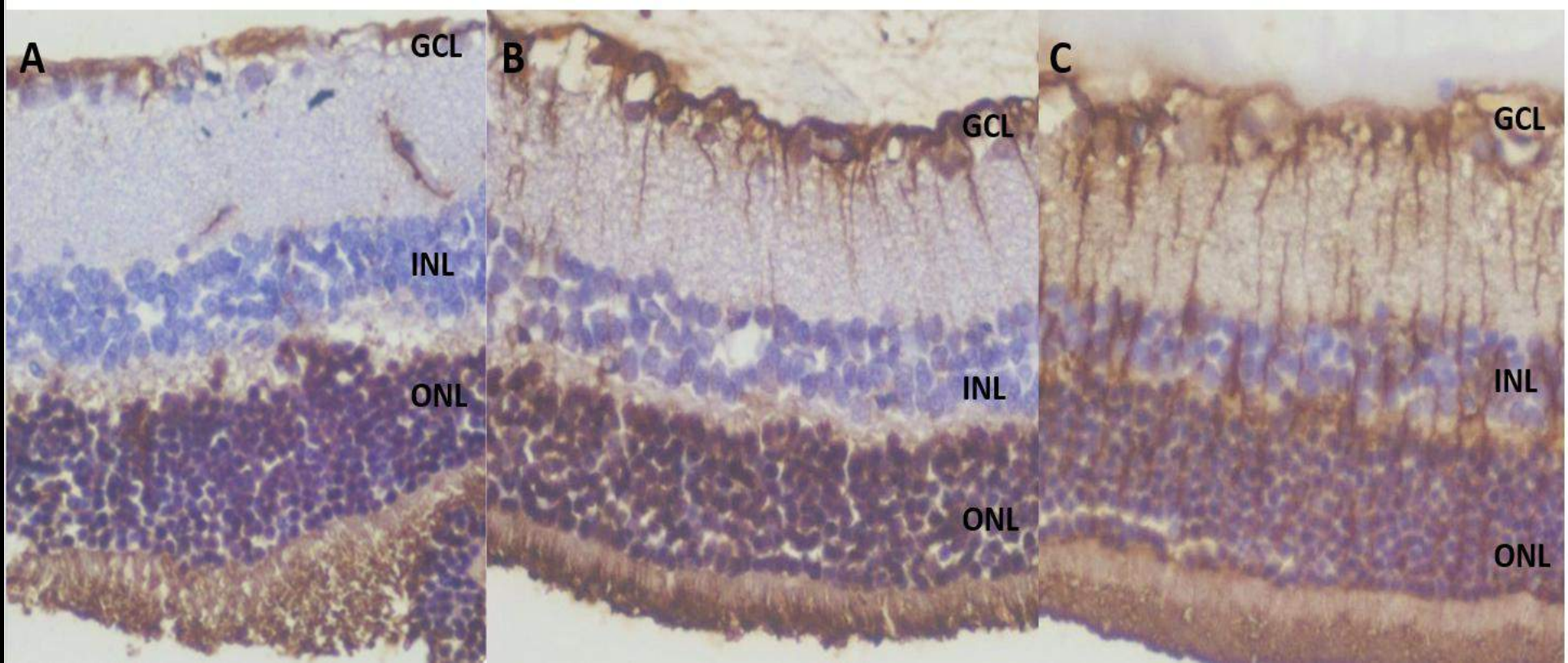


Işık yoğunluğuna göre
12 haftalık diyabet
boyunca yapılan 3
aylık takipte ERG
bulguları



