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Stress promotes lipid droplet accumulation in astrocytes

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Neuron-glia coupling of glucose and lipid metabolism in the brain

Regulation of **brain energy metabolism** – **bidirectional communication** between astrocytes and neurons.

Astrocytes metabolically support neurons with nutrients (e.g., glucose, lactate, free fatty acids).

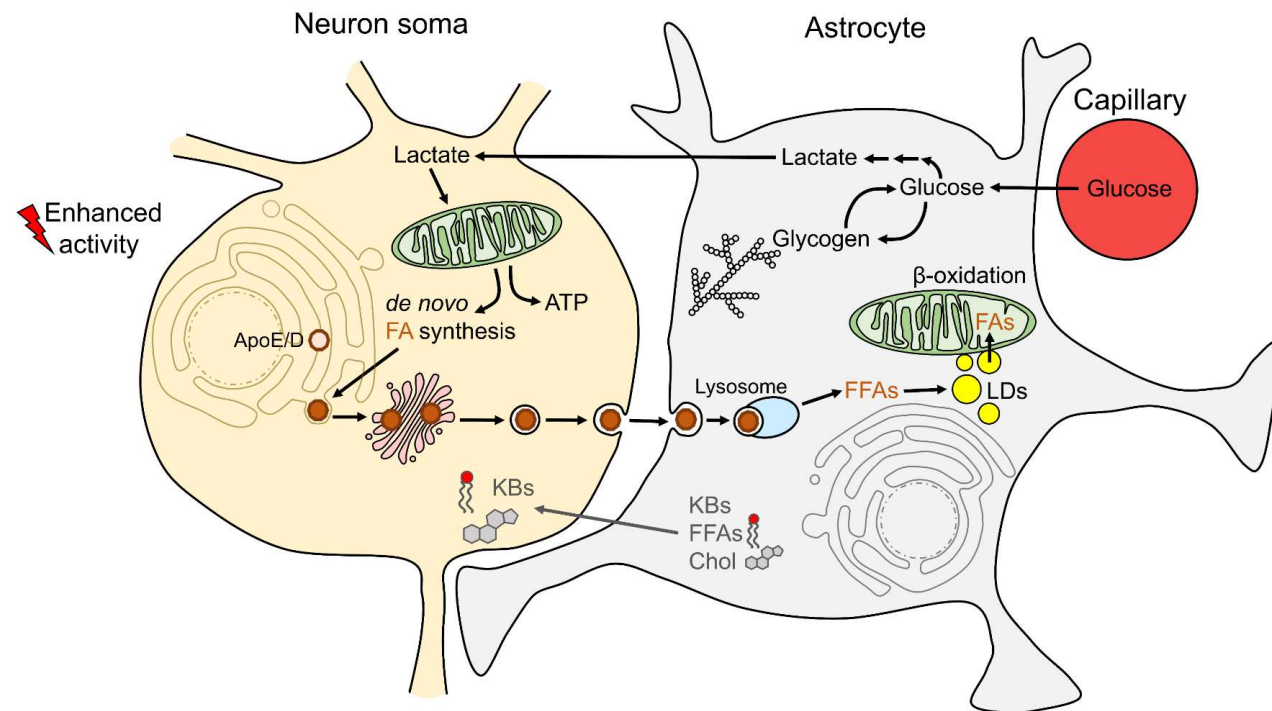
Astrocyte to neuron lactate shuttle (Pellerin, 1994)
→ glucose uptake and **lactate production** via **aerobic glycolysis**.

! The production of lactate in glial cells is regulated by neuronal activity.



In neurons, **lactate** can

- 1) be used as a **fuel in oxidative metabolism** (Pellerin and Magistretti, 2016) or
- 2) serve as a **substrate for *de novo* synthesis of FFAs** (Ioannou et al., 2019).



Smolič et al. 2021, Antioxidants

Lipid droplets are predominantly found in glial cells

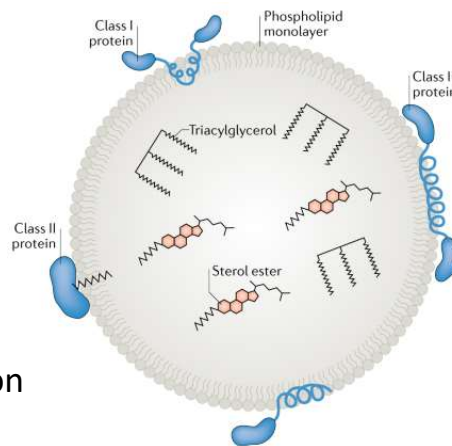
Excessive FFAs can be lipotoxic for **NEURONS**.



