14° CONGRESSO NAZIONALE SINut

SINut Società Italiana di Nutraceutica

12-14 settembre 2024

Bologna

«*Punica granatum l.*, Acido ellagico e Urolitine come modulatori delle vie noradrenergiche centrali: dagli effetti sistemici alle interazioni molecolari»

Veronica Torre

PhD in Medicina Sperimentale, Dipartimento di Farmacia, Università di Genova

Il sottoscritta ……Veronica Torre…….
ai sensi dell'art. 3.3 sul Conflitto di Interessi, pag. 17 del Reg. Applicativo dell'Accordo Stato-Regione del 5 novembre 2009,

dichiara

che negli ultimi due anni NON ha avuto rapporti diretti di finanziamento con soggetti portatori di interessi commerciali in campo sanitario

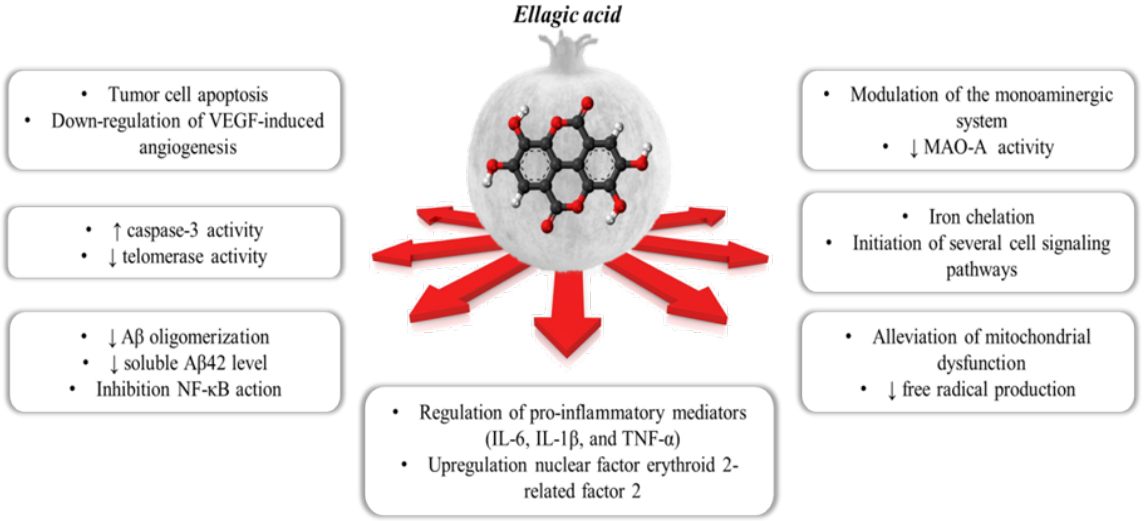
che negli ultimi due anni ha avuto rapporti diretti di finanziamento con i seguenti soggetti portatori di interessi commerciali in campo sanitario:

- *……*
- *……*
- *……*

Ellagic Acid (EA): sources and properties

Ellagitannins (ET) and their main hydrolysis product, ellagic acid (EA), are polyphenols naturally present in some fruits: pomegranates, black raspberries, raspberries, strawberries, but also in walnuts and almonds

Pharmacological effects of ellagic acid

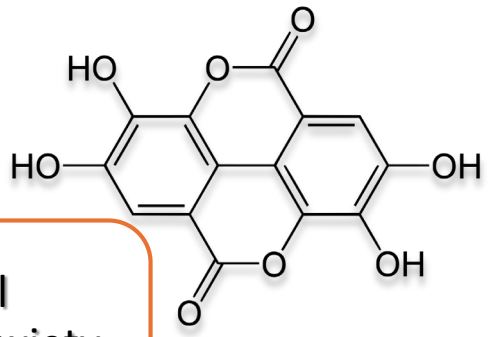


Romeo et al., 2021. Antioxidants, 10(11):1759

Several in vitro studies support the beneficial properties of ET and EA in peripheral and central diseases

Multi-target action → anti-angiogenic, anti-atherogenic, anti-carcinogenic, anti-obesity, anti-inflammatory, anti-oxidant and anti-thrombotic, anti-neurodegenerative properties

Ellagic Acid (EA) as Nutraceutical

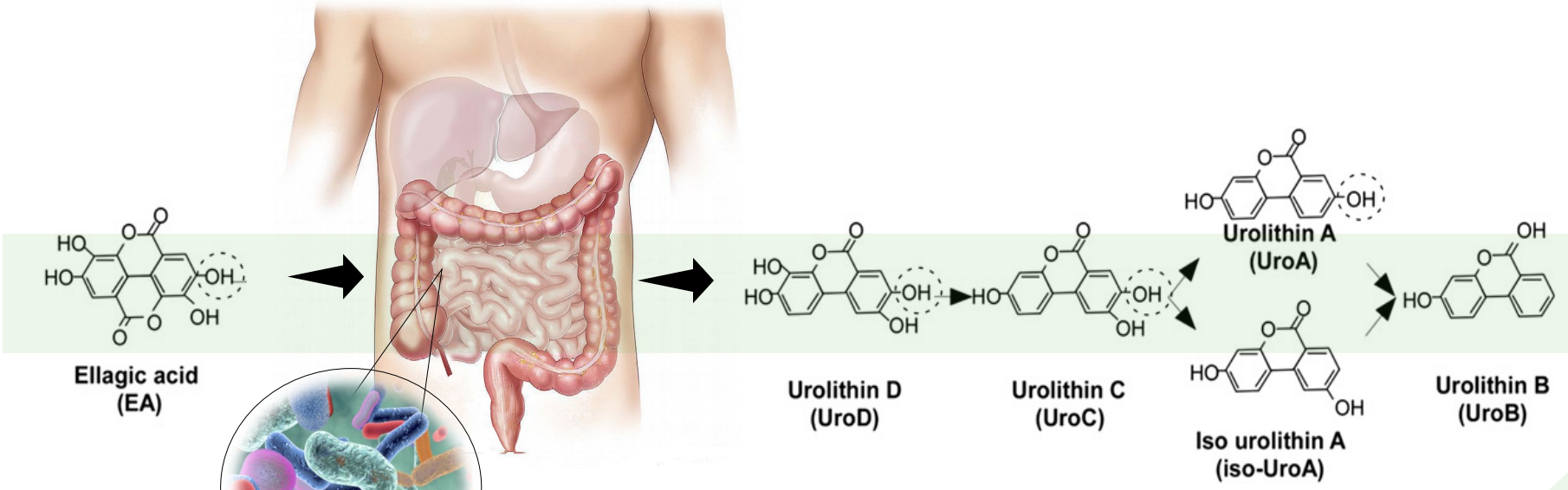


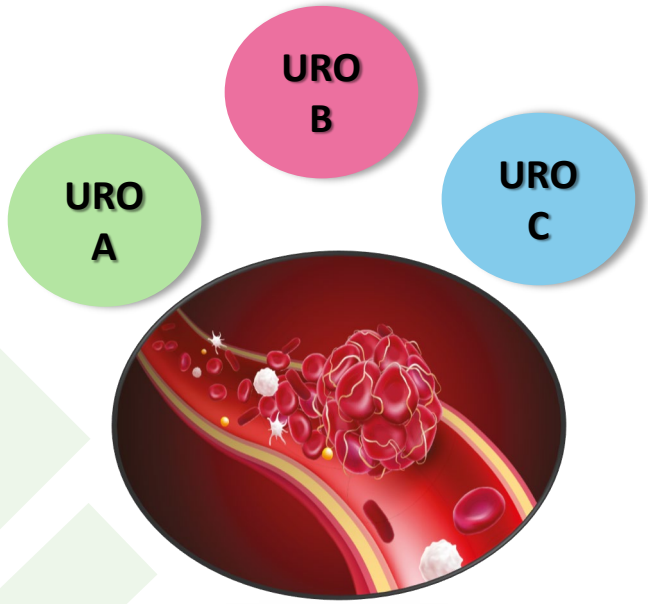
Special healthy role of EA in inflammatory and central (auto)immunological diseases, but also in depression, anxiety and age-related neurological disorders

EA's low water solubility and bioavailability (according to Lipinski's rule of 5) limits its intestinal absorption and its diffusion throughout the body and into the central nervous system (CNS).