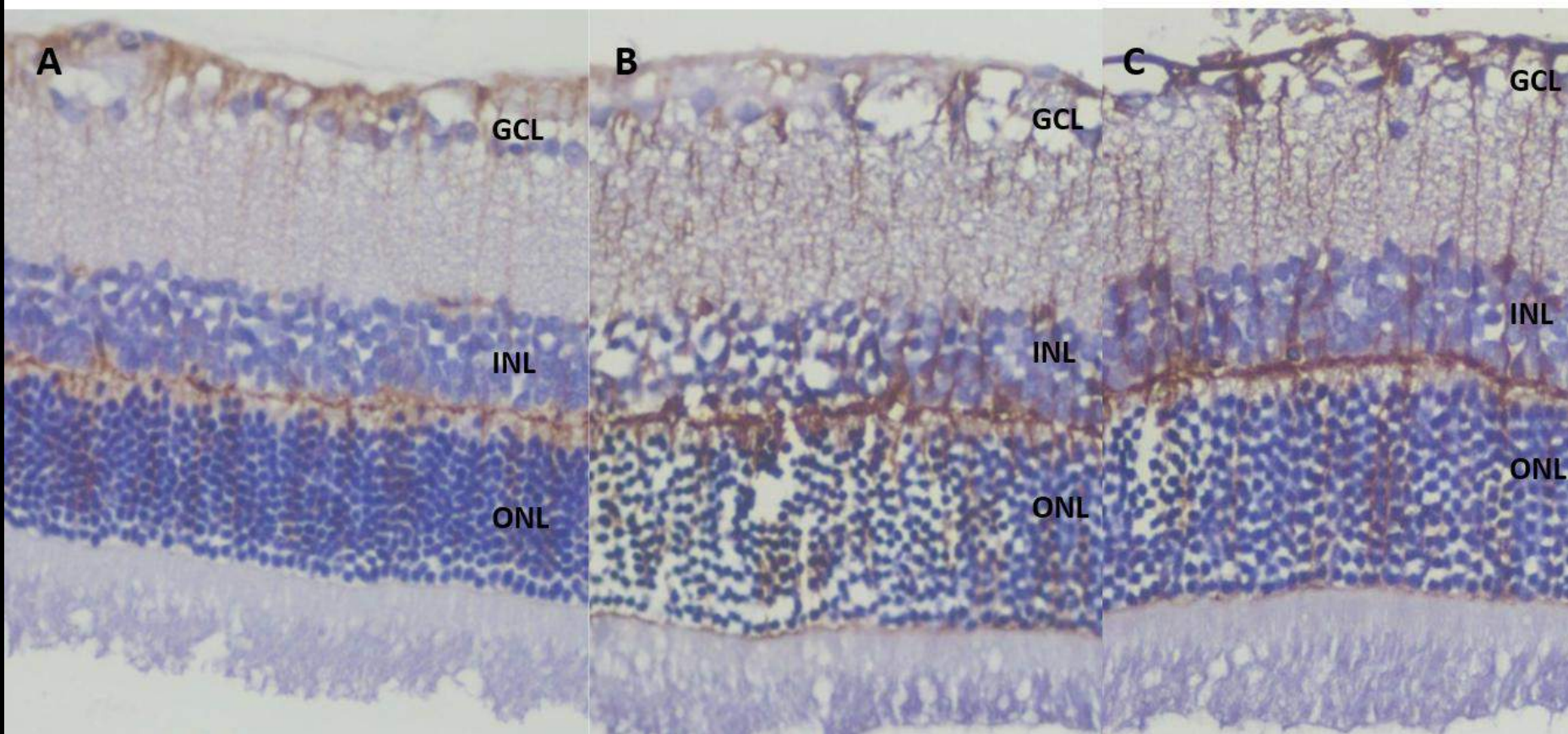


Immünohistokimya : Vimentin



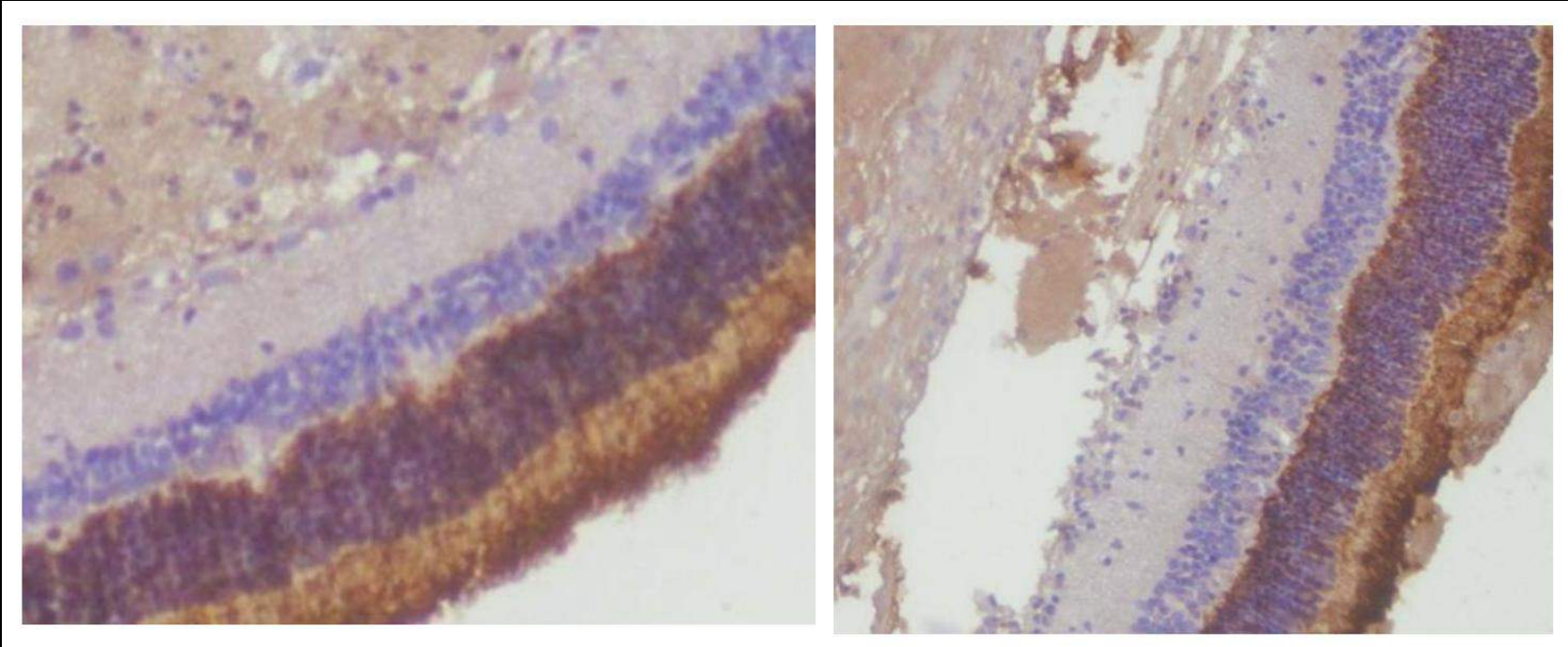
Diyabetik ratlarda anlamlı derecede yükselmiş vimentin ekspresyonu gözlendi
MKH enjekte edilen ratlarda hasta kontrole göre anlamlı fark gözlenmedi