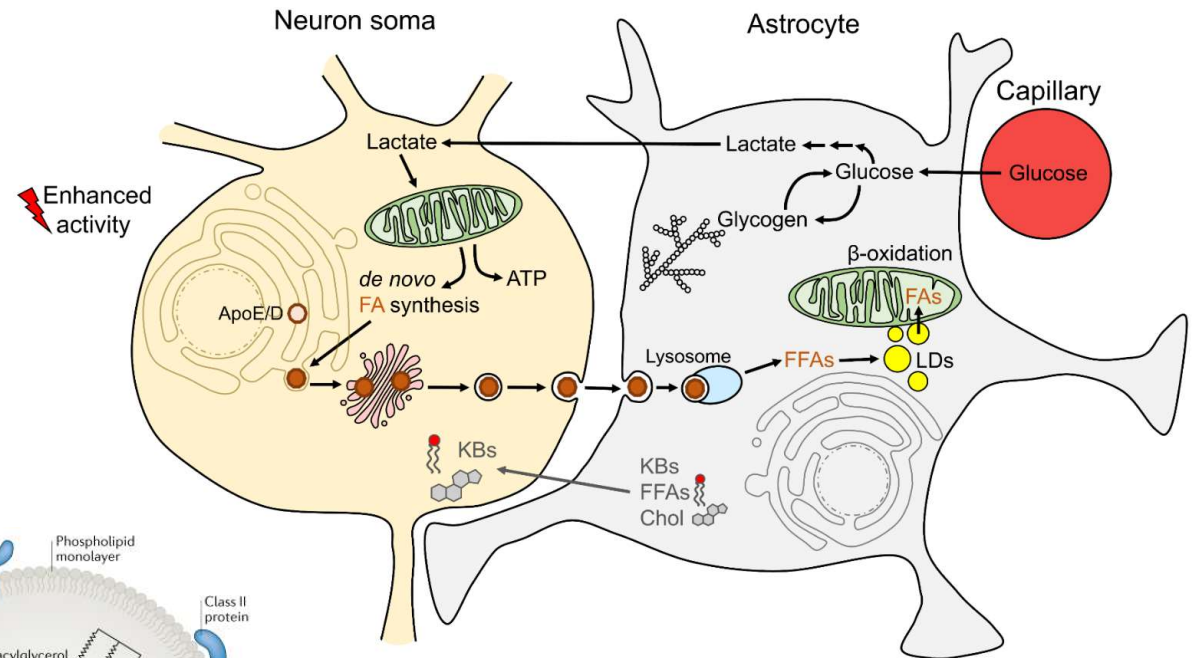
FFAs are **transferred to astrocytes** and stored in **LIPID DROPLETS** (LDs) (Ioannou et al., 2019).

LIPID DROPLETS

- **storage organelles** surrounded by a phospholipid monolayer and proteins
- composed of **neutral lipids**
- assembly involves **ER-resident proteins** (i.e., DGAT1 and DGAT2)
- provide substrates for energy metabolism, building blocks for membranes and protection against lipotoxicity



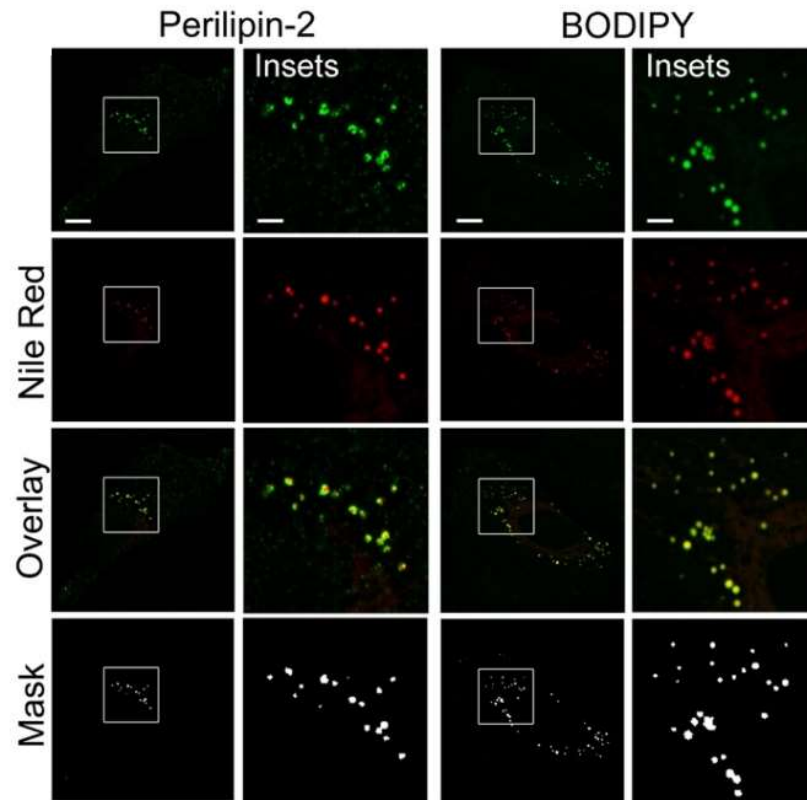
Olzmann and Carvalho 2019, Nature Reviews



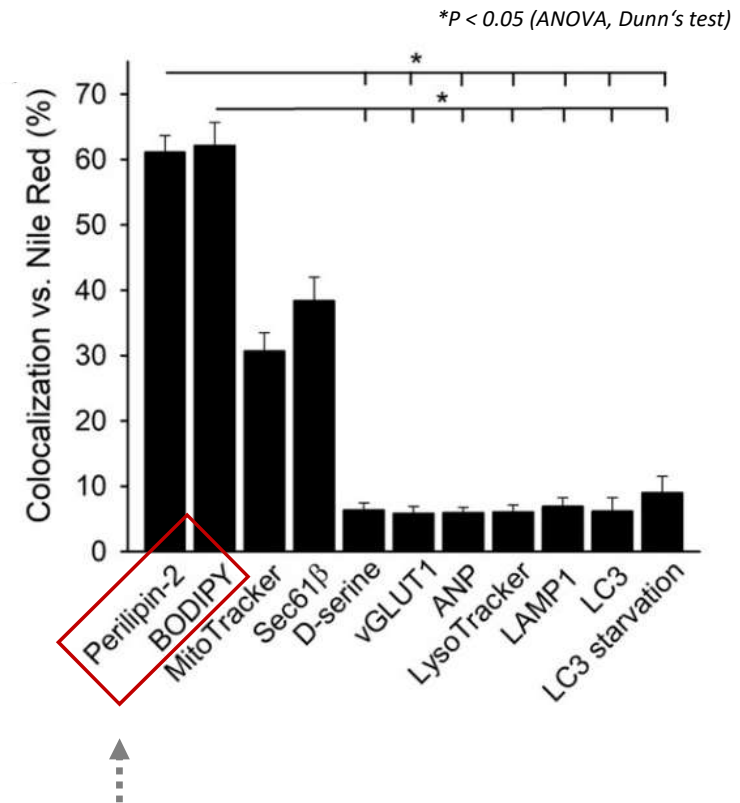
Smolič et al. 2021, Antioxidants

! The content of lipid droplets in the brain increases during aging and in neurodegenerative diseases (i.e., Alzheimer's disease, ALS), predominantly in glial cells.

