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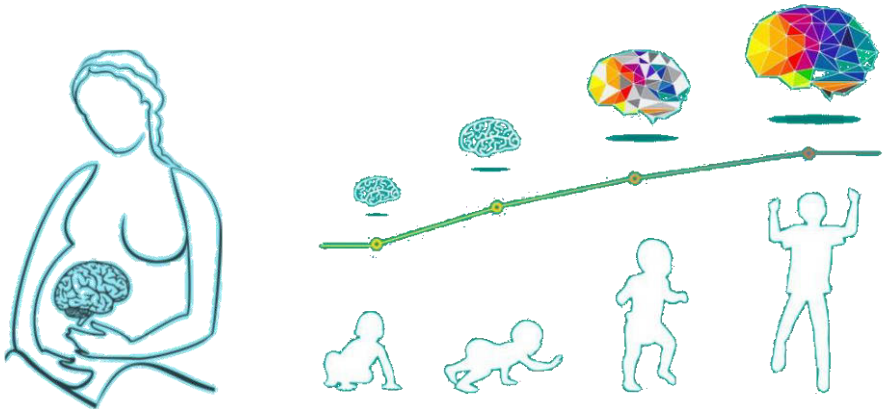
BOLOGNA
20-22 Febbraio 2023

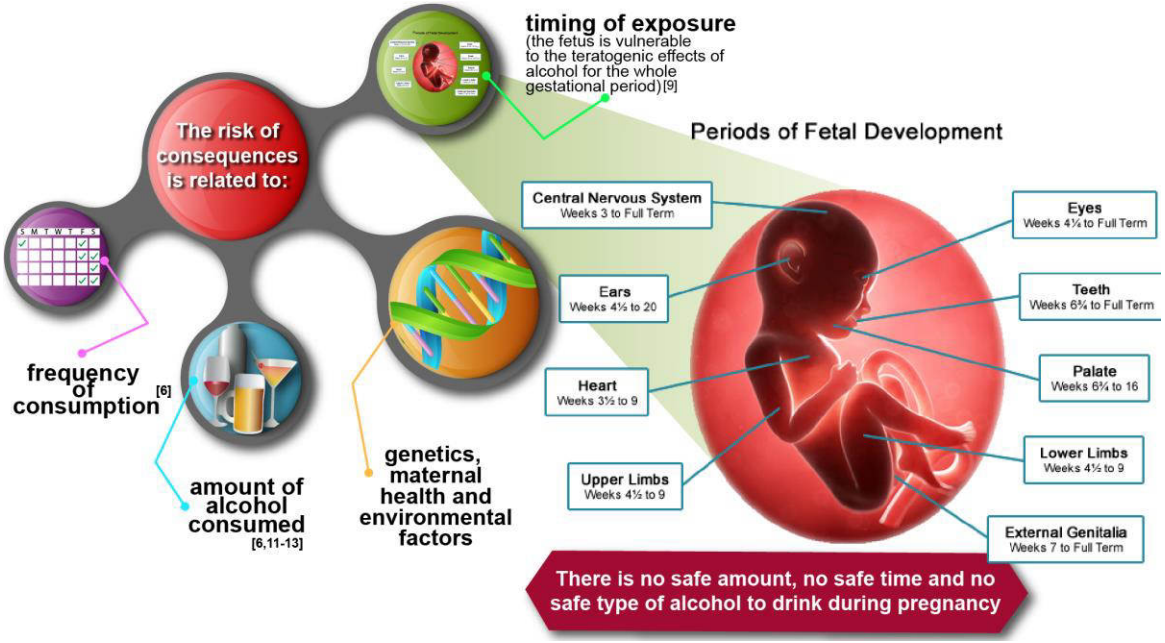
EFFETTI DELL'ALCOL SULLA TRASMISSIONE SINAPTICA DURANTE IL NEUROSVILUPPO

Elisabetta Gerace PhD

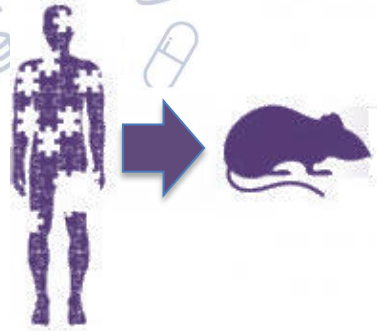
Università degli Studi di Firenze



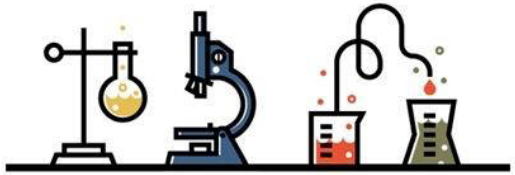
L'alcol altera il network neuronale e lo sviluppo del sistema nervoso centrale



I meccanismi alla base di questi processi sono ancora poco conosciuti



SCOPO DELLA RICERCA



Analizzare i meccanismi molecolari e funzionali indotti dall'esposizione all'etanolo durante il neurosviluppo

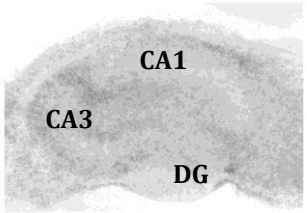
In vitro

Colture organotipiche ippocampali di ratto

In vivo

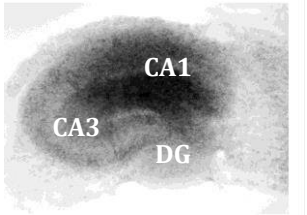
Esposizione prenatale all'alcol in topi C57/Bl6

Colture organotipiche ippocampali di ratto

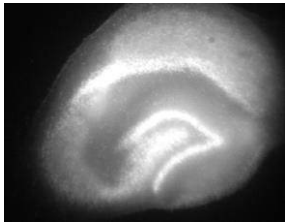


Control

Control



Toxicity



Toxicity

- Fettine ippocampali ottenute da ratti di 7-9 giorni e mantenute in coltura in filtri Millicell-CM per 10-15 giorni;

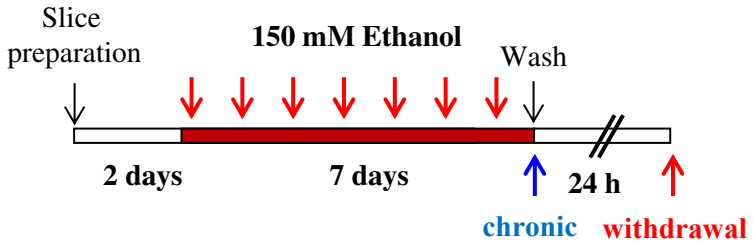
- Modello *in vitro* per applicare stimoli tossici e studiare i meccanismi e gli effetti dei farmaci;

- La morte cellulare è quantificata attraverso la misurazione dell'intensità di fluorescenza dello Ioduro di Propidio (PI);

Gerace et al., Methods Mol Biol.; (2012) 846:343-54.