Immünohistokimya : GFAP

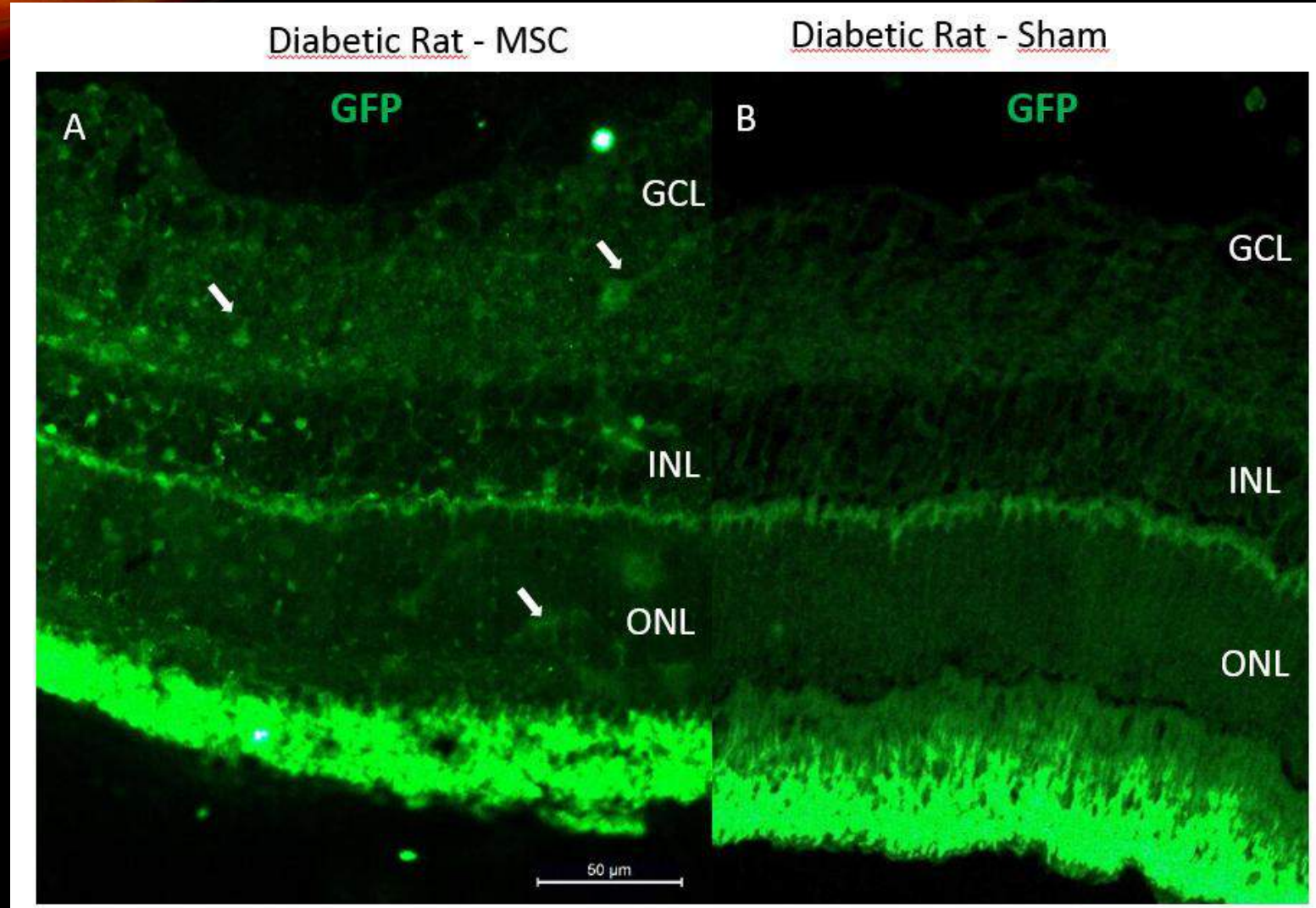


Diyabetik ratlarda anlamlı derecede yükselmiş GFAP ekspresyonu gözlemlendi
MKH enjekte edilen ratlarda hasta kontrole göre **anlamlı gliozis azalması** gözlemlendi ($p < 0.01$)