Lipid droplets are present in resting astrocytes

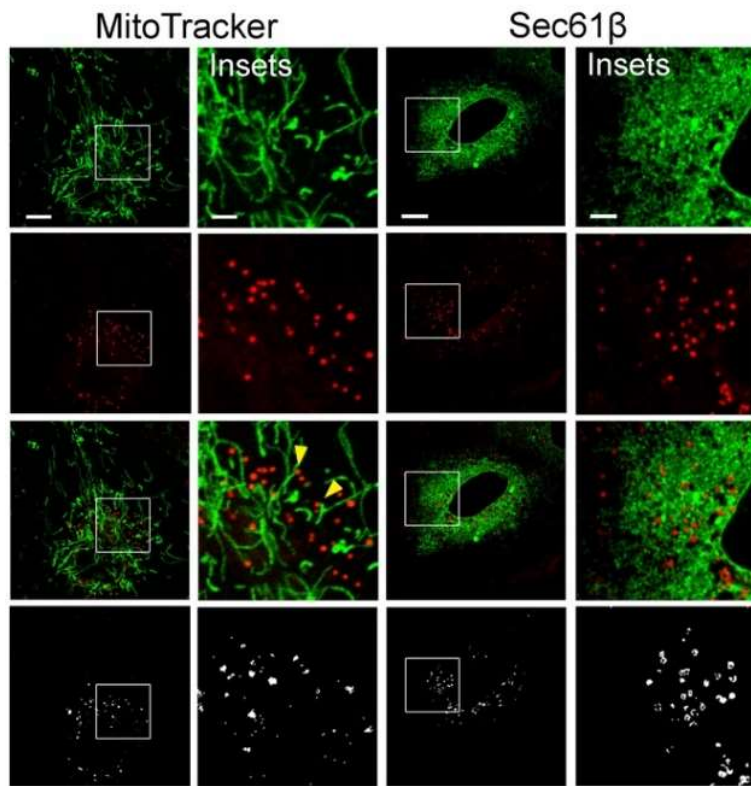


Scale bars: 10 μm
Scale bars (insets): 5 μm

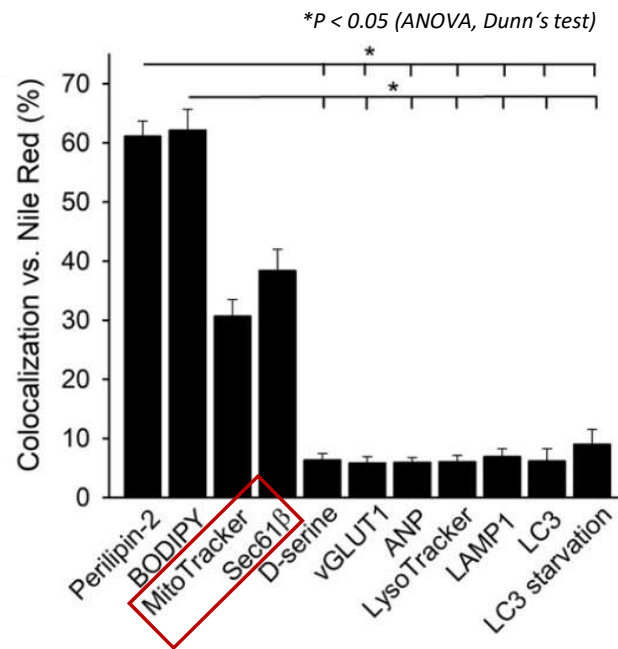


High colocalization of Nile Red-labelled
LDs with **perilipin-2** and **BODIPY**.

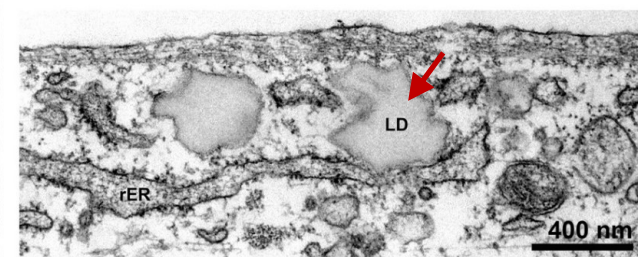
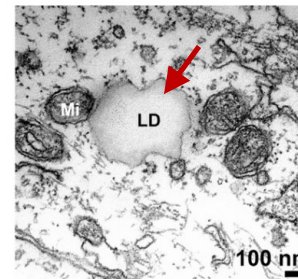
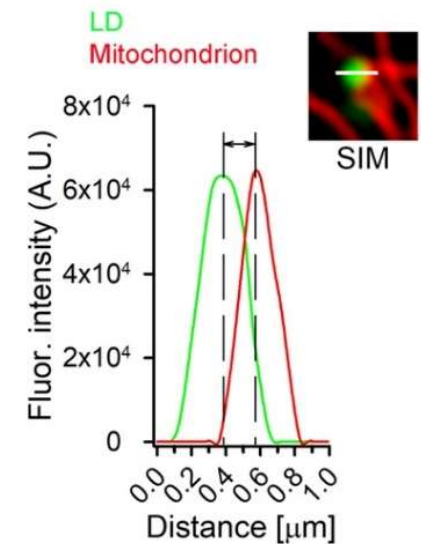
Astroglial lipid droplets are positioned near mitochondria and ER