EA is converted by the gut microbiota into Urolithins

From Ellagic Acid to Urolithins (URO)





Blood flow

Central Nervous System

Ellagic acid controls noradrenergic innervation

Eur J Pharmacol. 2012 May 5;682(1-3):118-25. doi: 10.1016/j.ejphar.2012.02.034. Epub 2012 Feb 24.

Evidence for the involvement of the monoaminergic system, but not the opioid system in the antidepressant-like activity of ellagic acid in mice

Chandrashekaran Girish [1], Vishnu Raj, Jayasree Arya, Sadasivam Balakrishnan

JOURNAL OF BIOCHEMICAL and MOLECULAR TOXICOLOGY

RESEARCH ARTICLE | Full Access

Prophylactic effects of ellagic acid and rosmarinic acid on doxorubicin-induced neurotoxicity in rats

Hanan A. Rizk, Marwa A. Masoud, Omar W. Maher

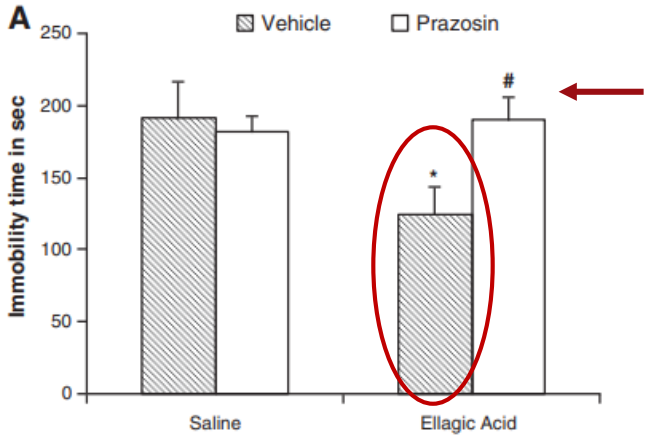
First published: 16 August 2017 | https://doi.org/10.1002/jbt.21977 | Citations: 42

International Journal of Molecular Sciences — MDPI

Int J Mol Sci. 2020 May; 21(10): 3631. PMCID: PMC7279224
Published online 2020 May 21. doi: 10.3390/ijms21103631 PMID: 32455600

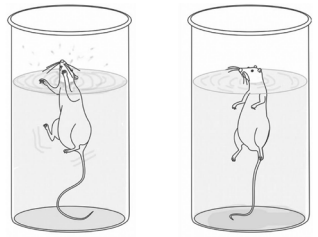
Neuroinflammation in Aged Brain: Impact of the Oral Administration of Ellagic Acid Microdispersion

Raffaella Boggia,[1] Federica Turrini,[1] Alessandra Roggeri,[1] Guendalina Olivero,[1] Francesca Cisani,[1] Tommaso Bonfiglio,[2] Maria Summa,[3] Massimo Grilli,[1] Gabriele Caviglioli,[1] Silvana Alfei,[1] Paola Zunin,[1] Rosalia Bertorelli,[3] and Anna Pittaluga[4,5,*]

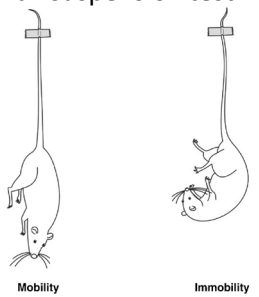


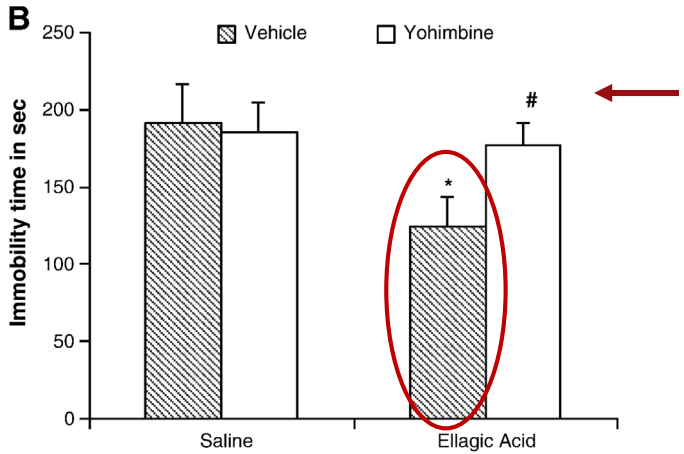
Reduction of immobility time of mice in both behavioral tests in EA-treated animals

Forced swim test



Tail suspension test





The decrease of immobility time was reverted by the following concomitant administration of selective noradrenergic receptor antagonists: Prazosin (a1 receptor antagonist) and Yohimbine (a2 receptor antagonist)

Ellagic acid controls noradrenergic innervation

Eur J Pharmacol. 2012 May 5;682(1-3):118-25. doi: 10.1016/j.ejphar.2012.02.034. Epub 2012 Feb 24.

Evidence for the involvement of the monoaminergic system, but not the opioid system in the antidepressant-like activity of ellagic acid in mice

Chandrashekaran Girish [1], Vishnu Raj, Jayasree Arya, Sadasivam Balakrishnan

JOURNAL OF BIOCHEMICAL and MOLECULAR TOXICOLOGY

RESEARCH ARTICLE | Full Access

Prophylactic effects of ellagic acid and rosmarinic acid on doxorubicin-induced neurotoxicity in rats

Hanan A. Rizk, Marwa A. Masoud, Omar W. Maher

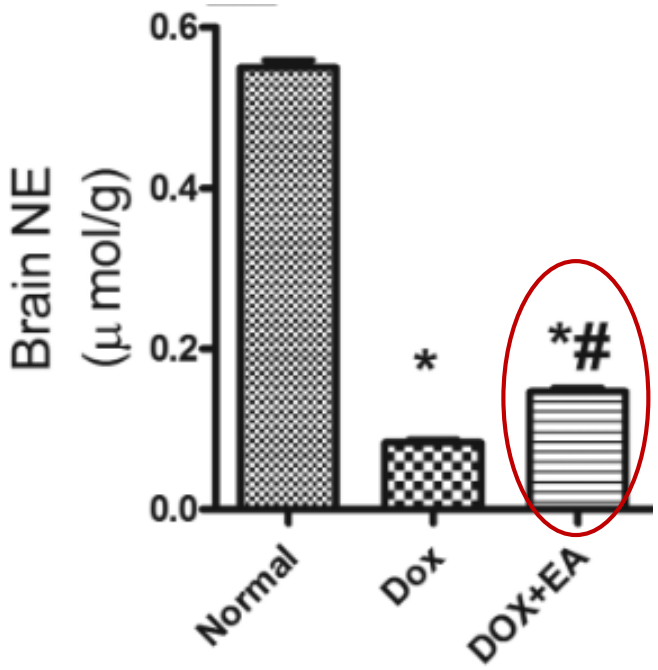
First published: 16 August 2017 | https://doi.org/10.1002/jbt.21977 | Citations: 42

International Journal of Molecular Sciences — MDPI

Int J Mol Sci. 2020 May; 21(10): 3631.
Published online 2020 May 21. doi: 10.3390/ijms21103631
PMCID: PMC7279224
PMID: 32455600

Neuroinflammation in Aged Brain: Impact of the Oral Administration of Ellagic Acid Microdispersion

Raffaella Boggia,[1] Federica Turrini,[1] Alessandra Roggeri,[1] Guendalina Olivero,[1] Francesca Cisani,[1] Tommaso Bonfiglio,[2] Maria Summa,[3] Massimo Grilli,[1] Gabriele Caviglioli,[1] Silvana Alfei,[1] Paola Zunin,[1] Rosalia Bertorelli,[3] and Anna Pittaluga[4,5,*]



Increased availability of central endogenous noradrenaline (NA) in doxorubicin-treated rats

Ellagic acid controls noradrenergic innervation

Eur J Pharmacol. 2012 May 5;682(1-3):118-25. doi: 10.1016/j.ejphar.2012.02.034. Epub 2012 Feb 24.