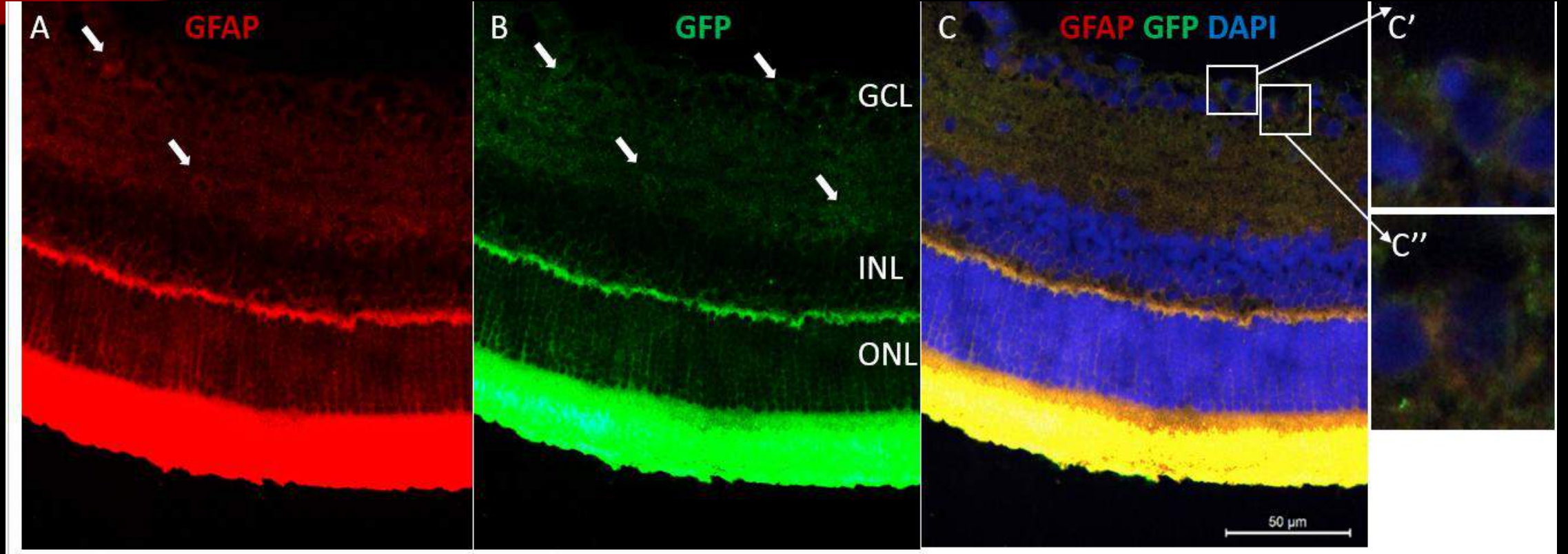
Immünohistokimya : Rodopsin



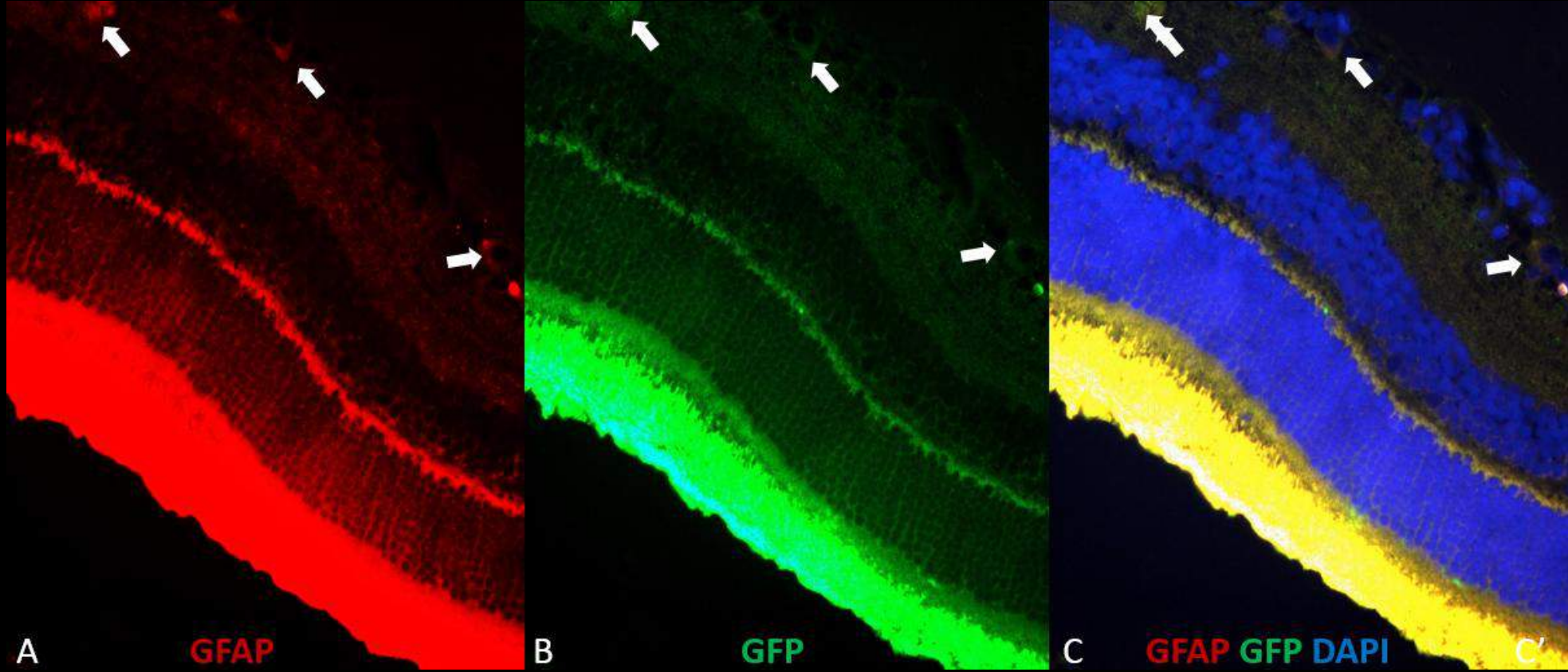
Diyabetik ratlar ile kontrol ratlar arasında anlamlı fark gözlenmedi.