Scale bars: 10 μm
Scale bars (insets): 5 μm

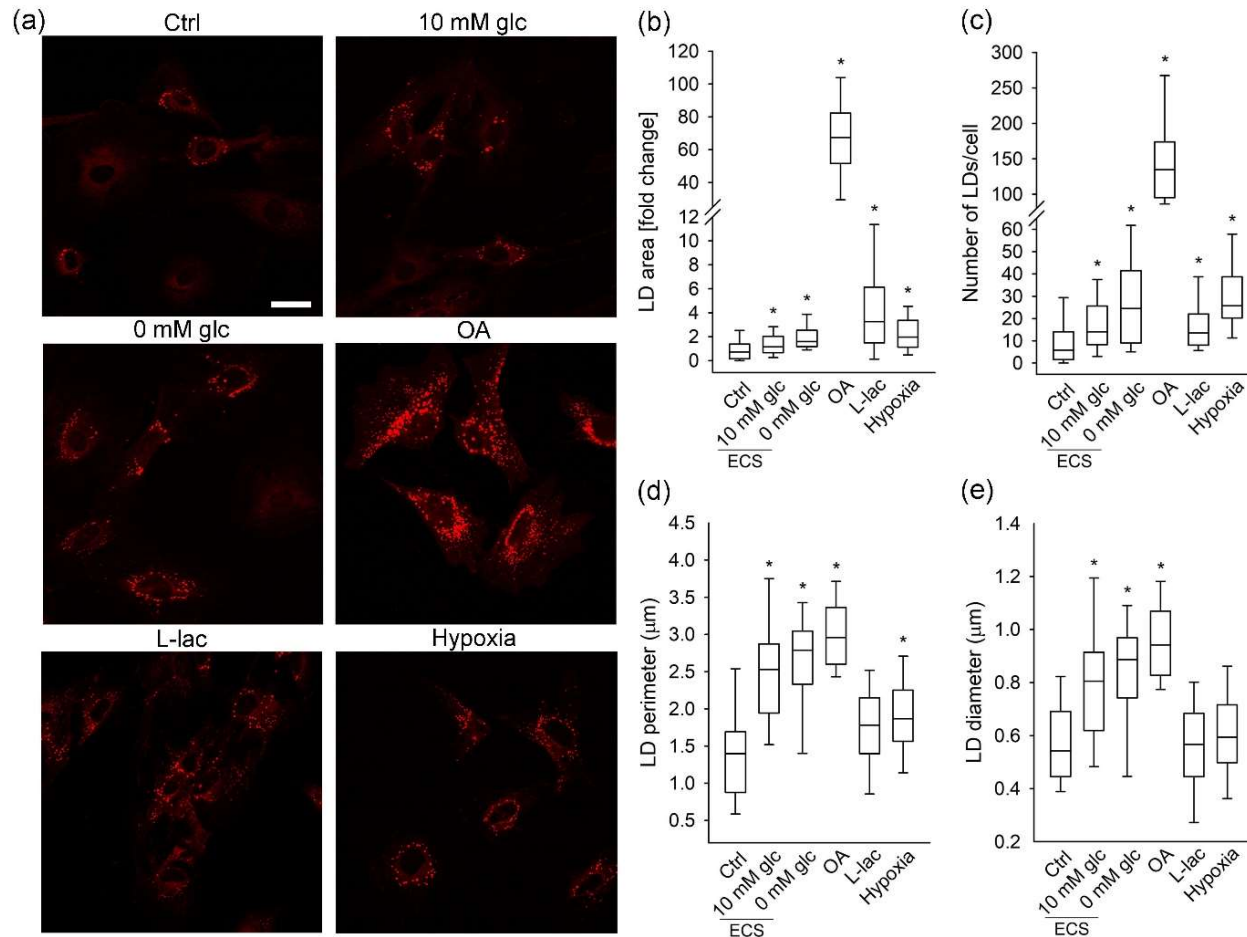


Relatively high colocalization of Nile Red-labelled LDs with MitoTracker and Sec61β.



Smolič et al. 2021, *Glia*

Nutrient and hypoxic stress trigger LD accumulation in astrocytes *in vitro*



Nutrient stress:

- partial/complete nutrient deprivation (10 and 0 mM glucose)
- excess FFAs (300 μM oleic acid (OA))
- Excess L-lactate (20 mM L-lac)

Hypoxic stress:

- 1% pO_2

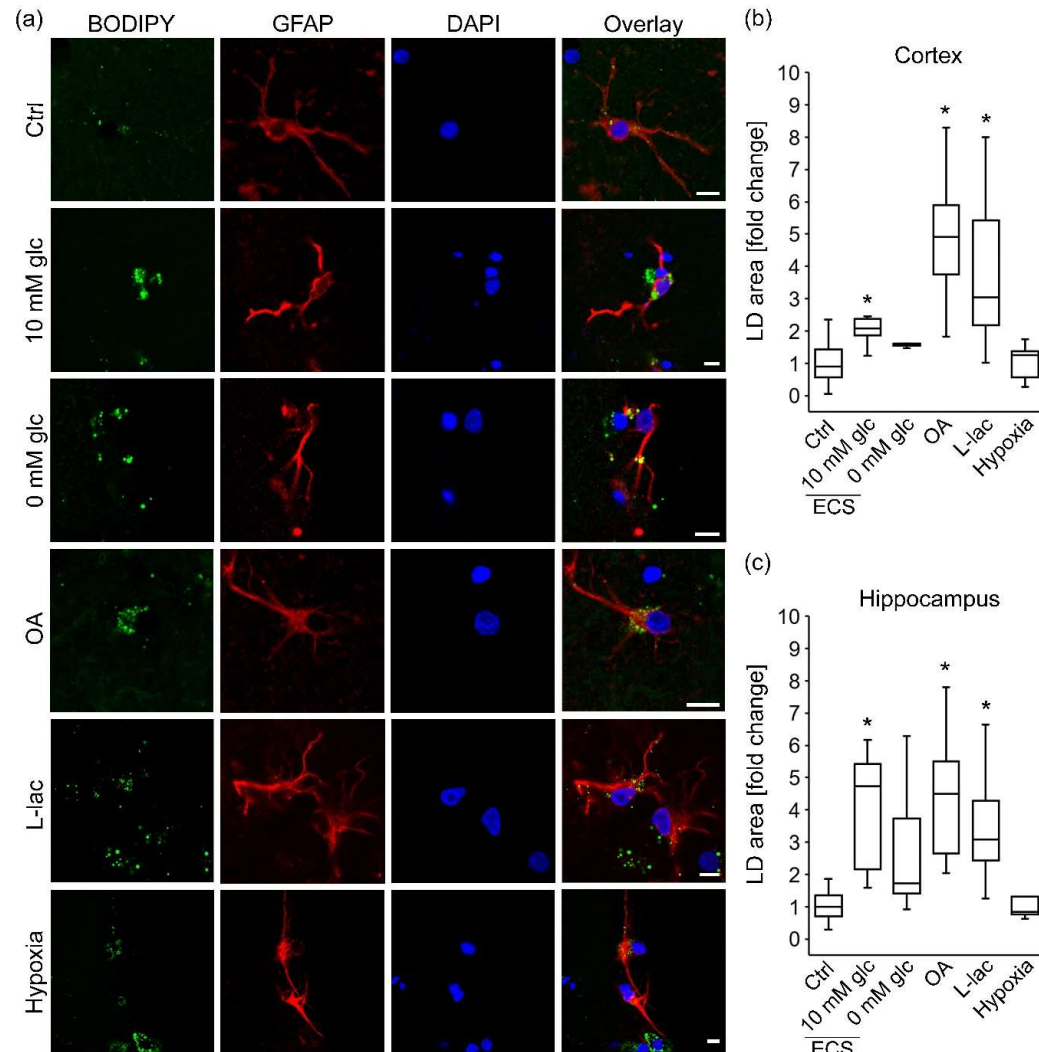
Nutrient and hypoxic stress (24 h exposure):

- 1) increased LD area
- 2) increased number of LDs/cell
- 3) increased LD perimeter and diameter (nutrient stress only)

Scale bar: 20 μm

* $P < 0.05$ (ANOVA, Dunn's test)

Nutrient stress triggers LD accumulation in astrocytes *in situ*



Nutrient stress:

- partial/complete nutrient deprivation (10 and 0 mM glucose)
- excess FFAs (300 μ M oleic acid (OA))
- Excess L-lactate (20 mM L-lac)

Hypoxic stress:

- 1% pO₂

Nutrient stress (24 h exposure):

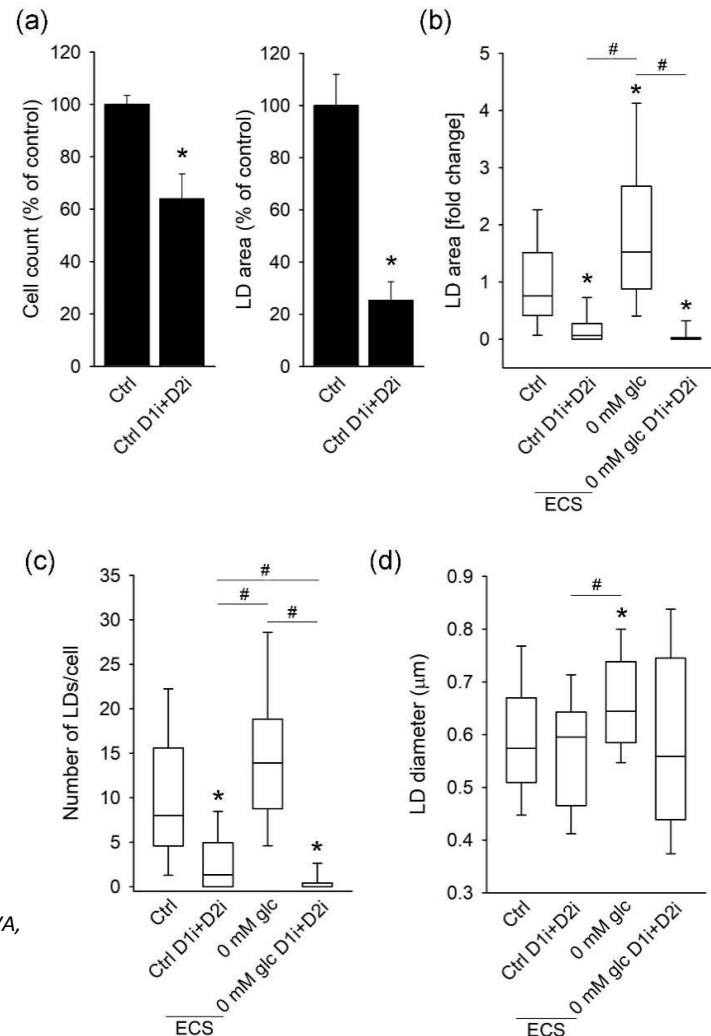
- increased LD area in hippocampal and cortical astrocytes.