Evidence for the involvement of the monoaminergic system, but not the opioid system in the antidepressant-like activity of ellagic acid in mice

Chandrashekaran Girish [1], Vishnu Raj, Jayasree Arya, Sadasivam Balakrishnan

JOURNAL OF BIOCHEMICAL and MOLECULAR TOXICOLOGY

RESEARCH ARTICLE | Full Access

Prophylactic effects of ellagic acid and rosmarinic acid on doxorubicin-induced neurotoxicity in rats

Hanan A. Rizk, Marwa A. Masoud, Omar W. Maher

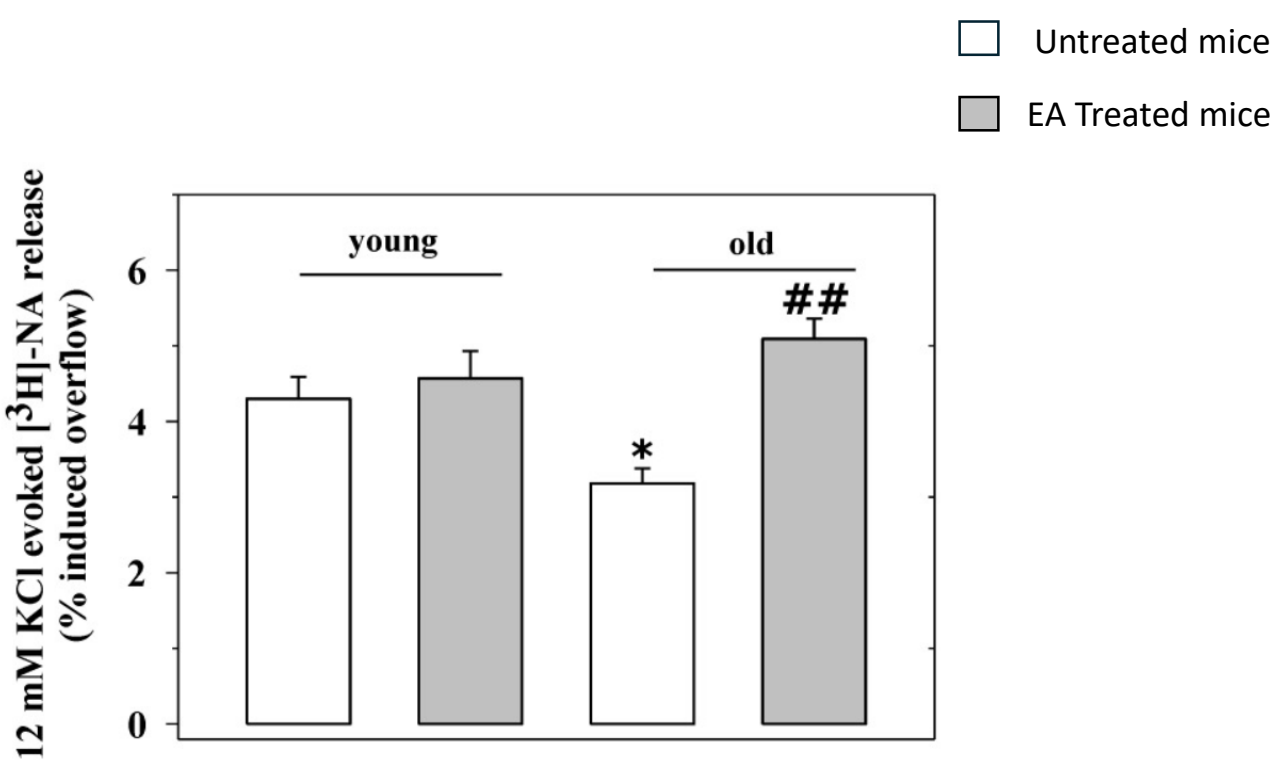
First published: 16 August 2017 | https://doi.org/10.1002/jbt.21977 | Citations: 42

International Journal of Molecular Sciences — MDPI

Int J Mol Sci. 2020 May; 21(10): 3631. PMCID: PMC7279224
Published online 2020 May 21. doi: 10.3390/ijms21103631 PMID: 32455600

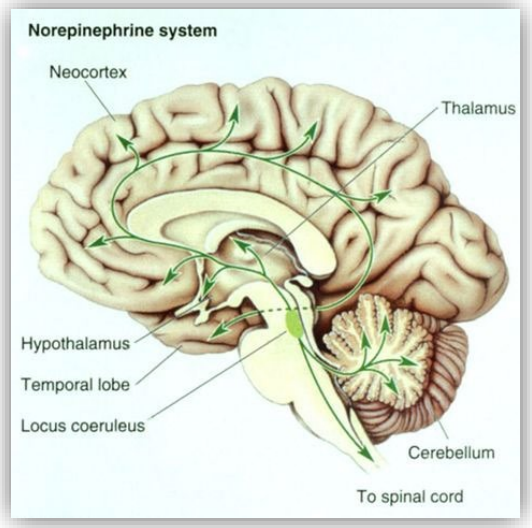
Neuroinflammation in Aged Brain: Impact of the Oral Administration of Ellagic Acid Microdispersion

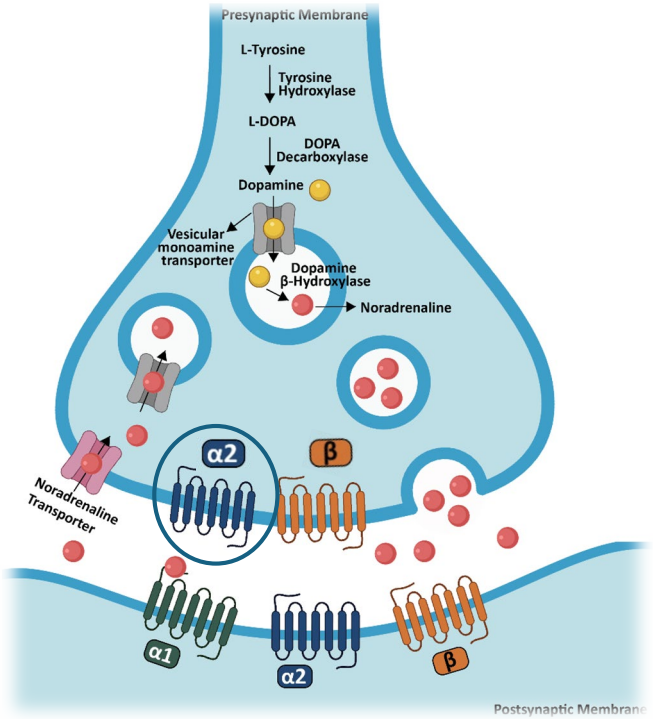
Raffaella Boggia,[1] Federica Turrini,[1] Alessandra Roggeri,[1] Guendalina Olivero,[1] Francesca Cisani,[1] Tommaso Bonfiglio,[2] Maria Summa,[3] Massimo Grilli,[1] Gabriele Caviglioli,[1] Silvana Alfei,[1] Paola Zunin,[1] Rosalia Bertorelli,[3] and Anna Pittaluga[4,5,*]