Piano sperimentale

In vitro
Colture organotipiche ippocampali

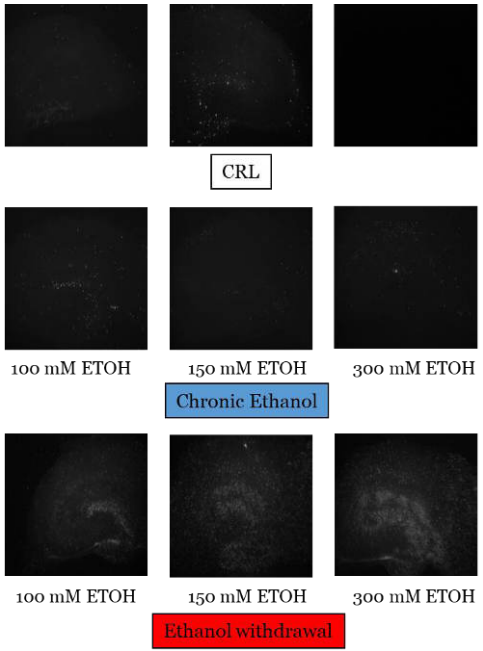


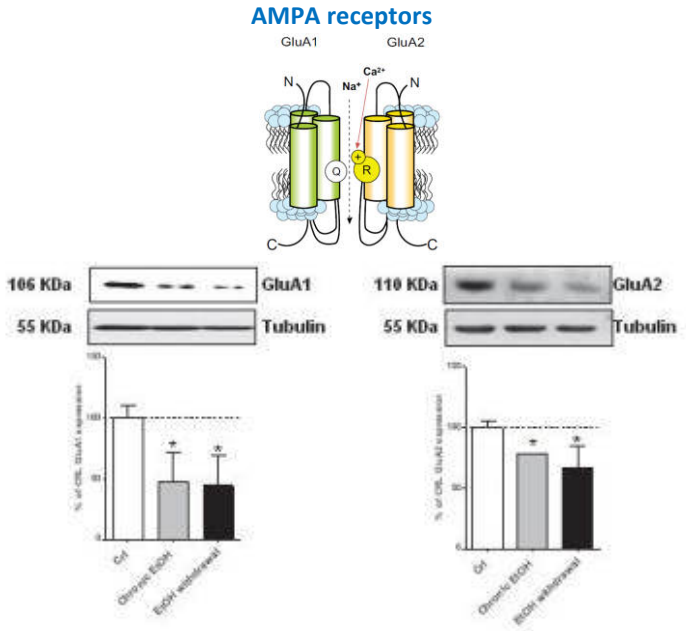
Background

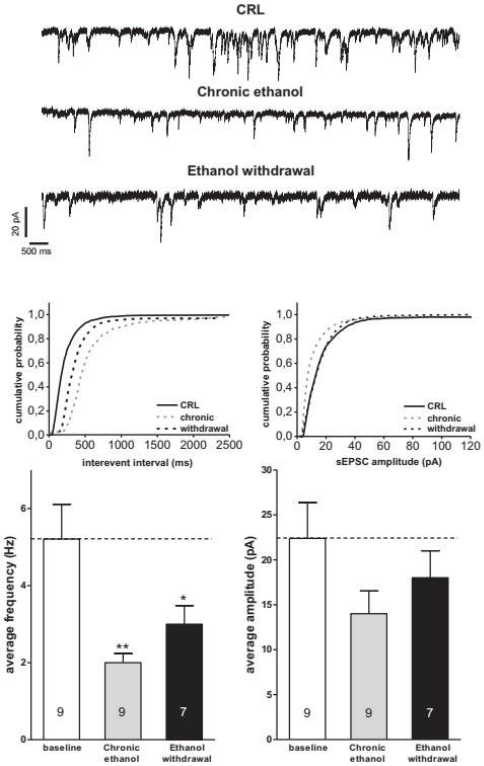
Alcoholism: Clinical and Experimental Research
Vol. 40, No. 4
April 2016

Ethanol Toxicity During Brain Development: Alterations of Excitatory Synaptic Transmission in Immature Organotypic Hippocampal Slice Cultures

Elisabetta Gerace, Elisa Landucci, Arianna Totti, Daniele Bani, Daniele Guasti, Roberto Baronti, Flavio Moroni, Guido Mannaioni, and Domenico E. Pellegrini-Giampietro





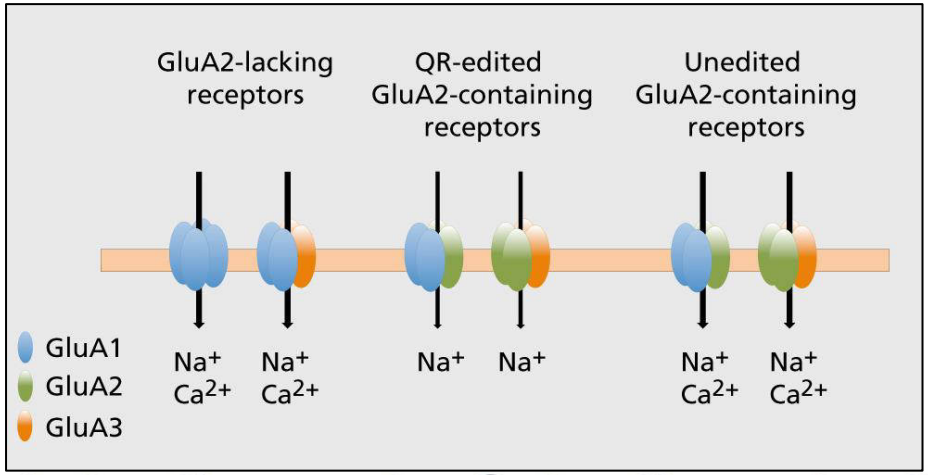


Gerace et al., Alcohol Clin Exp Res. 2016;40(4):706-16..

Recettori AMPA

- **Recettore** transmembrana **ionotropi** del glutammato che media la **neurotrasmissione sinaptica veloce**

- Composto da **quattro subunità** (GluA1, GluA2, GluA3 and GluA4) che si combinano per formare tetrameri
- La maggior parte degli AMPAR sono eterotetramerici, costituiti da dimeri 'simmetrici' di GluA2 e GluA1, GluA3 o GluA4



Ipotesi di lavoro

Ruolo dei microRNA e dei recettori mGlu5 nella regolazione della neurotrasmissione mediata dai recettori AMPA

OPEN ACCESS CellPress

Cell Reports
Report

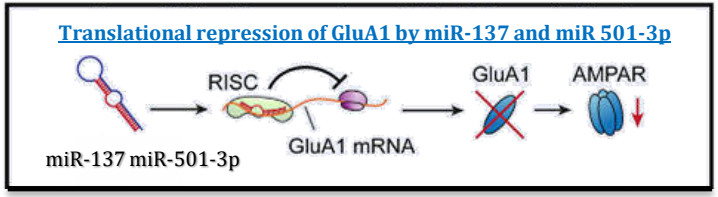
MicroRNA-137 Controls AMPA-Receptor-Mediated Transmission and mGluR-Dependent LTD

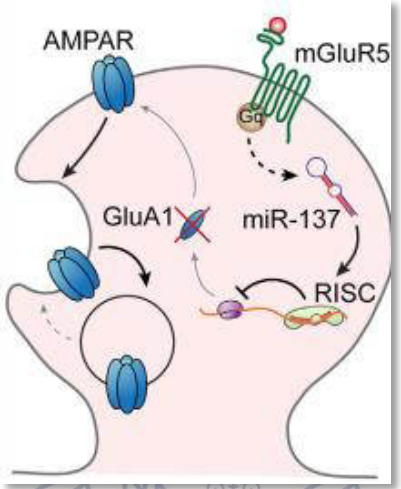
Nikkie F.M. Olde Loohuis,[1,5] Wei Ba,[4,5] Peter H. Stoerchel,[6] Aron Kos,[1,5] Amanda Jager,[1,5] Gerhard Schratt,[6] Gerard J.M. Martens,[3,5] Hans van Bokhoven,[1,4,5] Nael Nadif Kasri,[1,4,5,7,*] and Armaz Aschrafi[2,5,7,*]