Scale bars: 10 μ m

* $P < 0.05$ (ANOVA, Dunn's test)

Smolič et al. 2021, *Glia*

Inhibition of DGAT1 and DGAT2 decreases number of astrocytes and LD content



D1i – DGAT1 inhibitor (10 μM)

D2i – DGAT2 inhibitor (10 μM)

Attenuation of *de novo* LD biogenesis by inhibitors of DGAT1 and DGAT2 enzymes (24 h exposure):

- 1) reduced number of astrocytes by ~40%
- 2) decreased LD content by ~80%
- 3) decreased LD content in cells deprived of glucose (starvation)



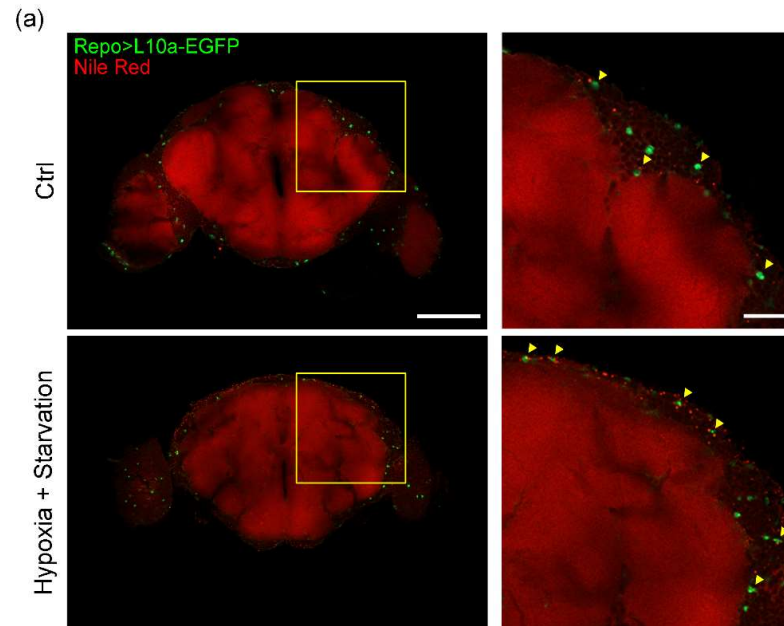
Accumulation of LDs in astrocytes is the result of *de novo* biogenesis of LDs.

LD turnover is important for cell survival and/or proliferation.

* $P < 0.05$ vs. Ctrl (ANOVA, Dunn's test)

$P < 0.05$ all pairwise (ANOVA, Dunn's test)

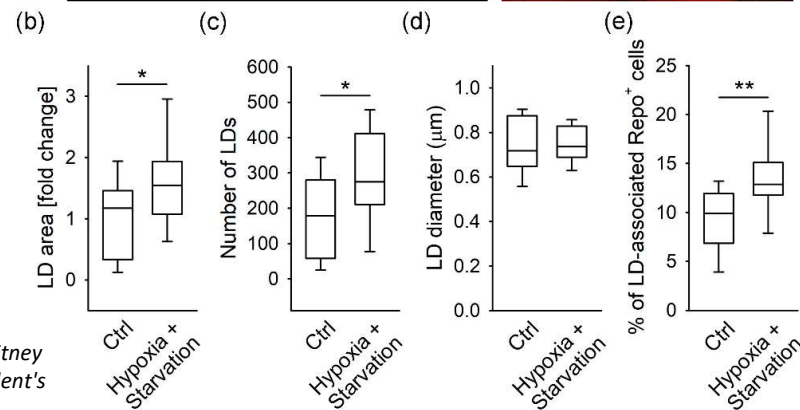
In vivo nutrient and hypoxic stress trigger LD accumulation in the fly brain



Scale bar: 100 μ m
Scale bar (insets): 20 μ m

***In vivo* exposure of *Drosophila melanogaster* to starvation and hypoxic stress (24 h):**

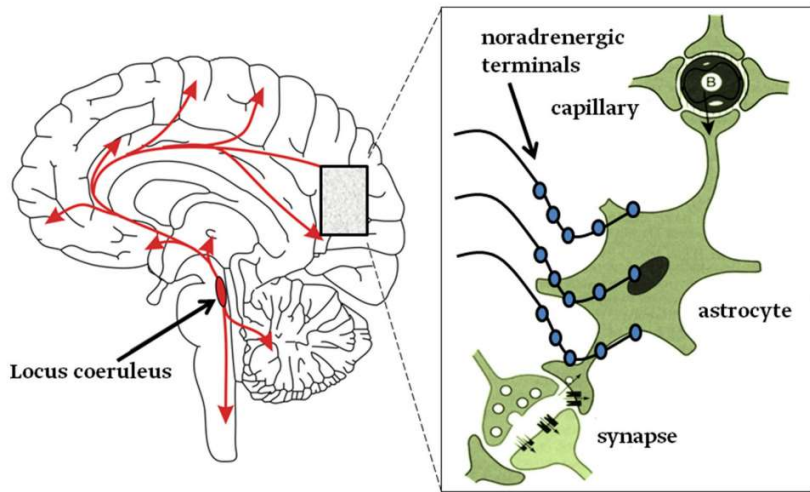
- 1) increased LD area and number in the fly brain
- 2) the percentage of LD-associated glial cells increased by 46%



