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3 The influence of subretinal injection pressure on the microstructure of the monkey retina

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5 Short title: The influence of subretinal injection pressure on the monkey retina

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26 **Abstract**

27 **PURPOSE:** To investigate the influence of subretinal injection pressure on the
28 microstructure of the retina in a monkey model.

29 **METHODS:** After vitrectomy, balanced salt solution was injected subretinally into one
30 eye each of four cynomolgus monkeys while controlling the injection pressure. Initially,
31 a pressure of 2 psi was used, and this was gradually increased to determine the
32 minimum required pressure. Subsequent injections were performed at two pressures:
33 minimum (n=13) and high (n=6). To compare the influence of these injection pressures
34 on retinal structure, optical coherence tomography (OCT) was performed before
35 surgery and every week afterwards. The monkeys were euthanized and their eyes were
36 enucleated at 1 or 6 weeks after the injections. The eyes were processed for light
37 microscopy and transmission electron microscopy (TEM) as well as for TdT-mediated
38 dUTP nick end labeling.

39 **RESULTS:** The minimum pressure required to perform subretinal injection was 6 psi.
40 After injection at this pressure, both OCT and microscopy showed that the retinal
41 structure was well-preserved throughout the experimental period at all injection sites.
42 Conversely, after injection at high pressure (20 psi) OCT images at all injection sites
43 showed disruption of the ellipsoid zone (EZ) after 1 week. Microscopy indicated damage
44 to the photoreceptor outer segment (OS) and stratification of the retinal pigment
45 epithelium (RPE). After 6 weeks, OCT demonstrated that the EZ had become
46 continuous and TEM confirmed that the OS and RPE had recovered. Photoreceptor
47 apoptosis was absent after subretinal injection at both pressures.

48 **CONCLUSIONS:** The retinal damage caused by subretinal injection increases
49 depending on pressure, indicating that clinicians should perform subretinal injection at
50 pressures as low as possible to ensure safety.