Increased NA exocytosis in aged mice

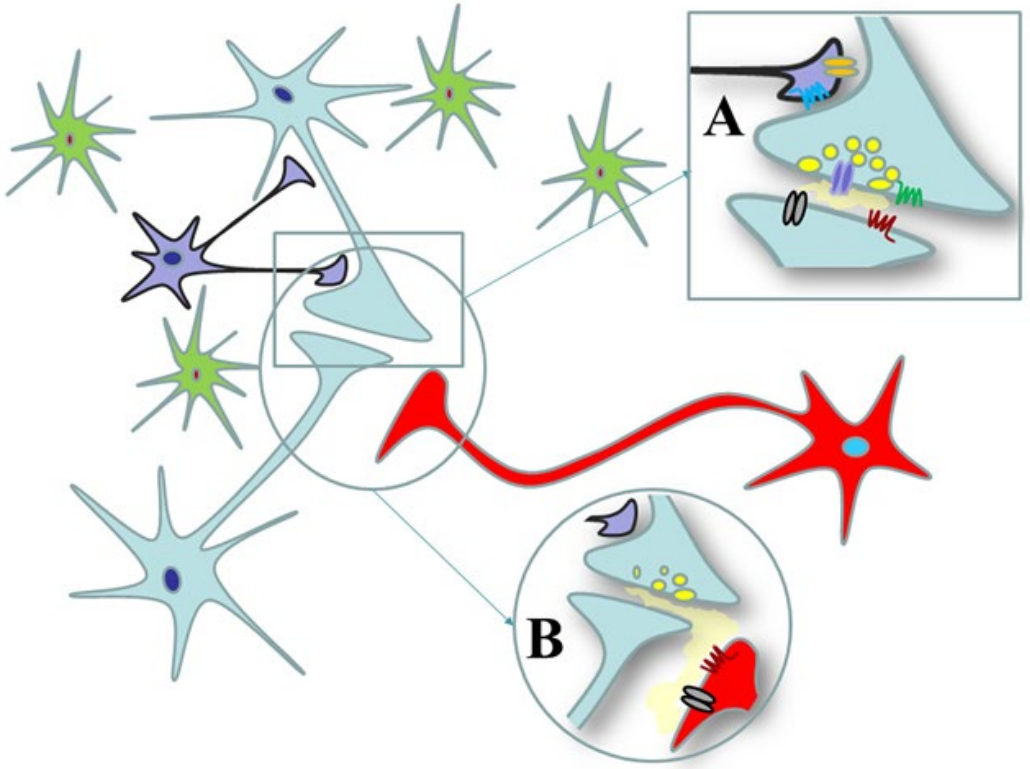
Norepinephrine and the noradrenergic system





Mercan D, Heneka MT. The Contribution of the Locus Coeruleus–Noradrenaline System Degeneration during the Progression of Alzheimer's Disease. *Biology*. 2022; 11(12):1822. https://doi.org/10.3390/biology11121822

Synaptic and non-synaptic communications in the central nervous system



frontiers in Pharmacology
Front Pharmacol. 2016; 7: 295. PMCID: PMC5006178
Published online 2016 Aug 31. doi: 10.3389/fphar.2016.00295 PMID: 27630571

Presynaptic Release-Regulating mGlu1 Receptors in Central Nervous System

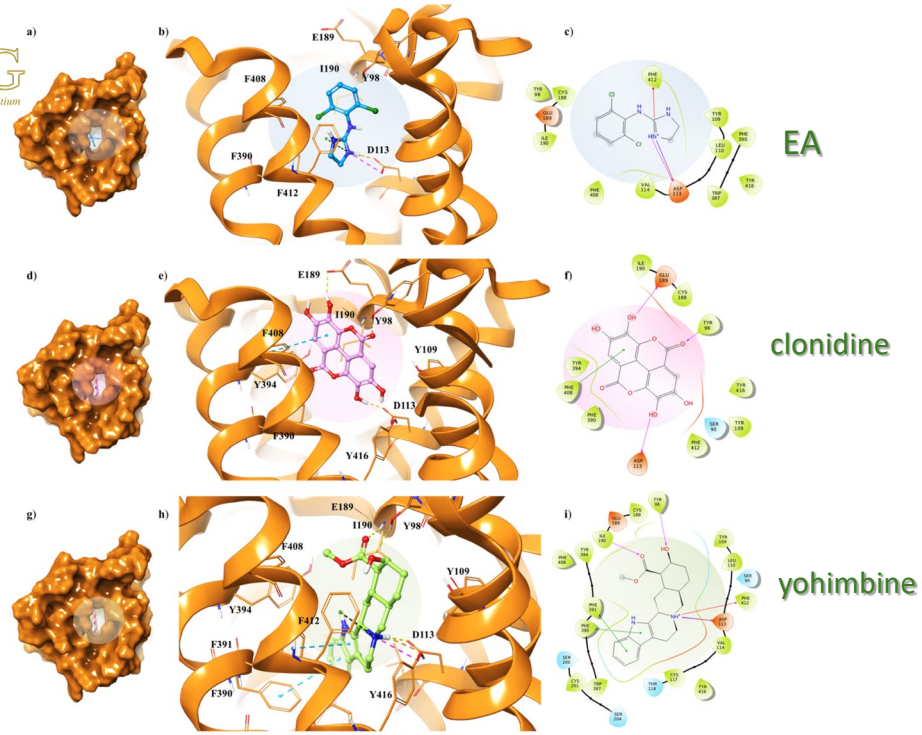
Anna Pittaluga[1,2,*]