* $P < 0.05$, ** $P < 0.01$; Mann–Whitney Rank Sum Test (b and e) and Student's *t*-test (c)

Adrenergic activation triggers LD accumulation in astrocytes *in vitro*

Noradrenergic system in the human brain

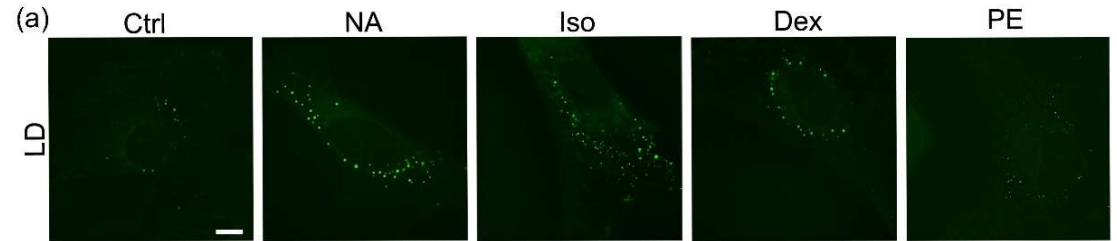


Leanza, Gulino and Zorec 2018, *Frontiers in Molecular Neuroscience*

Adrenergic receptor (AR)	Agonist	Antagonist
α_1 -AR, α_2 -AR, β -AR	NA	
β -AR (G_s proteins, \uparrow cAMP)	Iso	Prop
α_2 -AR (G_i proteins, \downarrow cAMP)	Dex	Atip
α_1 -AR (G_q proteins, \uparrow Ca ²⁺)	PE	Tera

Noradrenaline and selective β -AR agonist isoprenaline (24 h exposure):

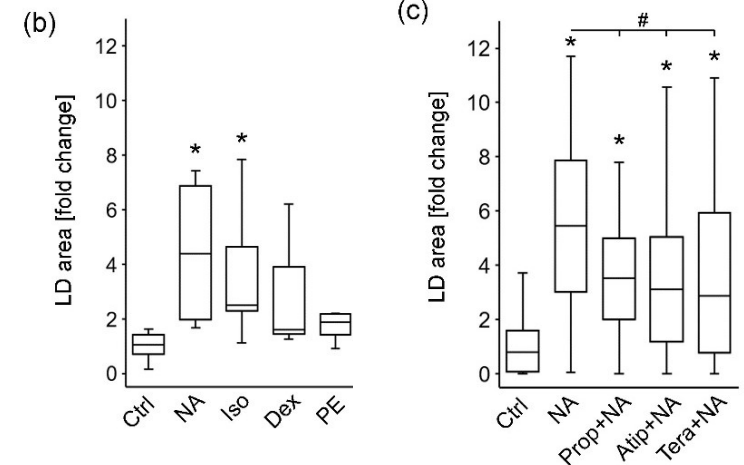
- 1) increased LD area
- 2) NA-mediated increase was attenuated in the presence of AR antagonists



Scale bar: 10 μ m

* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. Ctrl (ANOVA, Dunn's test)

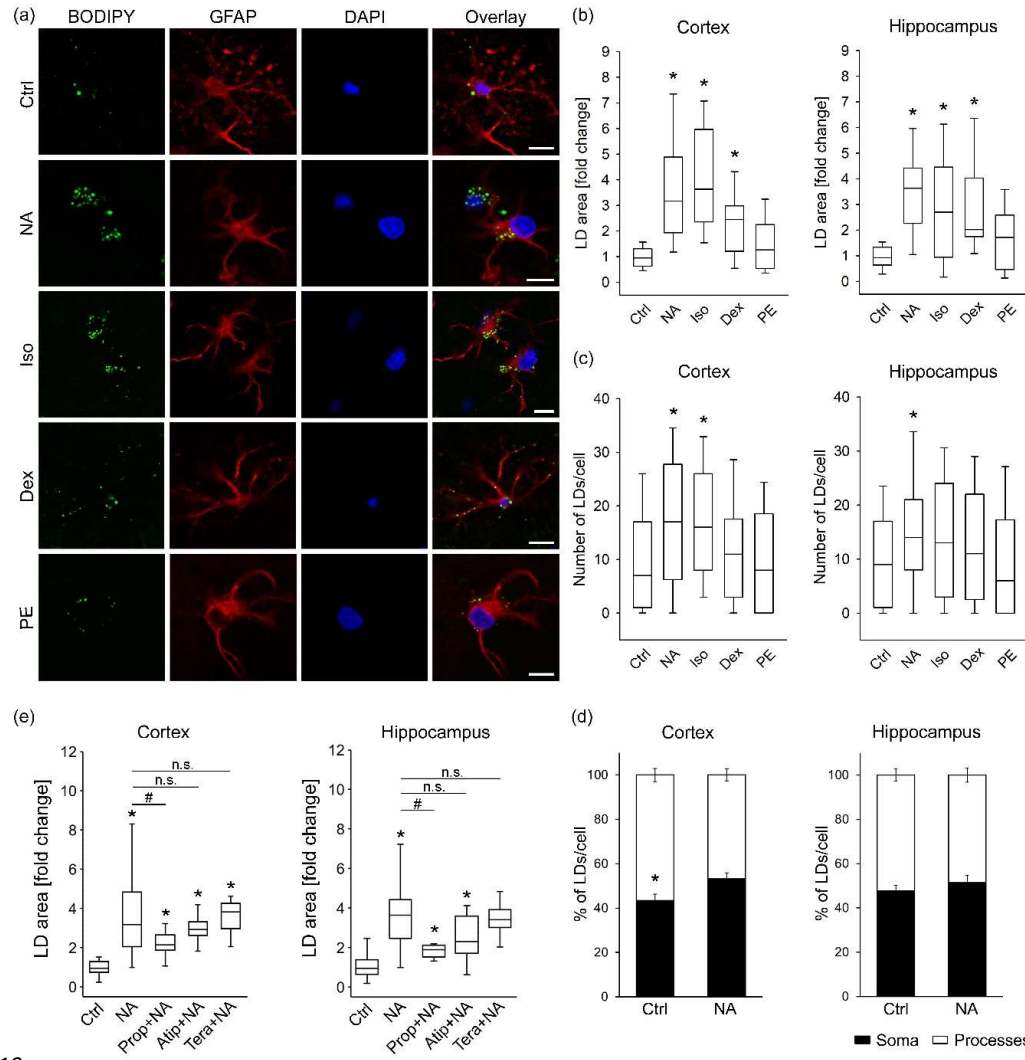
$P < 0.05$ vs. NA (ANOVA, Dunn's test)