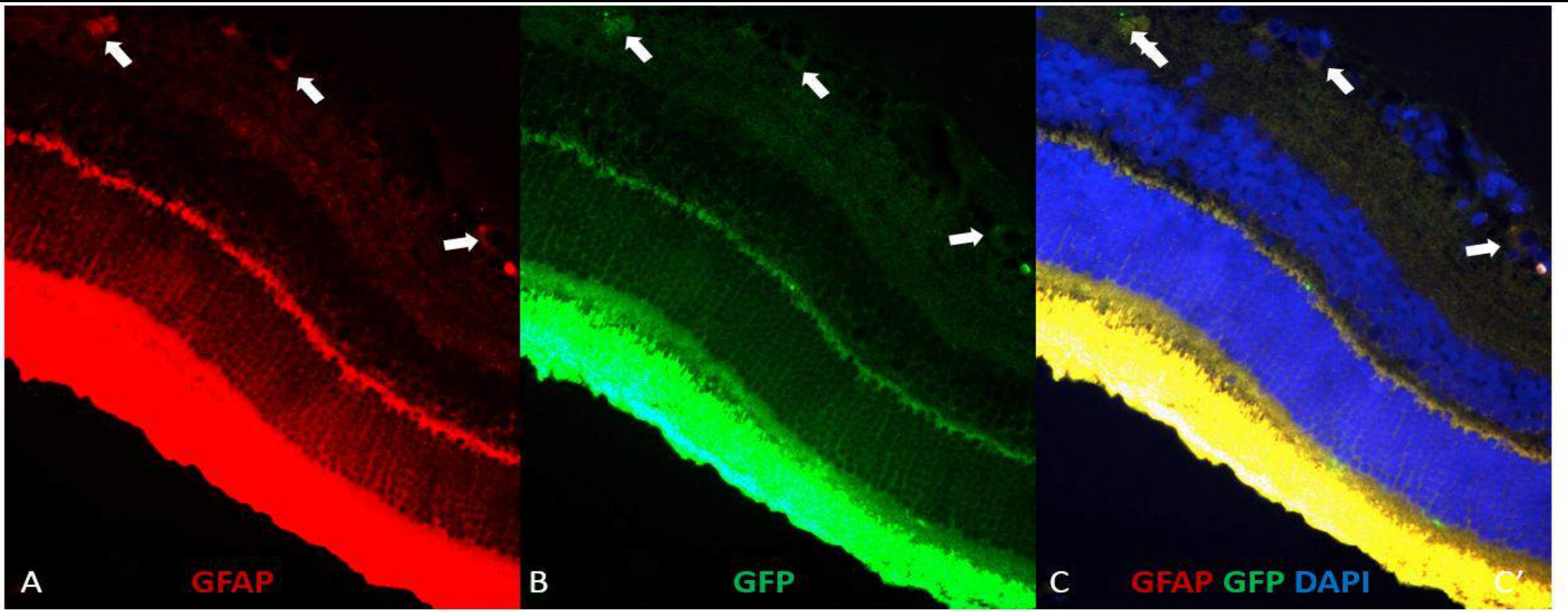
Shame enjekte edilen ratlarla GFP işaretli MKH enjekte edilen rat retinasında GFP ekspresyonu