JCB: Article

miR-501-3p mediates the activity-dependent regulation of the expression of AMPA receptor subunit GluA1

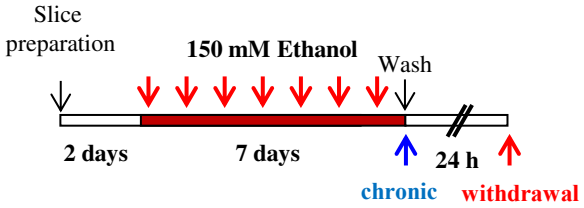
Zhonghua Hu,[1] Jun Zhao,[1] Tianyi Hu,[1] Yan Luo,[2] Jun Zhu,[2] and Zheng Li[1]





Piano sperimentale

In vitro
Colture organotipiche ippocampali



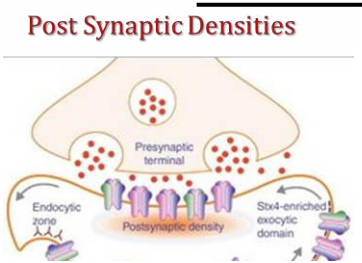
Analisi:

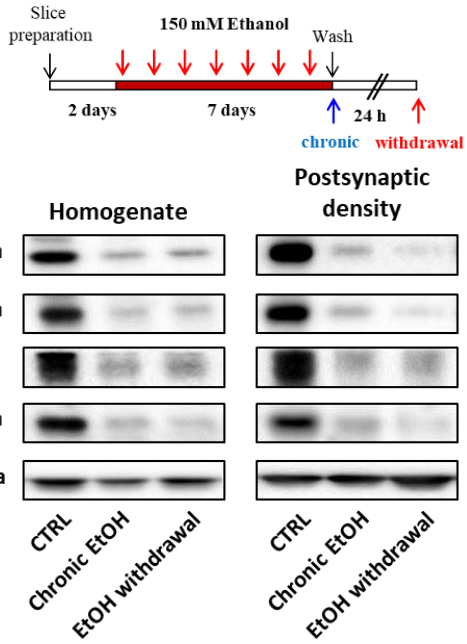
- Espressione delle subunità AMPA e relative proteine di ancoraggio tramite western blotting
- Dosaggio dei miRNA 137 e 501-3p tramite RT-PCR
- Registrazioni elettrofisiologiche nei neuroni piramidali della CA1 dell'ippocampo tramite patch clamp

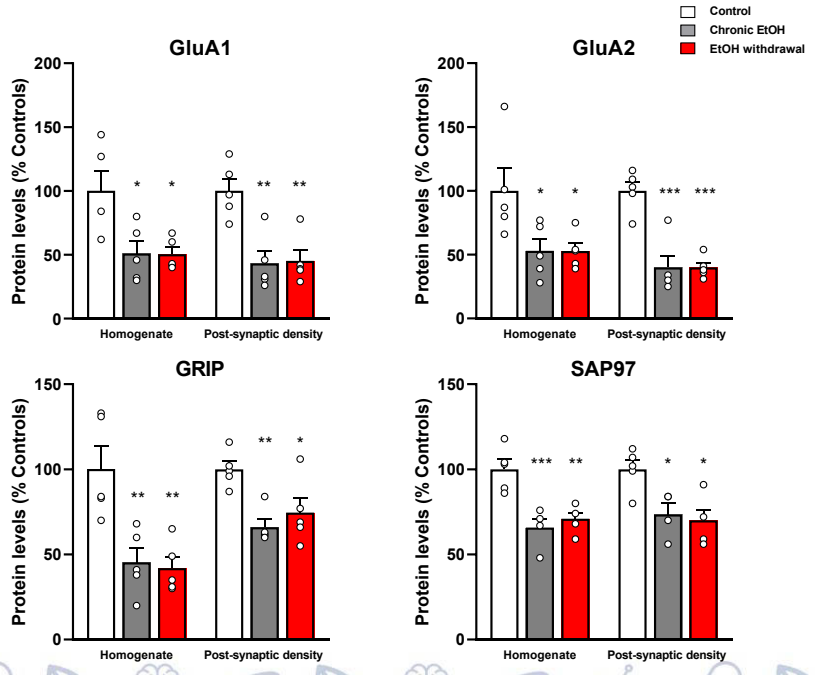
Francesca Mottarlini

Risultati

Localizzazione e trafficking dei recettori AMPA nelle densità post sinaptiche







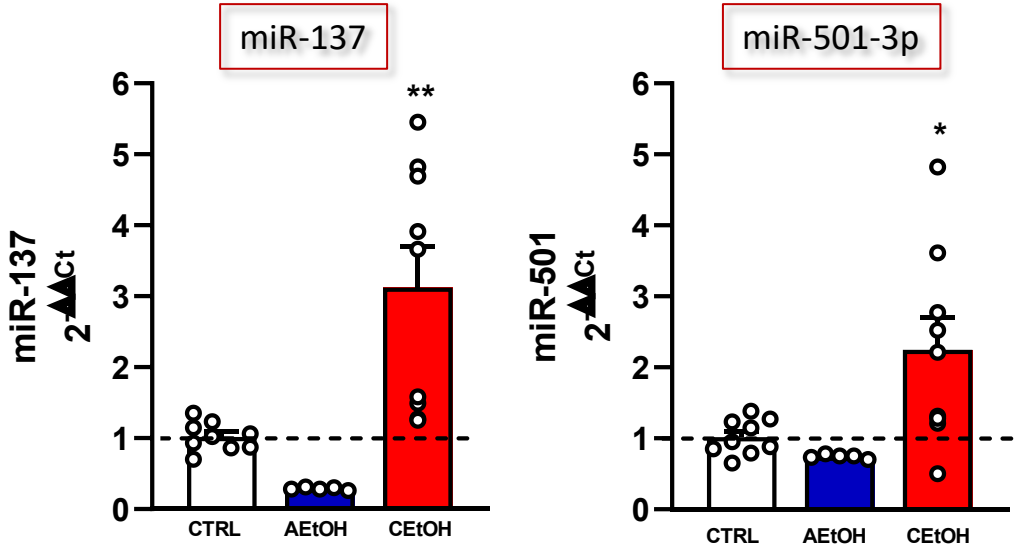
Under review


Cristina Luceri

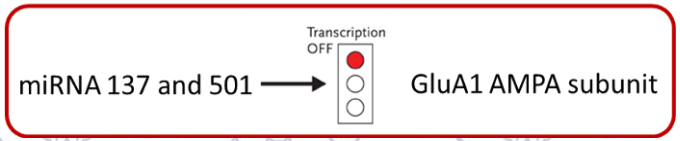

Elisabetta Bigagli

Risultati

miR-137 e miR-501-3p vengono upregolati dall'esposizione cronica ma non acuta di etanolo

