Metabolic impacts of cigarette smoke on the retina of complement-compromised mice

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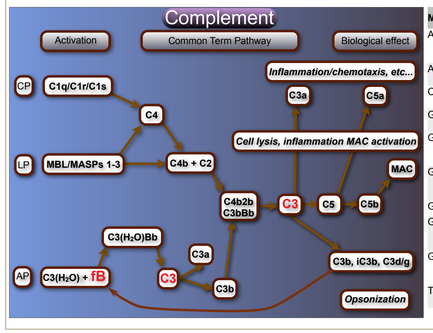
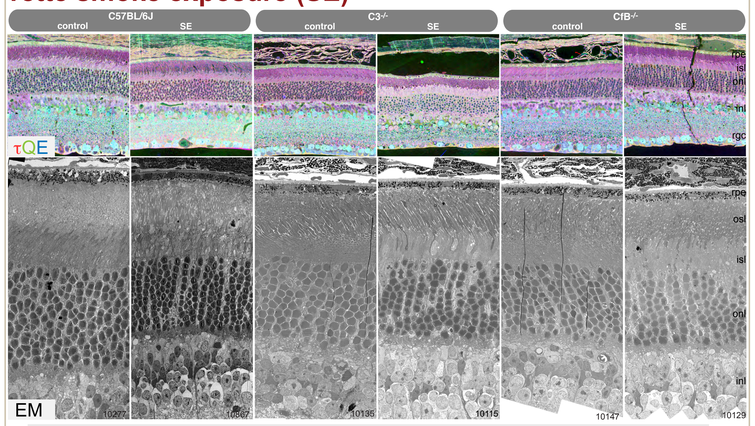
We examined the metabolic and ultrastructure effects of cigarette smoke on retinas of mice deficient in either the alternative pathway (complement factor B, CfB) or the common terminal pathway (complement component 3, C3).



Molecule	Symbol	Major functions
Arginine	R	Activation of mTOR signaling; gene expression regulation; protein methylation/ deimination; immune modulation
Aspartate	D	Purine, pyrimidine, asparagine, and arginine synthesis; transamination; urea cycle
CRABLP	CRABLP	Retinoid binding protein in RPE and Müller glia
GABA	γ	Excitatory neurotransmitter; inhibition of T-cell response and inflammation
Glutamate	E	Glutamine, citrulline, and arginine synthesis; bridging the urea cycle with the Krebs cycle; transamination; ammonia assimilation
Glutamine	Q	Regulation of protein turnover through cellular mTOR signaling, gene expression, a major fuel for rapidly proliferating cells
Gln Synth	GS	glutamate recycling; Müller glia cell marker
Glutathione	J	Free radical scavenger; antioxidant; signal transduction; cellular redox; immune response
Glycine	G	Calcium influx through a glycine-gated channel in the cell membrane; purine and serine synthesis
Taurine	τ	Antioxidant; osmoregulation; anti-inflammation,

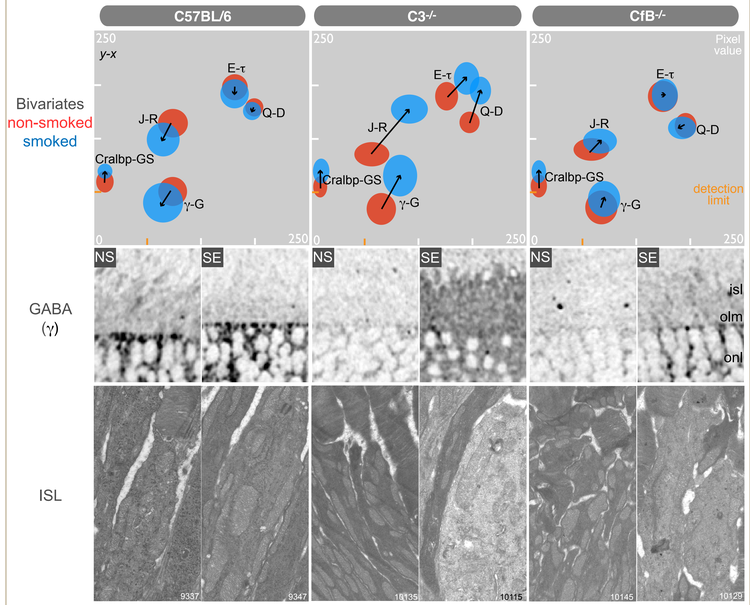
C3$^{-/-}$ retinas display greater metabolic and morphological features of stress than CfB$^{-/-}$ retinas in response to cigarette smoke exposure (SE)