Noradrenergic α_2 receptors are a molecular target of EA


Prof. Stefano Alcaro

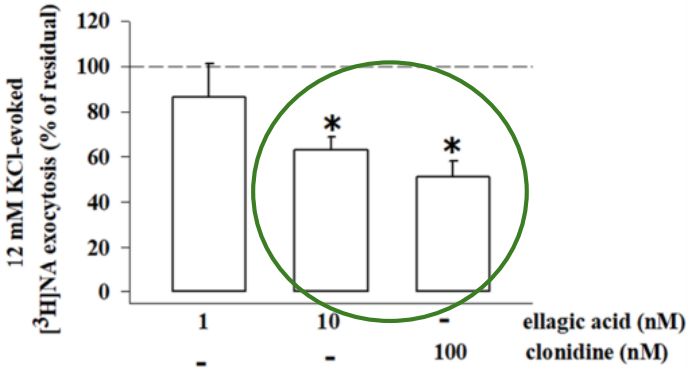

Dr. Giosuè Costa

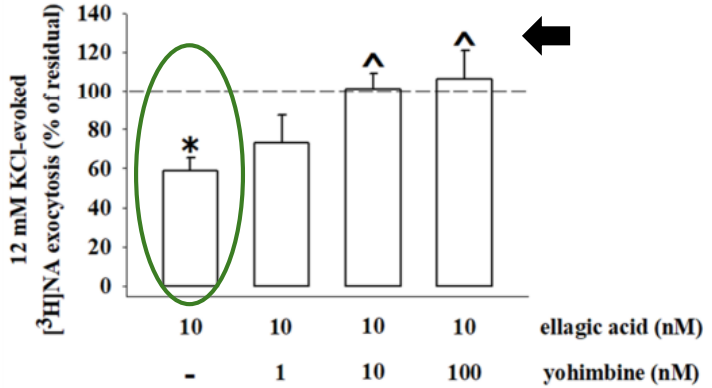

Dr. Isabella Romeo



EA binds predominantly to α_{2A} receptors showing partial agonist behaviour

NA release experiments on synaptosomes





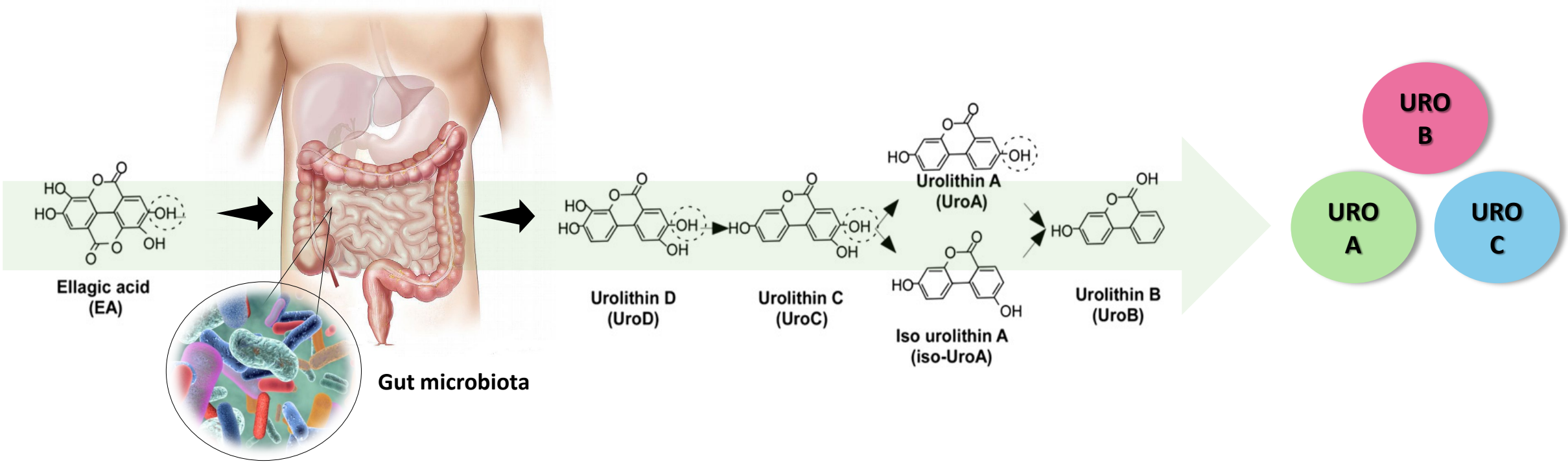
antioxidants
MDPI
Article
Presynaptic Release-Regulating Alpha2 Autoreceptors: Potential Molecular Target for Ellagic Acid Nutraceutical Properties
Isabella Romeo [1,2,3,*], Giulia Vallarino [4], Federica Turrini [4], Alessandra Roggeri [4], Guendalina Olivero [4], Raffaella Boggia [4], Stefano Alcaro [1,2,3], Giosuè Costa [1,2,3,*] and Anna Pittaluga [4,5]
Published: 4 November 2021

Ellagic acid (10 nM) inhibits NA release

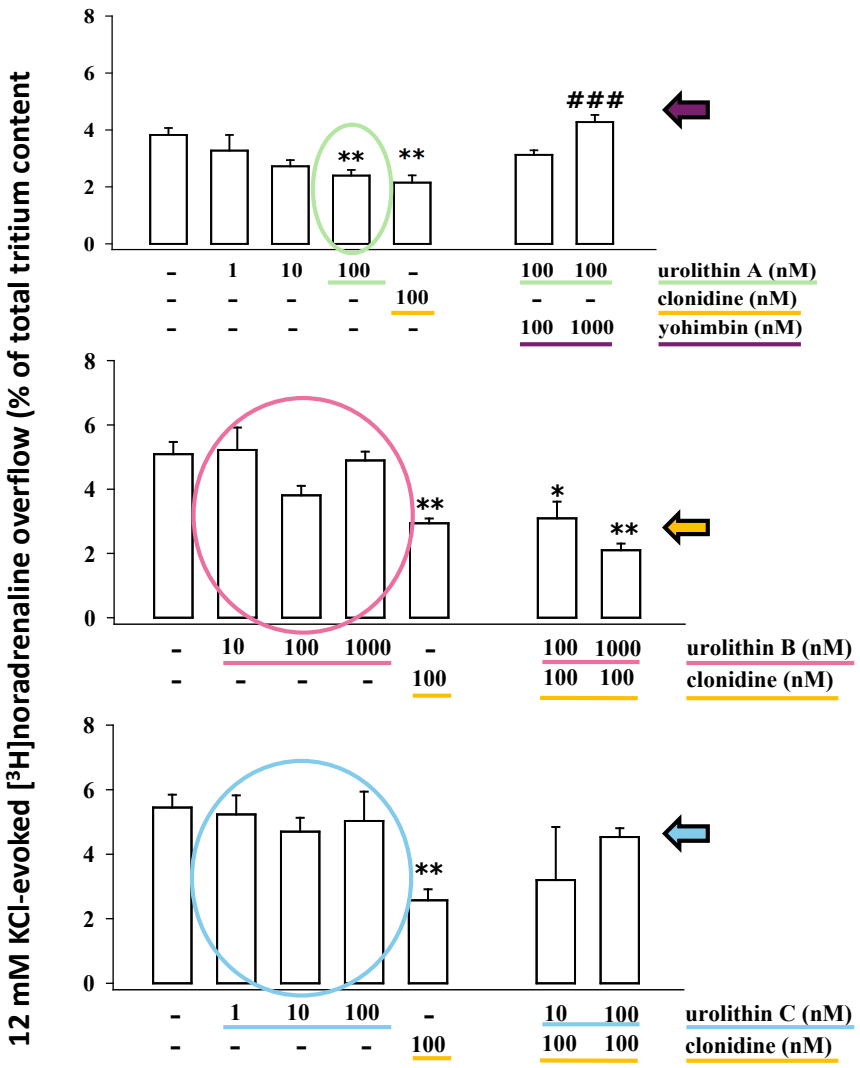
The effect is comparable to that induced by the agonist clonidine (100 nM)

The α_2 antagonist Yohimbine reverses the inhibition caused by ellagic acid (10 nM)

From Ellagic Acid to Urolithins (URO)



Effect of URO A, B and C on noradrenaline release in nerve endings of adult mouse hippocampus: comparison with clonidine and effect of yohimbine



URO A → It mimics the agonist behaviour of clonidine and ellagic acid by inhibiting NA release.
Its effect is reversed by the antagonist yohimbine.