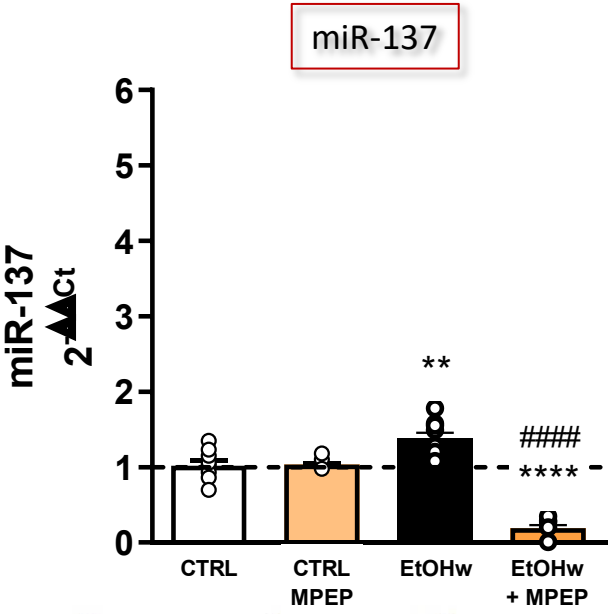


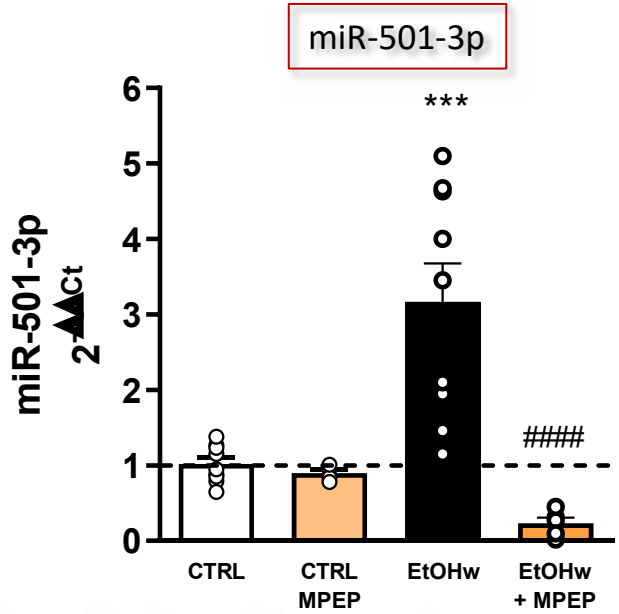
Under review

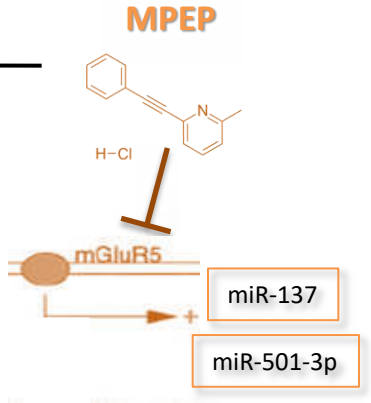


Risultati

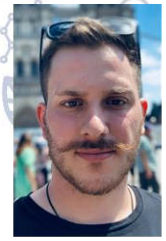
L'upregolazione dei miR-137 e miR-501-3p indotta dall'Etanolo è prevenuta dall'antagonista mGlu5 MPEP





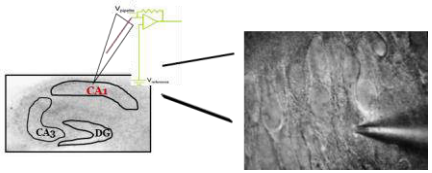


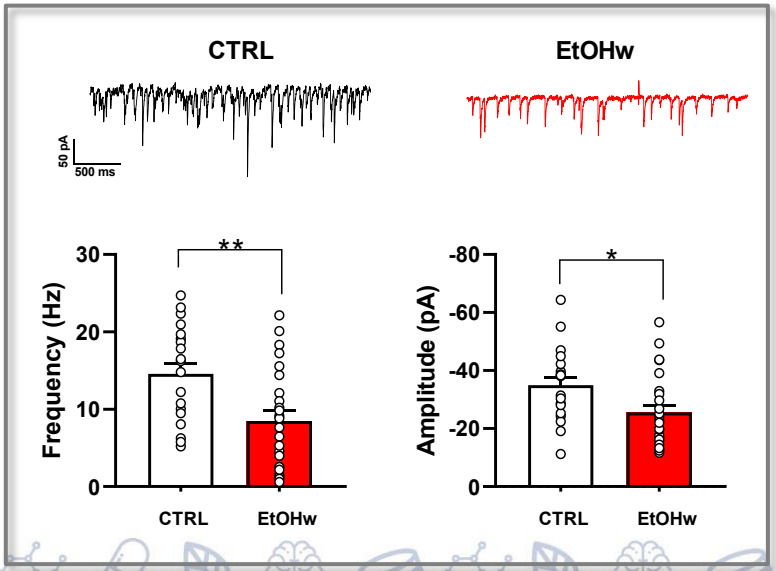
Under review

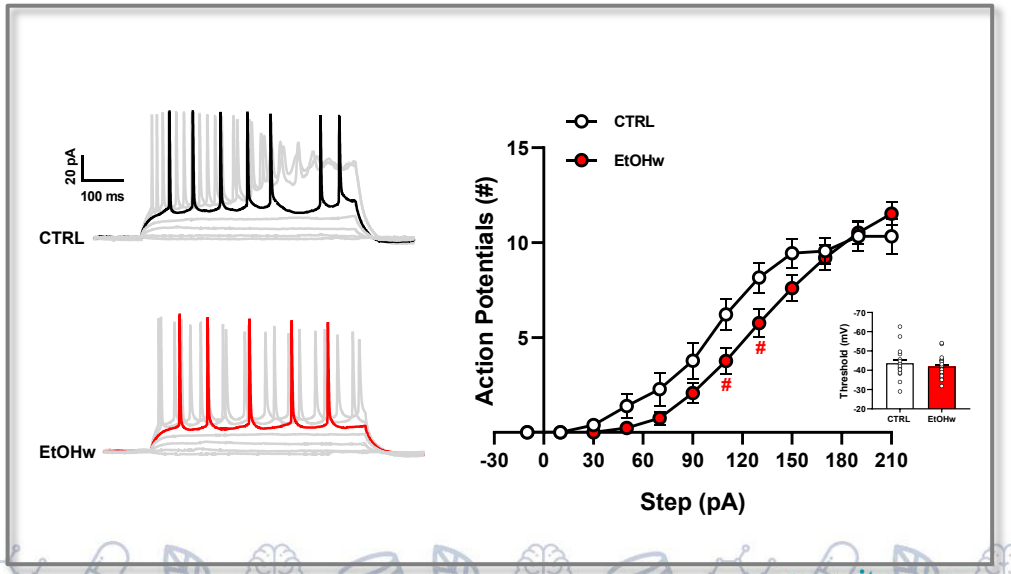

Lorenzo Curti

Risultati

L'etanolo induce una riduzione delle correnti spontanee eccitatorie e dell'eccitabilità dei neuroni della CA1 dell'ippocampo