Morphological index	WT NS	WT SE	*T-test*	C3 NS	C3 SE	*T-test*	CfB NS	CfB SE	*T-test*
RPE nuclei/mm	31 ± 6	34 ± 13	0.59	37 ± 6	26 ± 7	0.02	37 ± 14	41 ± 10	0.72
Melanocyte area/mm	7410 ± 3402	5445 ± 1737	0.33	7940 ± 2714	8140 ± 2424	0.92	19515 ± 2964	19372 ± 3119	0.96
Melanocytes/mm	3828 ± 1832	2579 ± 675	0.24	3347 ± 240	3052 ± 725	0.54	5850 ± 286	5546 ± 279	0.32
ISL electron density	122 ± 0	109 ± 24	0.52	136 ± 4	94 ± 1	0.00	133 ± 8	96 ± 2	0.02
PR/mm	2308 ± 320	2059 ± 310	0.17	2500 ± 176	2171 ± 141	0.04	3414 ± 253	3326 ± 321	0.77
Pyknotic bodies/mm	2 ± 3	1 ± 3	0.75	3 ± 2	14 ± 11	0.14	4 ± 5	0 ± 0	0.27
cones/mm	64 ± 14	60 ± 13	0.64	71 ± 12	80 ± 13	0.39	96 ± 24	84 ± 13	0.51
CRALBP at ONL area/mm	9922 ± 3132	17082 ± 2927	0.04	5774 ± 478	15077 ± 2498	0.04	7719 ± 1190	16359 ± 98	0.01
MG somal electron density				125 ± 16	89 ± 7	0.03			

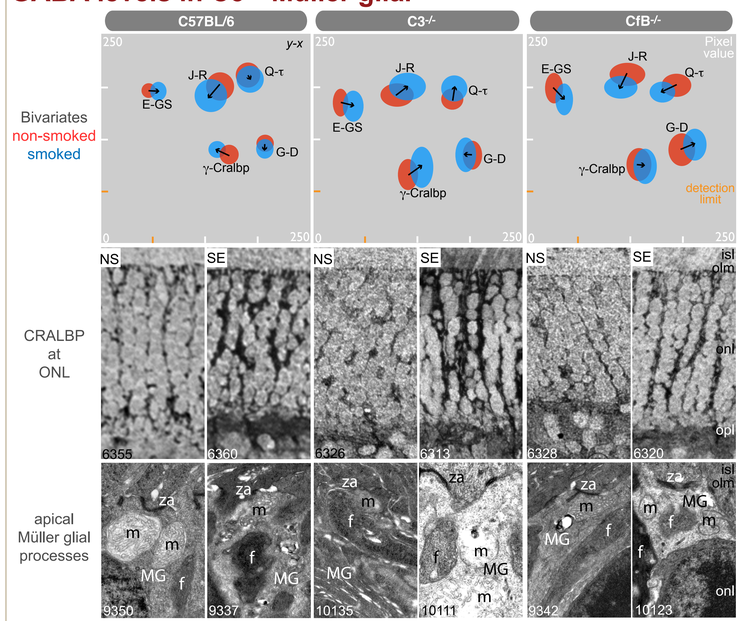
Mice were exposed to cigarette smoke for 6 h/day, 5 day/wk for 6 months using an automated machine. Eyes were fixed in glutaraldehyde. Metabolic maps were generated using adjacent ultrathin sections (vertical stack 10309) that were probed with specific anti-hapten IgGs, visualized with silver-intensification, and overlayed in Photoshop. Electron microscope (EM) mosaics represent ~200 tiles at 5000x. Morphological quantitation was obtained from biological replicates and EM mosiacs expanding ~300 µm of retina.

SE enhances photoreceptor cell loss, mitochondrial swelling and GABA levels in C3$^{-/-}$ photoreceptors.