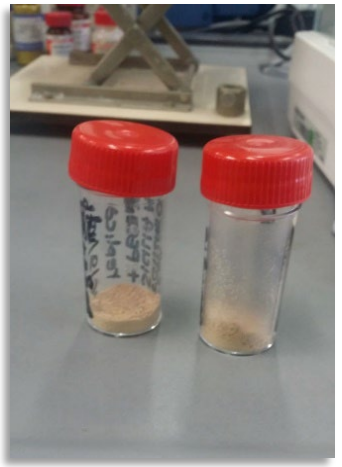
URO B → It has no effect on NA release

URO C → Acts as an antagonist, reversing clonidine-induced inhibition of NA release

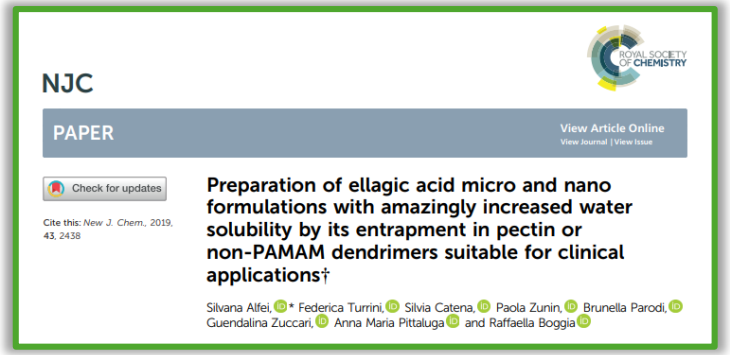
In-vivo treatment with EAm on healthy adult mice

Microdispersion of EA (EAm) by spray-drying technique

Spray-dryer



Microdispersion of Ellagic Acid (EAm)



DIFAR

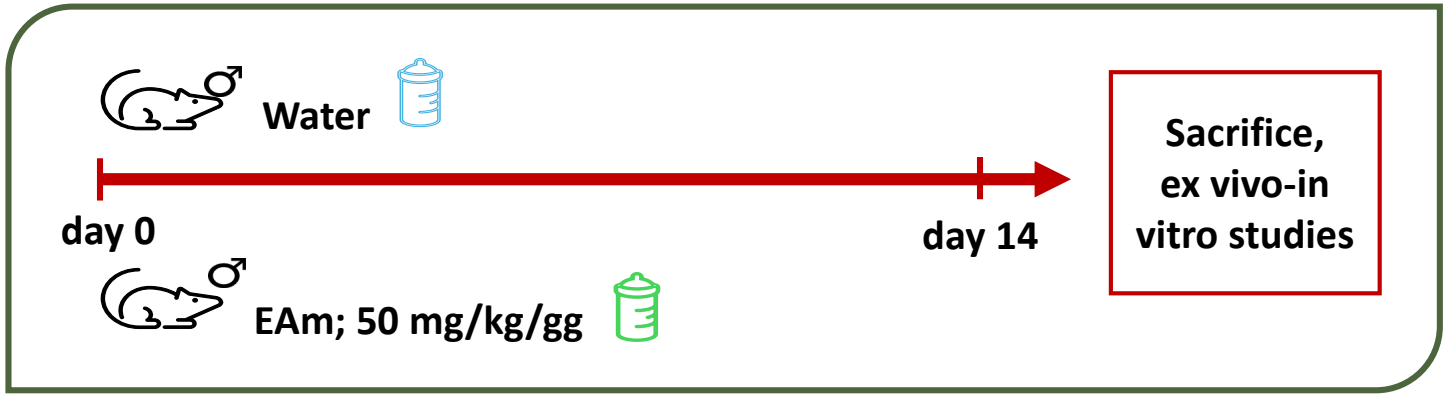
Prof. Raffaella Boggia

Dr. Federica Turrini

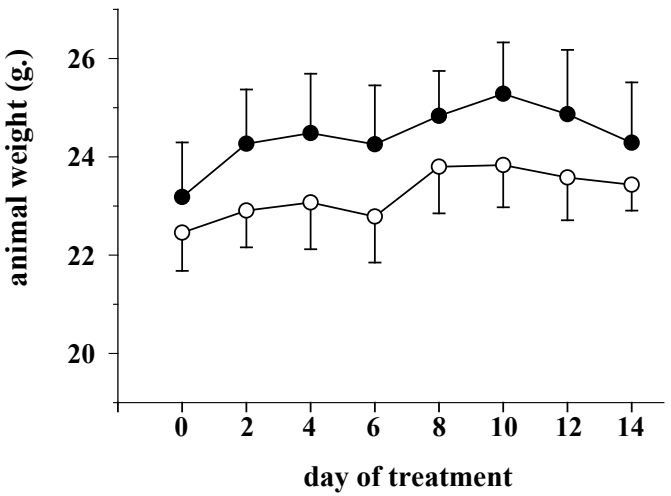
EAm, prepared with low-methoxyl pectin using spry-drying technology, increased the solubility of EA in water from 9.7 µg/mL to 300 µg/mL.

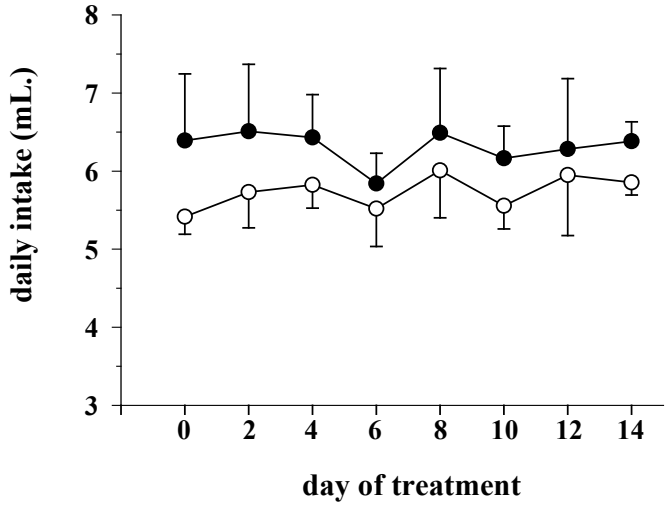
30 times greater solubility in water!

Impact of in vivo treatment with EAm on adult male mice



Treatment	Δ weight
EAm	0,22±0,20
Ctrl	0,10±0,31

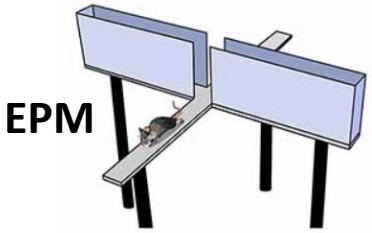




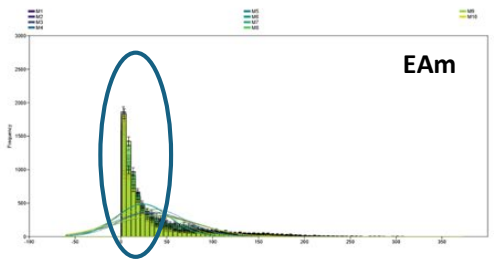
Impact of in vivo treatment with EAm on behaviors of adult male mice

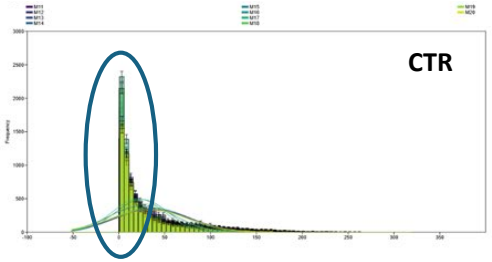
Data obtained from the analysis of two behavioural tests: hole board maze (HBM) and elevated plus maze (EPM)

Treatment	Δ Av. Speed (mm/s)	Δ Mob. Av. Speed (mm/s)	Δ Av. Accel (mm/s^2)	Δ Mobility Rate (%)	Δ Exploration Rate (%)	Δ Total Distance (mm)	Δ Frozen Events	Δ Tot. Time Frozen (m:s)	Δ Avg. Time Frozen (m:s)	Δ Max Instant Speed	Δ Max Instant Acceleration	Δ Head Dips	Δ CentreHead Dips	Δ 140-180 (centre)	Δ 20-40 (edge)
EAm	-9,64±1,97	-8,84±12,07	-16,13±3,43	-3%±0,01	-6%±0,01	-3237,93±630,22	0,80±0,33	3,55±1,76	2,02±0,69	-3,94±17,79	-1,33±27,29	**-9,8±3,15 *p≤0,05**	-0,58±0,74	-3,60±1,73	2,50±10,57
Ctrl	-7,28±1,78	-7,11±1,77	-13,12±2,63	-1%±0,01	-9%±0,02	-2443,42±584,15	0,10±0,38	1,06±1,67	1,21±0,93	13,24±7,55	11,86±13,38	**-16,78±2,29**	-1,25±0,38	2,40±2,01	12,80±15,89