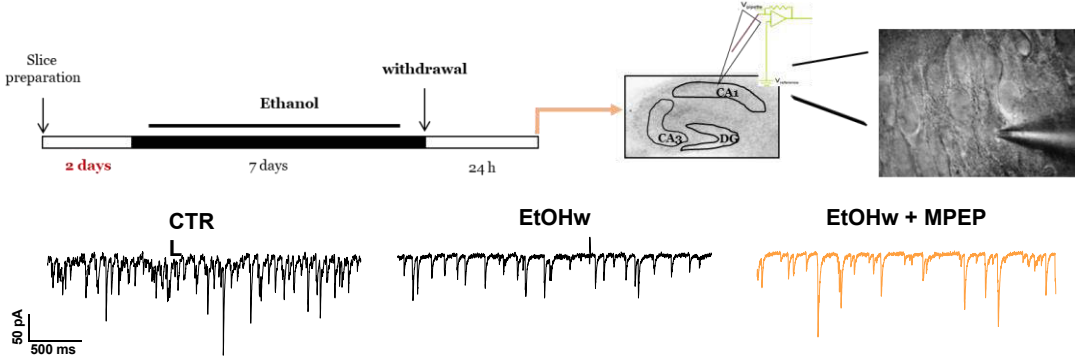


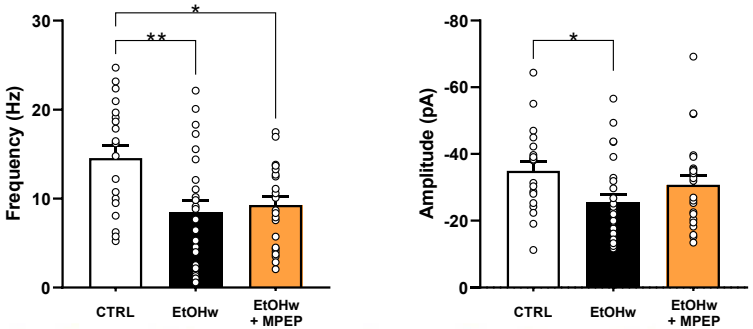


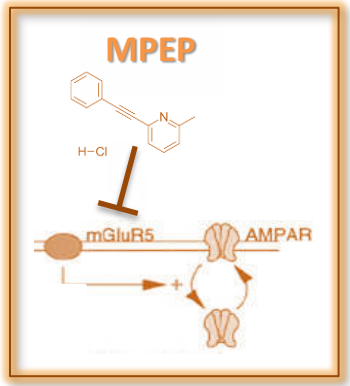


Risultati

La downregolazione AMPA indotta dall'Etanolo è mediata dai recettori metabotropi mGlu5



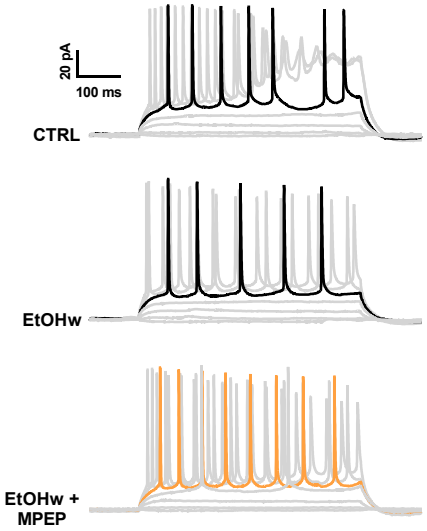


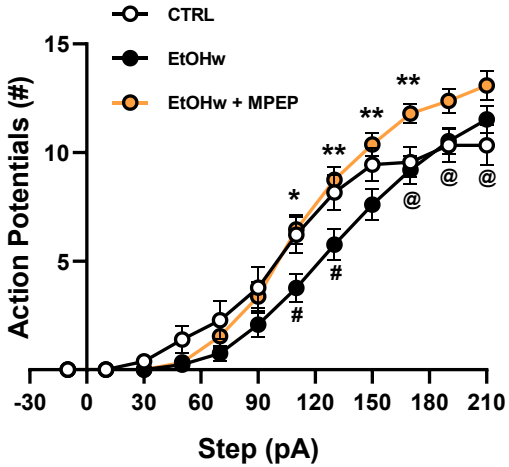


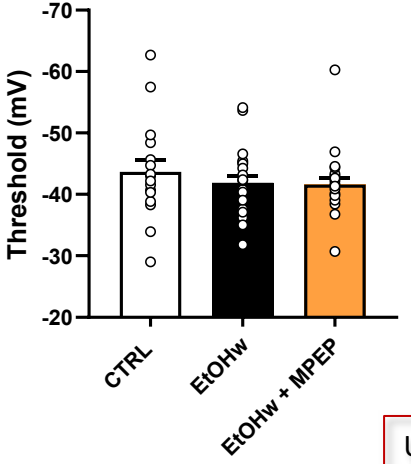
Under review

Risultati

La ridotta eccitabilità dei neuroni piramidali della CA1 dell'ippocampo indotta dall'Etanolo è prevenuta dall'antagonista mGlu5 MPEP