Ganglion hücre tabakasında GFP ve GFAP koekspresyonu

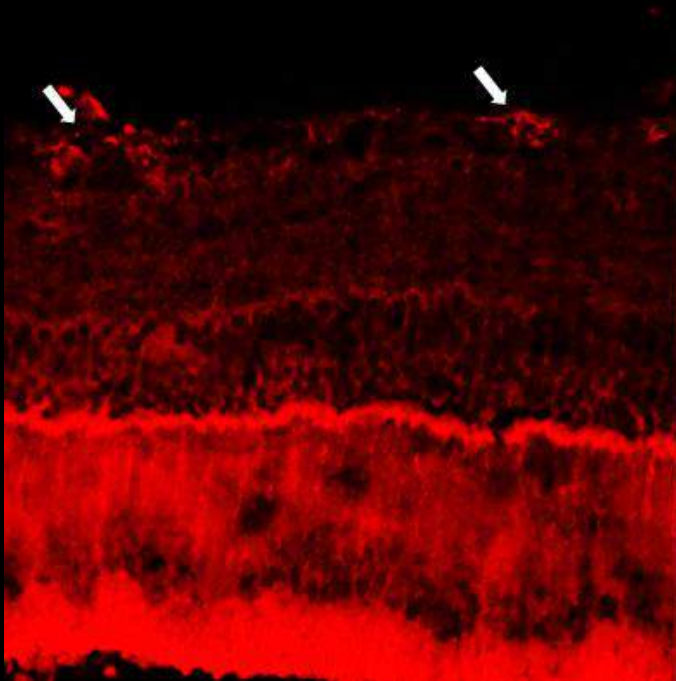


- Ganglion hücre tabakasında GFP ve GFAP koekspresyonu



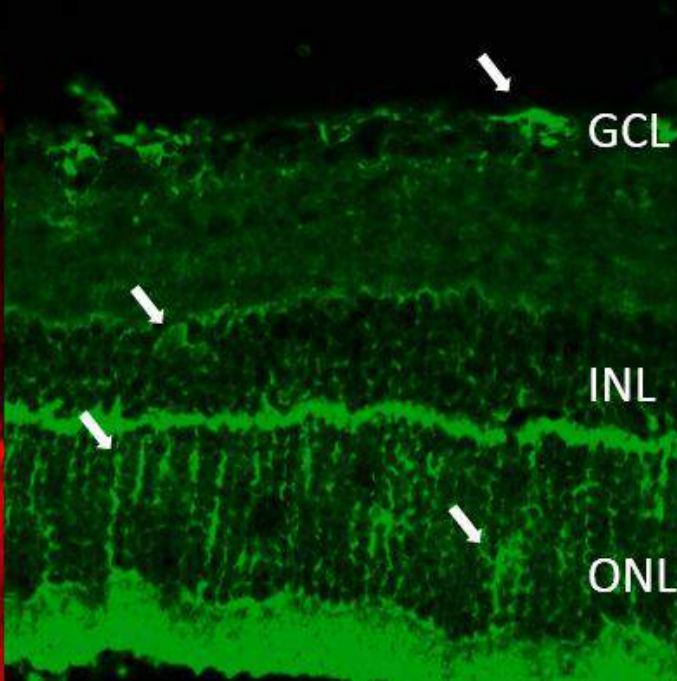
A

GFAP



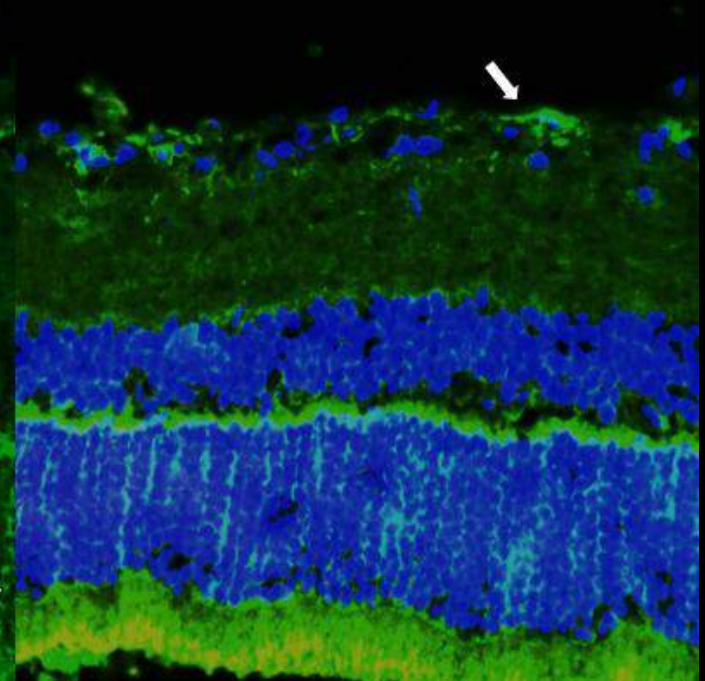
B

GFP

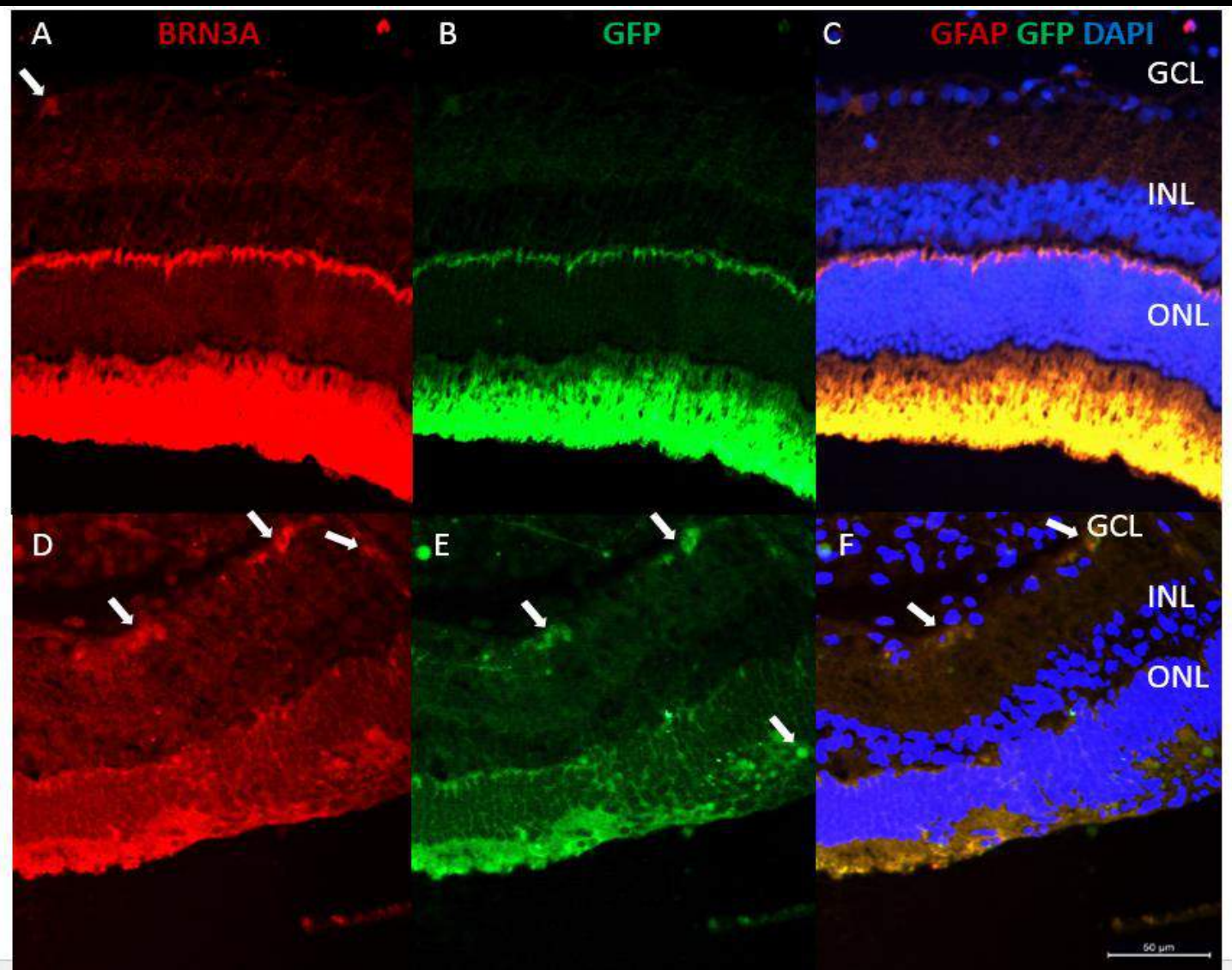


C

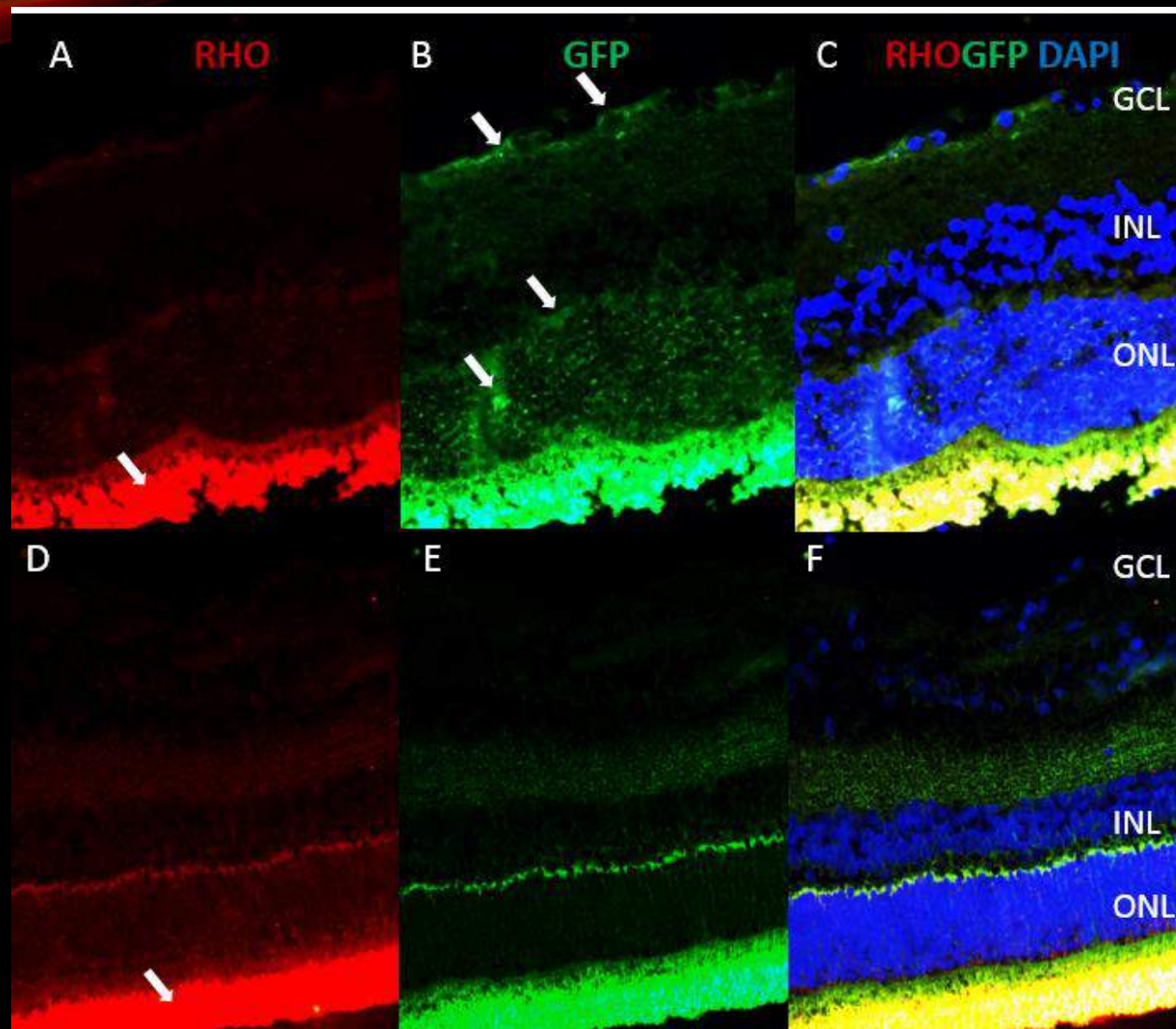
GFAP GFP DAPI