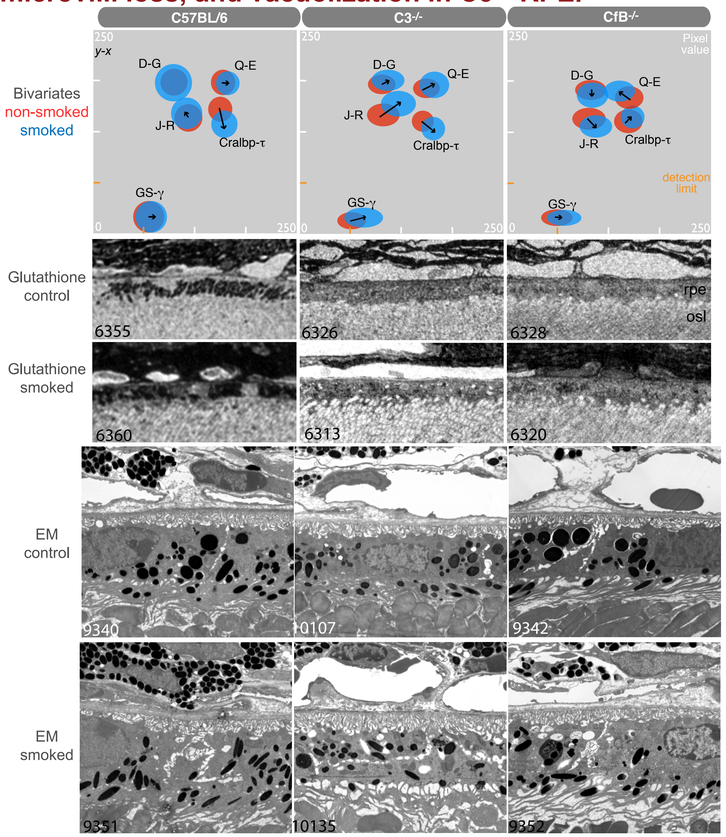
Stacked bivariate histograms represent ten signals mapped on x-y pixel values from the analyses of vertical stack 10309. The metabolic function of photoreceptors can be monitored using their classic signature (E-τ), stress markers (J-R), energy/synthetic metabolites (Q-D), moderate levels of γ-G, and low levels of glial markers (CRALBP-GS). EM of ISL show either pyknosis of ellipsoids or swelling of mitochondria.

SE enhances hypertropy, mitochondrial swelling, and GABA levels in C3$^{-/-}$ Müller glia.



Müller cell function is monitored using their classic signature (τQ), stress markers (J-R), glutamate (E-GS) and GABA (γ) metabolizing capacity, and retinoid binding protein CRALBP. CRALBP shows hypertrophy of apical MG processes at the ONL. Ultrastructure also shows mitochondrial swelling and electron density decrease. Abreviations: za, zonula adherens; m, mitochondria; MG, Müller glia; f, outer fiber.

SE induces nuclei loss as well as enhanced glutathione, microvilli loss, and vacuolization in C3$^{-/-}$ RPE.



RPE function is monitored using readouts of retinoid binding (CRALBP), taurine transport/osmoregulation (τ), glutamate metabolism/transport (DEQ), stress markers (J-R), and low GABA and GS levels. Glutahione is elevated in CSE C3$^{-/-}$ and less variable in CfB$^{-/-}$ RPE. Ultrastructure also enhaced vacuolization and microvilli loss. Our previous work showed that smoke increases RPE mitochondrial size concomitant with an apical shift in mitochondrial distribution within the RPE and a thickening of Bruch's membrane. CfB$^{-/-}$ mice were protected from developing these SE-mediated alterations (Woodell et al., PLoS ONE, 2013).

1. **SE effects are widespread including mitochondrial swelling in RPE, photoreceptors and Müller glia as well as bipolar cell swelling and vacuolization of amacrine cell layer.**
2. **Complement activation is necessary to maintain homeostasis, as the absence of all terminal pathway signaling in C3$^{-/-}$ retinas enhanced susceptibility to SE.**
3. **GABA is a novel marker of retinal stress. Potential mechanisms:**

1. GABA and GAD immunoreactivity of photoreceptors of primate retina, Nishimura et al., *Nature* 1986. **2**. Localization and metabolism of Glu, Asp, and GABA in the rat retina, Voaden et al., *Neurochemistry* 1980. **3**. Function of taurine transporter as a GABA transporting protein, Tomi et al., *BBA* 2008. **4**. Effects of acute ethanol exposure on polyamine and GABA metabolism in the regenerating liver, Luo et al., *Alcohol* 1999.

NIH Grants EY015128 (BWJ), EY014800 Vision Core (BWJ), RPB CDA (BWJ), Thome Foundation, Bank of America (BWJ), RPB (unrestricted grant to the Moran Eye Center), NIH training grant HD07491 (FVC), IRRF (FVC), KTEF (FVC), FFS (FVC), UofU (AB). Special thanks to Drew Ferrell, for technical support.