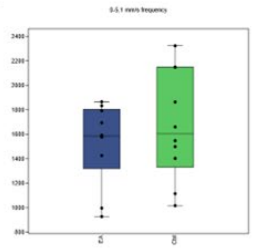


Speed profile of each animal at the treatment endpoint

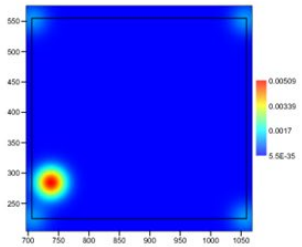




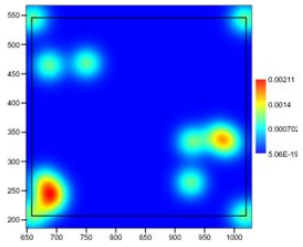
Analysis of low-speed frequencies (≤ 5.1 mm/s)



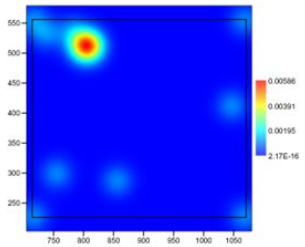
Static profile of animals at day 0 and day 14 represented by heatmaps of frozen events



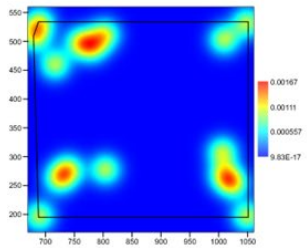
CTR day 0



CTR day 14

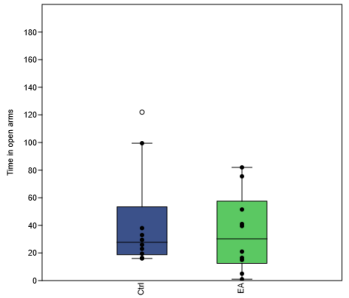


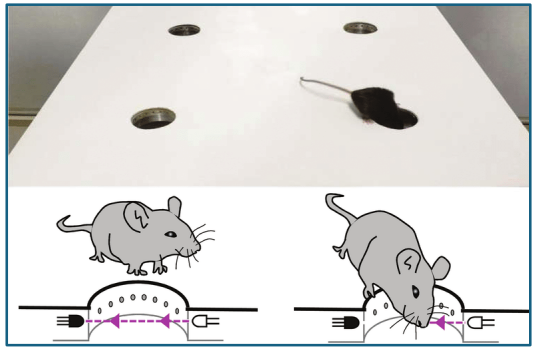
EAm day 0



EAm day 14

Analysis of time spent in open arms during EPM





↑ Head- dippings

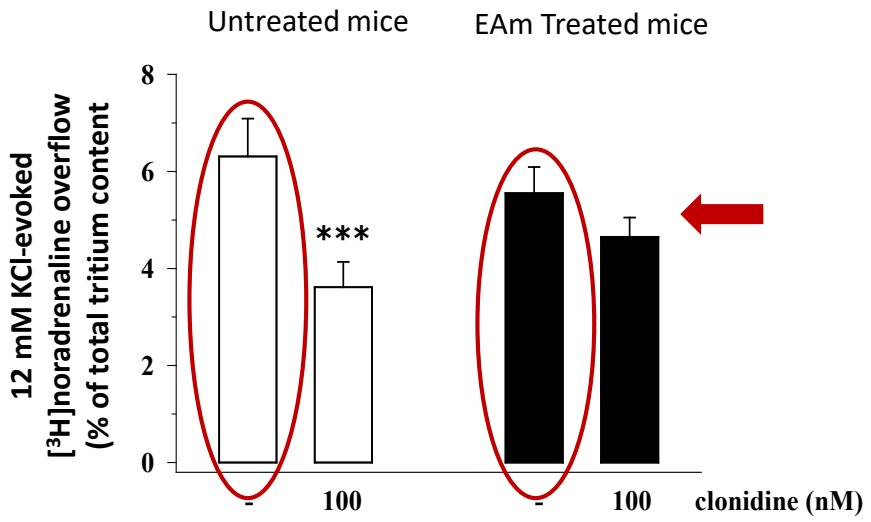
↓ Animal's anxiety-like state

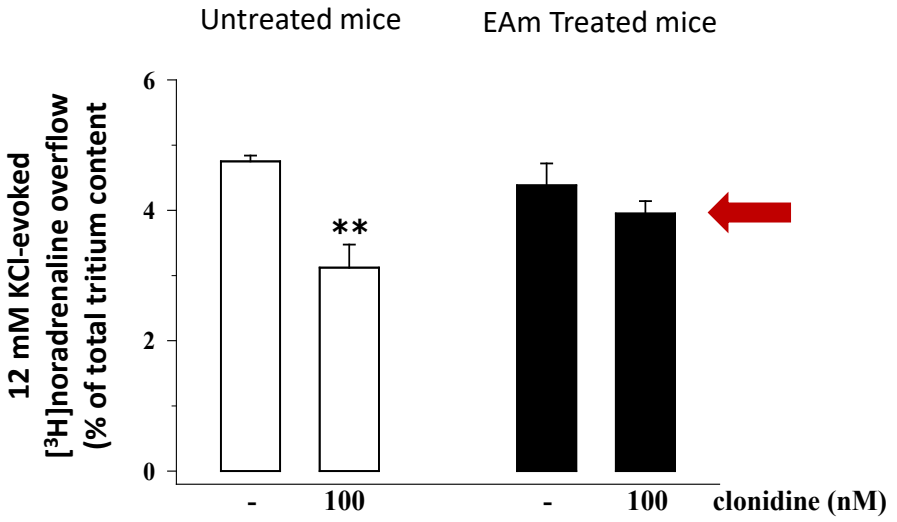
Ex vivo-in vitro experiments:

- Release efficiency of [^{3} H]NA and inhibition of clonidine in synaptosomes isolated from in vivo EAm-treated mice

Hippocampus

Cortex

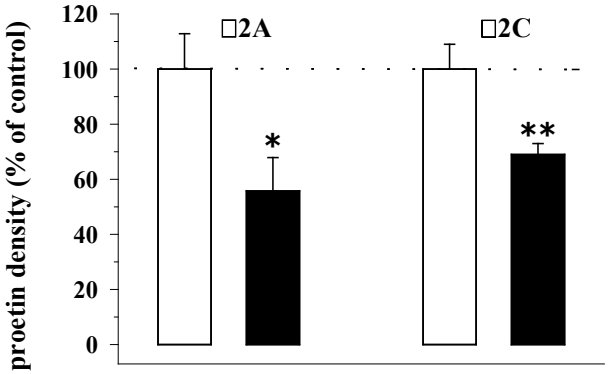


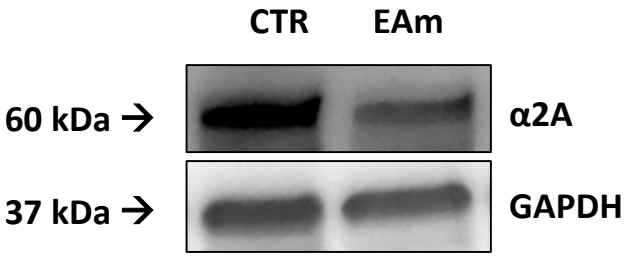


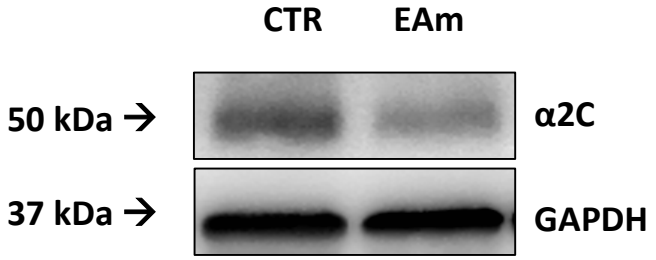
Desensitization of the presynaptic α_2 autoreceptors