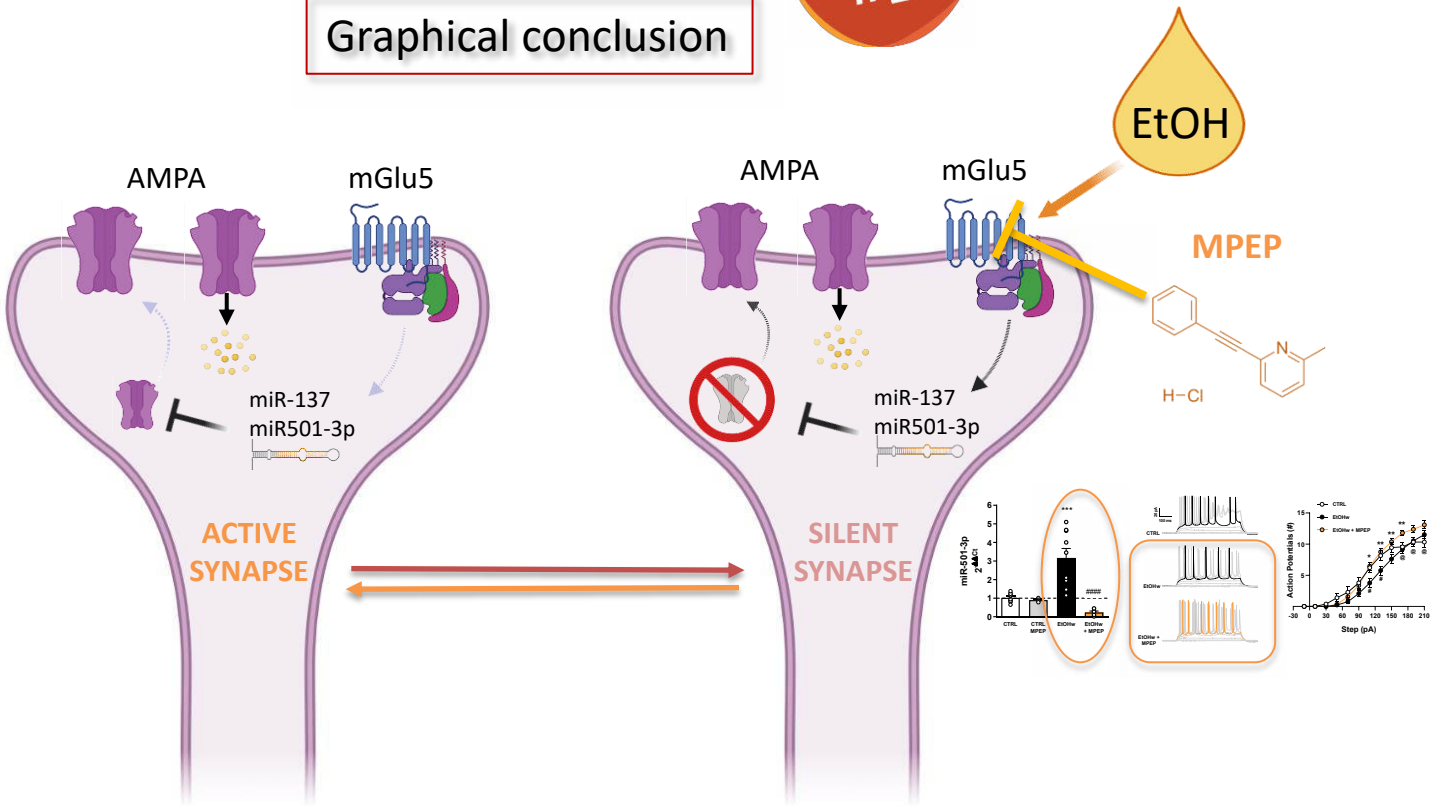




Under review

PART #1

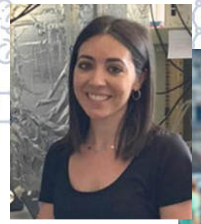
Graphical conclusion



SIGNIFICATO E IMPLICAZIONI

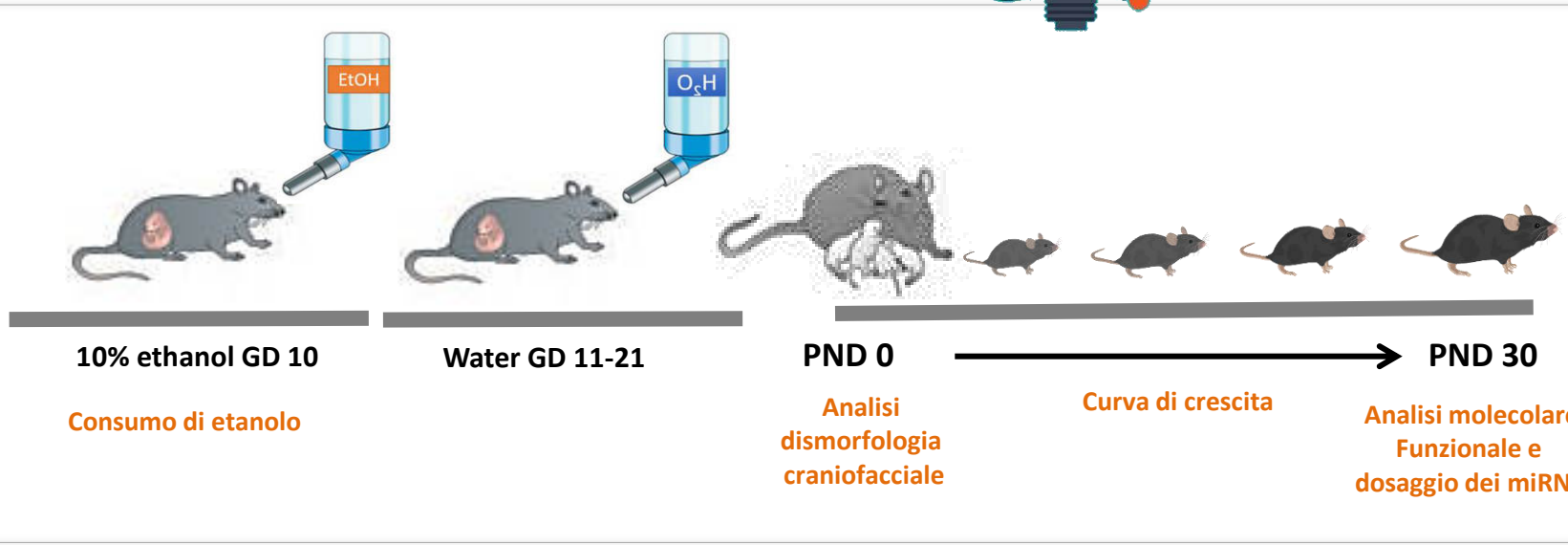
Potenziali target terapeutici per la FASD