Sham



MSC





Bulgularımız vitre içine enjekte edilen kemik iliđi kaynaklı mezenkimal kök hücrelerin ađırlıklı olarak retinanın Müller ve Mikroglia gibi gliyal hücrelerine dönüştüklerini göstermektedir.

Living optical fibers

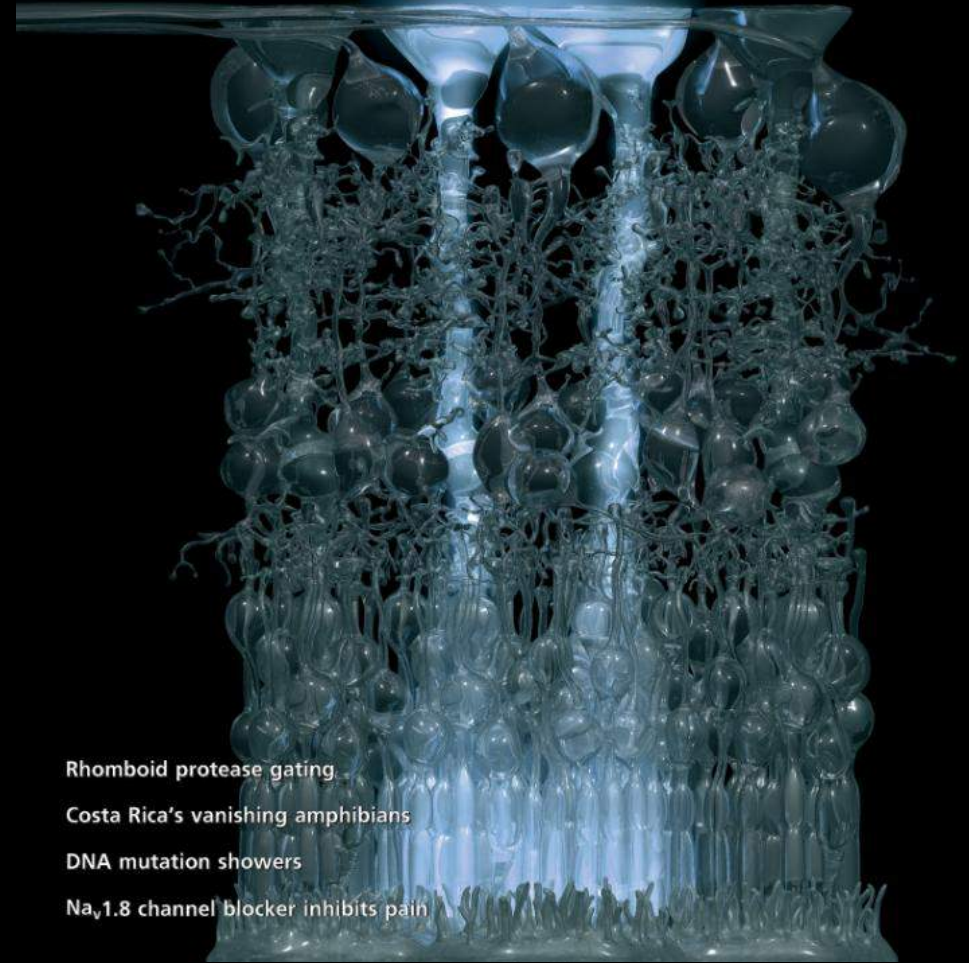
Diyabette Müller hücre **apoptozu**

Müller hücrelerinin diyabette artmış GFAP ekspresyonu yaptığı (**reaktif gliosis**)

Müller hücrelerinin invert retinada ışığı alt tabakalara ileten **optik fiber** gibi davrandığı

Sinapslarda **kılıf** görevi gördüğü

Müller hücrelerinin balıklarda yeniden programlanarak **kök hücrelere dönüştüğü** ve daha sonra retinanın diğer hücrelerine dönüşebildiği gösterilmiştir.





Teşekkürler...