Noradrenaline increases LD accumulation in cultured astrocytes through β -AR activation.

Smolič et al. 2021, *Glia*

Adrenergic activation triggers LD accumulation in astrocytes *in situ*



Noradrenaline and selective β - and α_2 -AR agonist isoprenaline and dexmedetomidine, respectively (24 h exposure):

- 1) increased LD area
- 2) NA-mediated increase was attenuated in the presence of β -AR antagonist

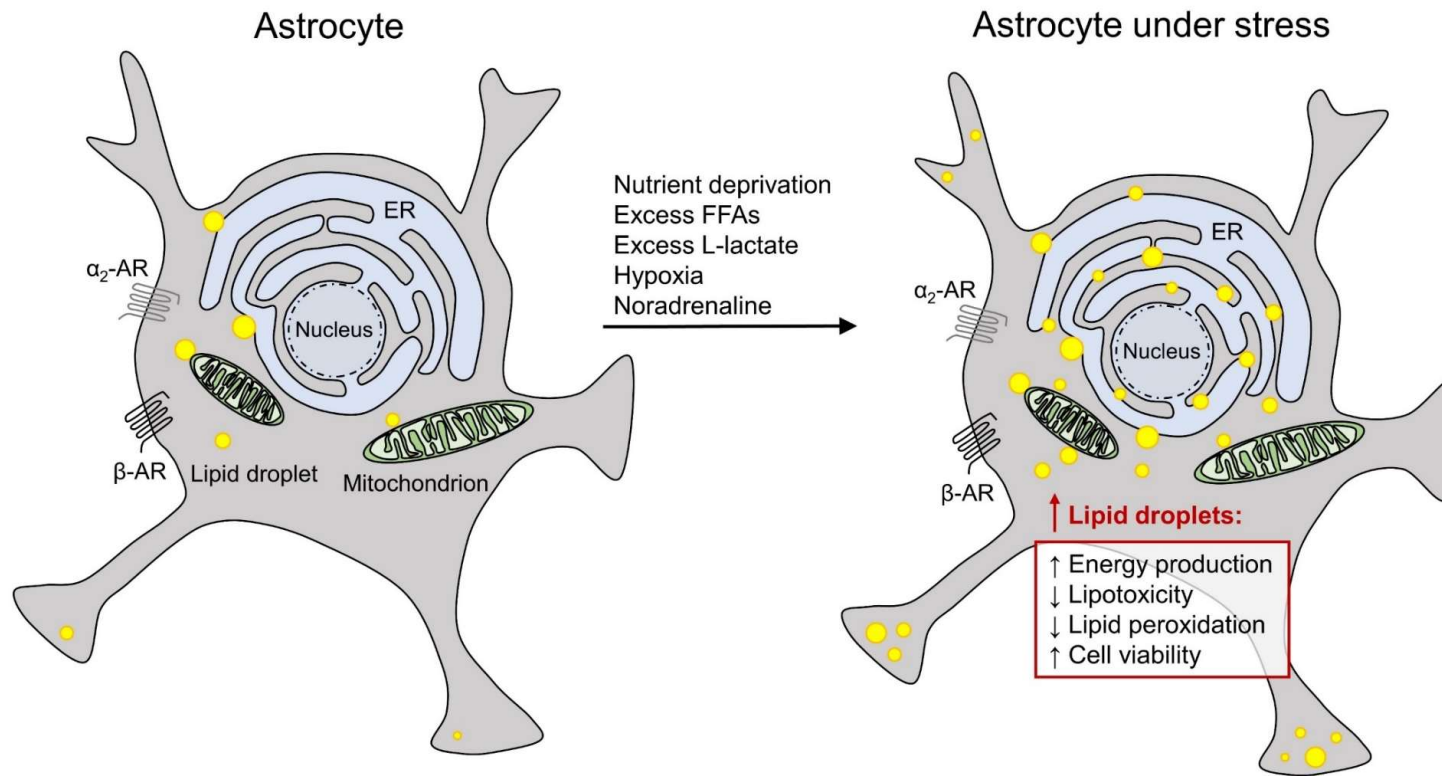


Noradrenaline increases LD accumulation in brain tissue astrocytes through β - and α_2 -AR activation.

* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. Ctrl (ANOVA, Dunn's test)

$P < 0.05$ vs. NA (ANOVA, Dunn's test)

Conclusions



Thank you for your attention!

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