- Effect of in vivo EAm treatment on α2A and α2C receptor protein density in cortical and hippocampal homogenates

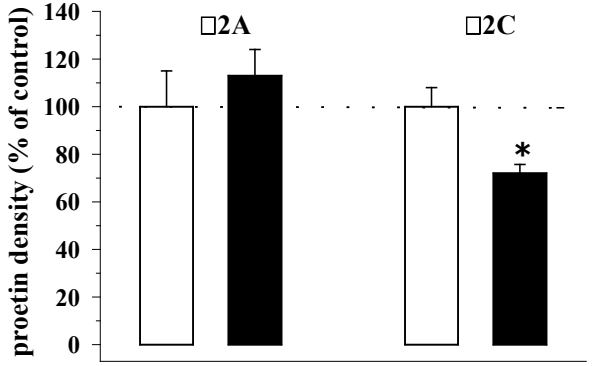
Hippocampus

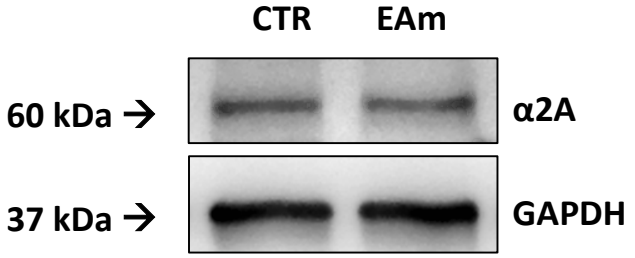


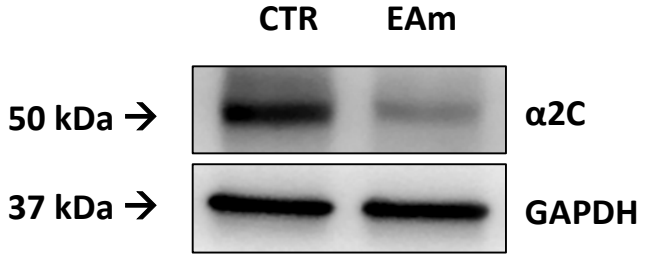




Cortex

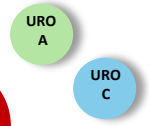






CONCLUSION

1



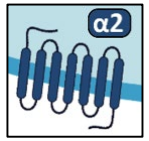
Urolithins modulate noradrenergic transmission, targeting the inhibitory presynaptic autoreceptor α_2.

From *In vitro* experiments:
- Urolithin A acts as an α_2 receptor agonist
- Urolithin C as an α_2 receptor antagonist

2

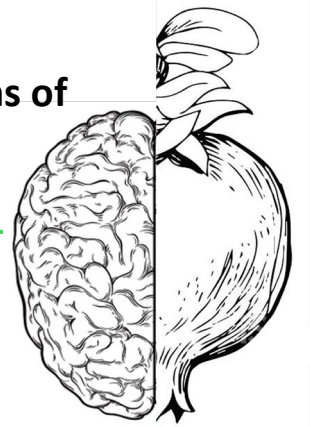
The *in vivo* EAm chronic administration desensitizes the presynaptic α_2 autoreceptors, since clonidine could not further modify the [^{3}H]NA exocytosis in EA-treated animals as observed in the untreated ones.

3

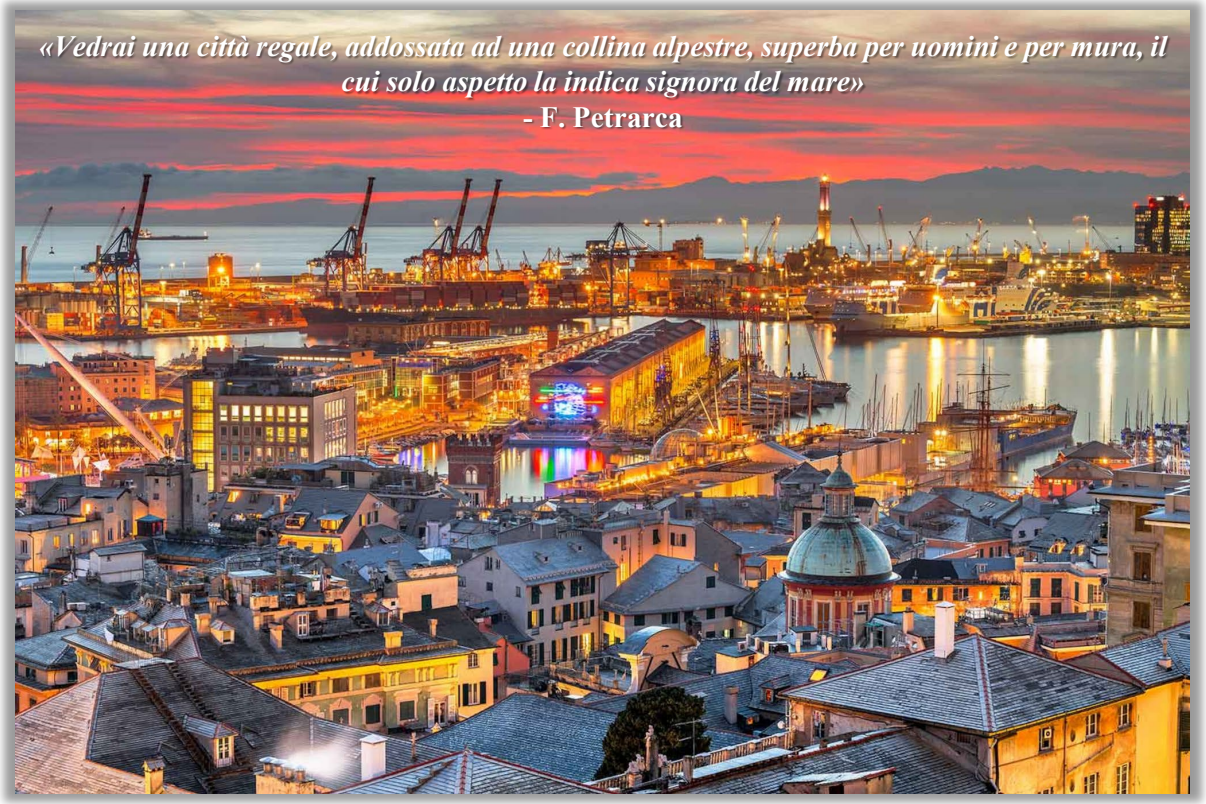


Western blot data showed reduced α_{2A}R and α_{2C}R density in EAm treated mice compared to controls.

Our results confirm that EA supplementation regulates noradrenergic system innervation, possibly by means of the main metabolites urolithins.



ACKNOWLEDGEMENTS



Anna Pittaluga
Guendalina Olivero
Alice Taddeucci
Nicole Rosenwasser

Raffaella Boggia
Federica Turrini
Massimo Grilli
Martina Monaco

DIPARTIMENTO DI FARMACIA
UNIVERSITA' DI GENOVA
DIFAR

PRIN
MIUR