- Recettori AMPA
- miRNA 137 e 501-3p
- Recettori mGlu5



Alice Ilari
Lorenzo Curti

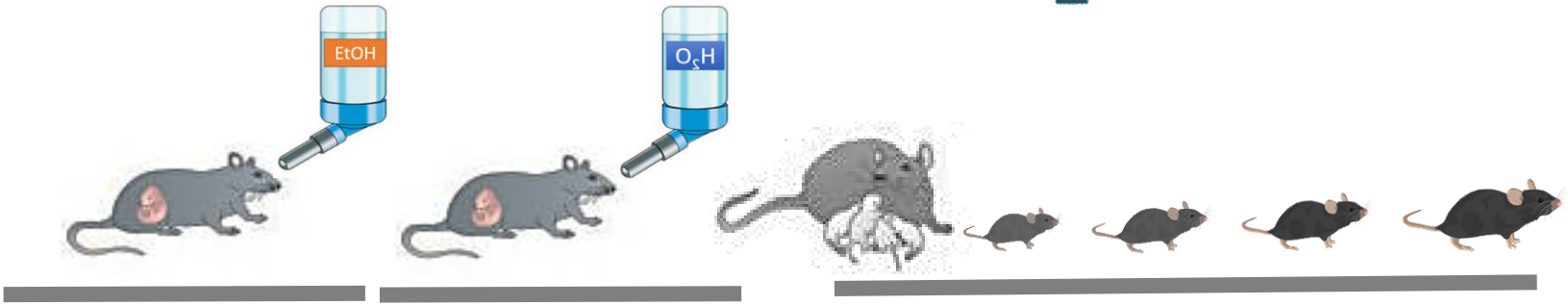
Piano sperimentale



Adapted from Kaminen-Ahola et al., 2010

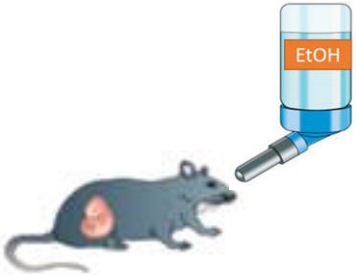
Unpublished

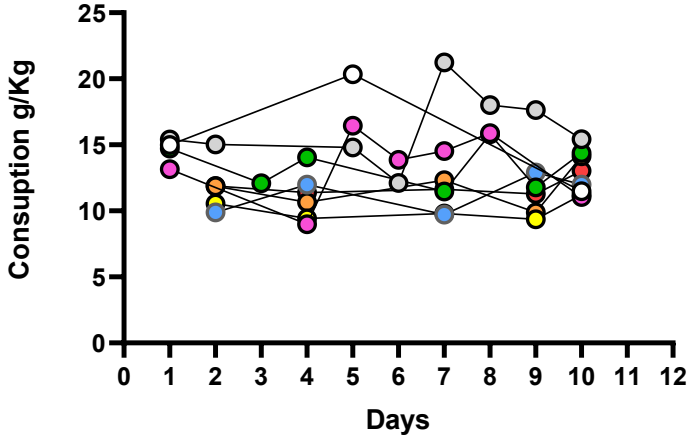
Piano sperimentale

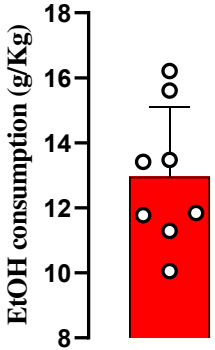


Risultati

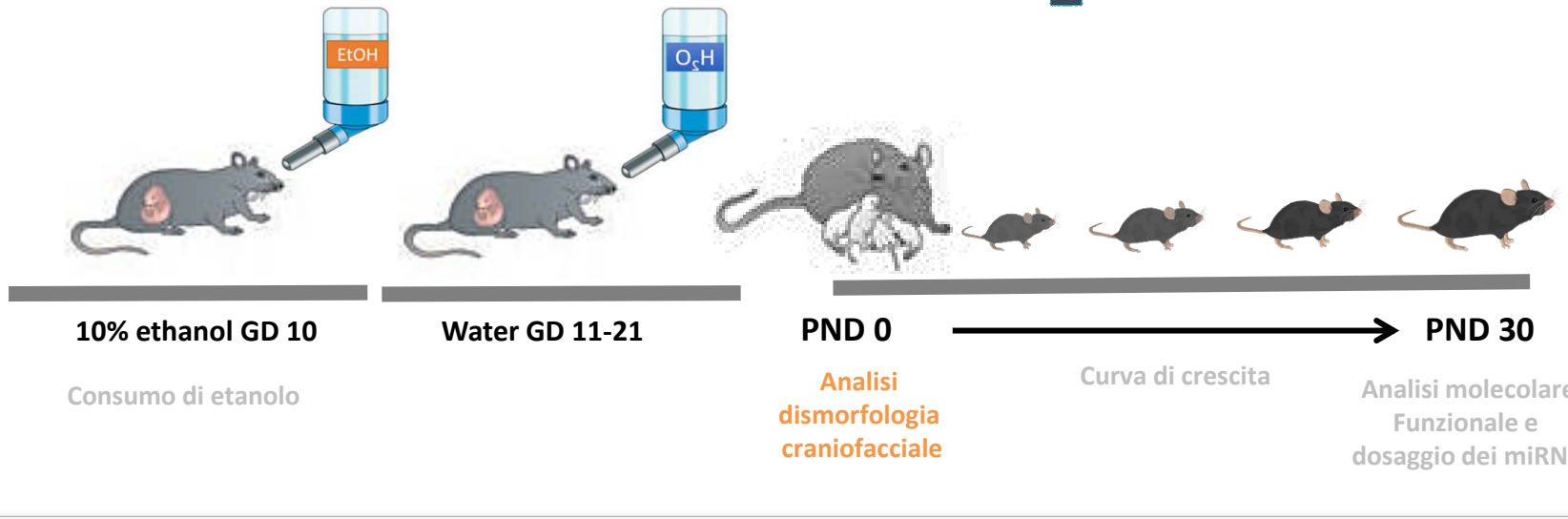
Consumo di etanolo nei topi femmina durante il primo trimestre di gestazione







Piano sperimentale

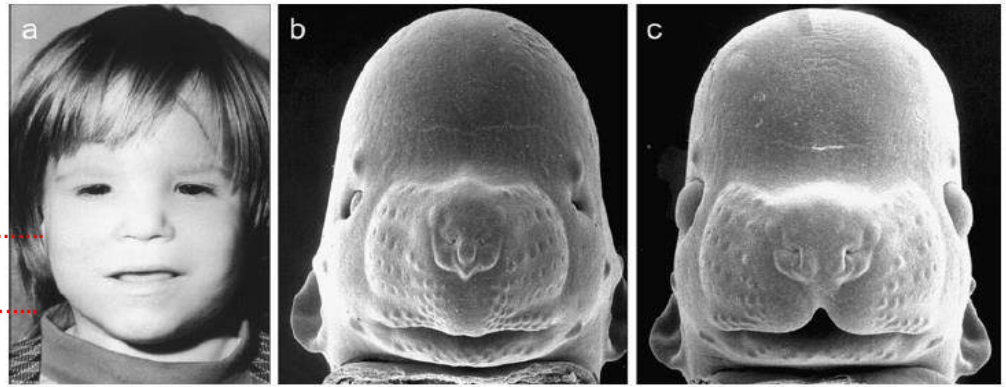


L'esposizione prenatale all'etanolo causa caratteristiche dismorfiche craniofacciali

Small eye opening.....

Short nose.....

Thin upper lip.....



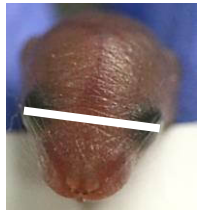
Child with FASMouse with FAS Normal mouse

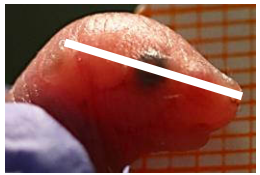
Genesis of Alcohol-Induced Craniofacial Dysmorphism.
Sulik KK. (2005) *Exp Biol Med* **230:** *366-375*

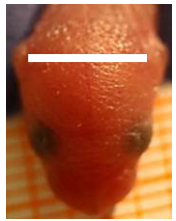
Giuseppe Ranieri

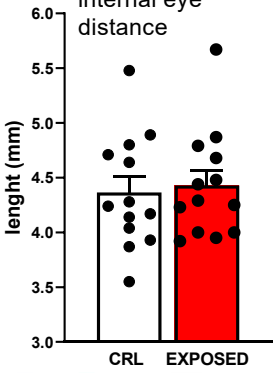
Risultati

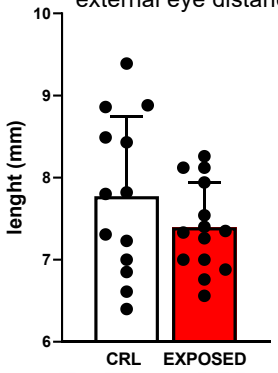
Caratteristiche dismorfiche craniofacciali in PAE C57/Bl6 mice

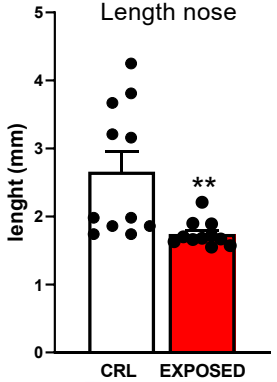


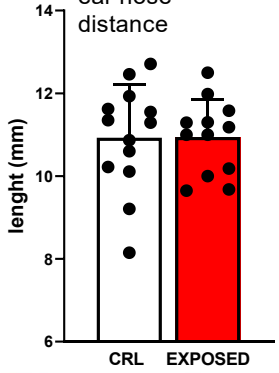


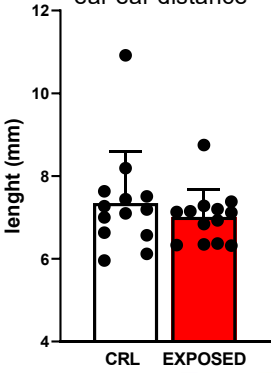




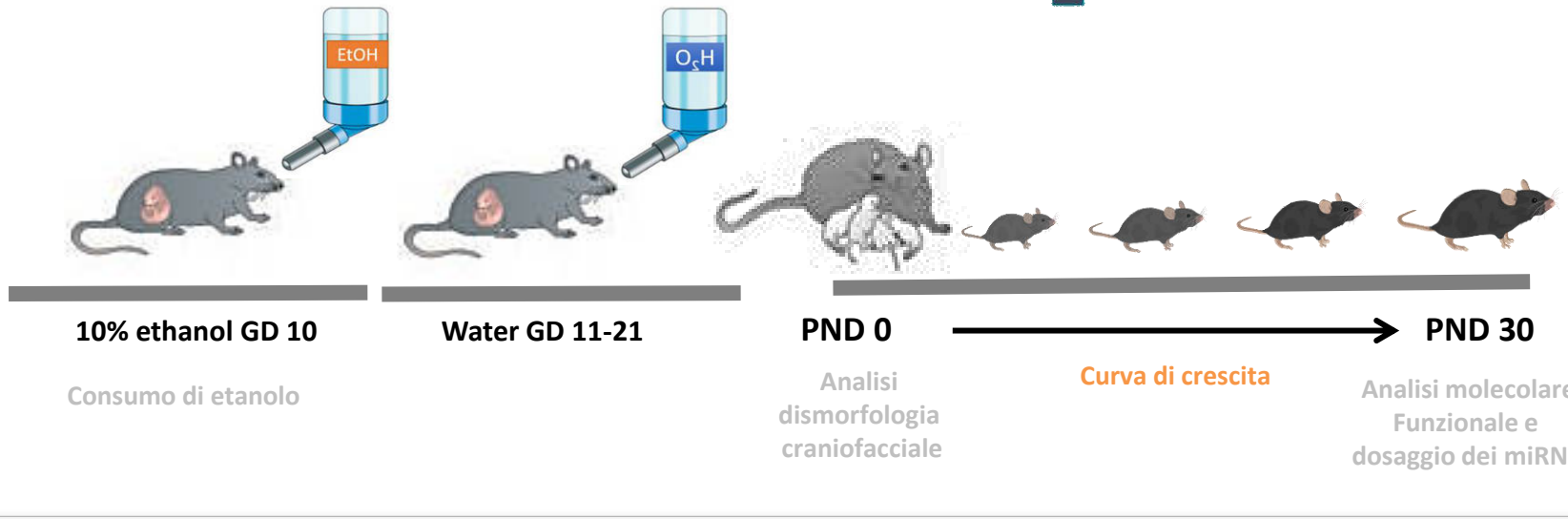


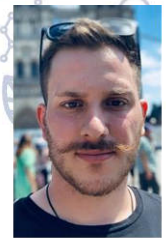






Piano sperimentale





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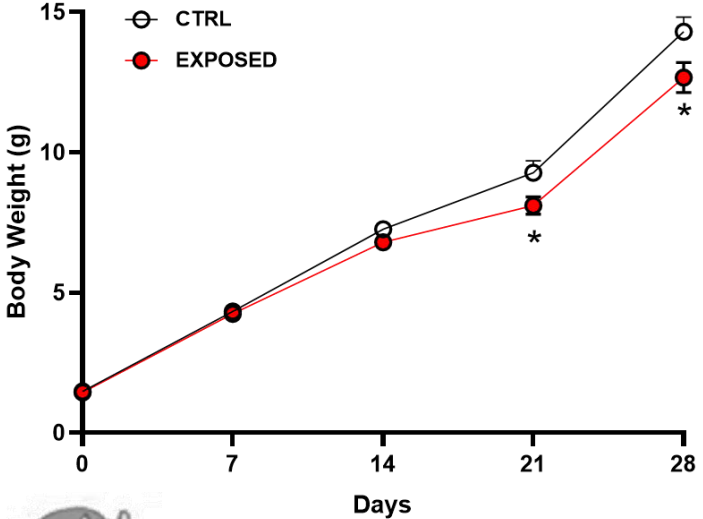
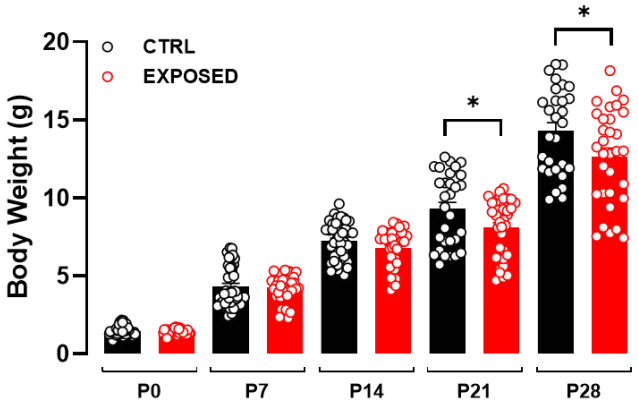
Risultati

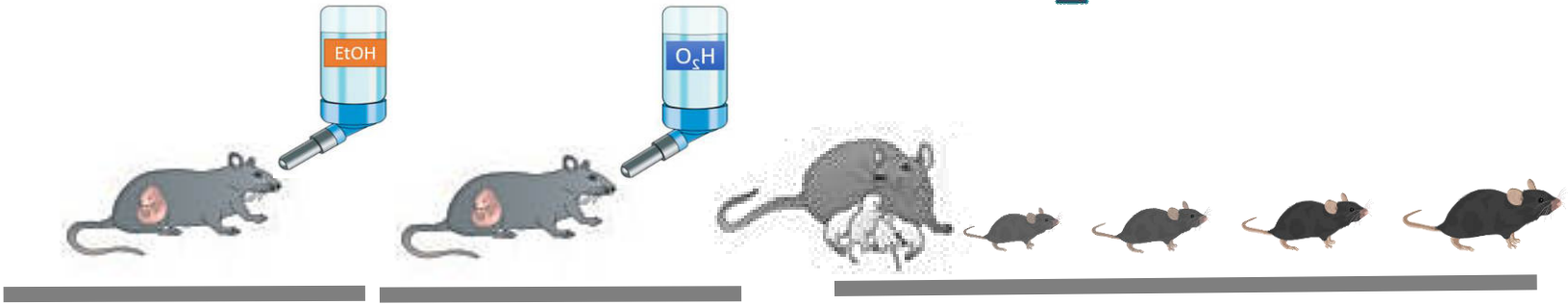
WEIGHT SCALE

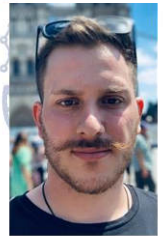
Curva di crescita espressa come peso corporeo





Piano sperimentale

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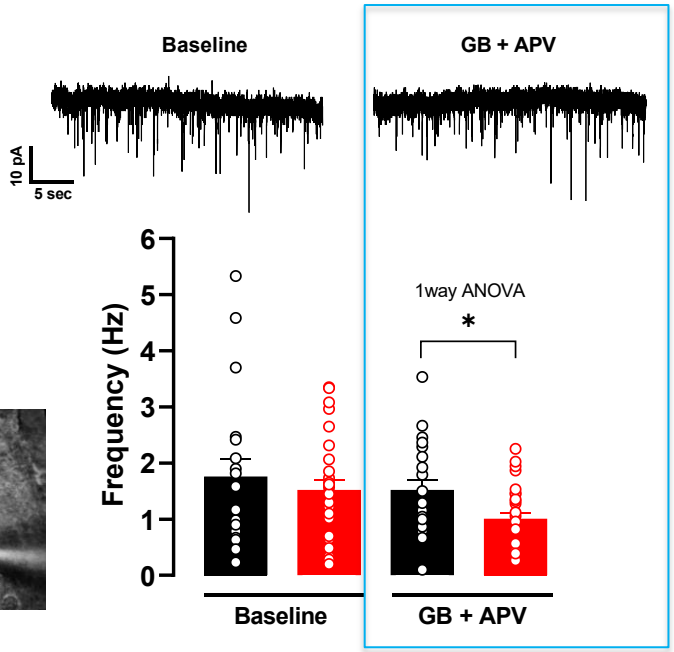
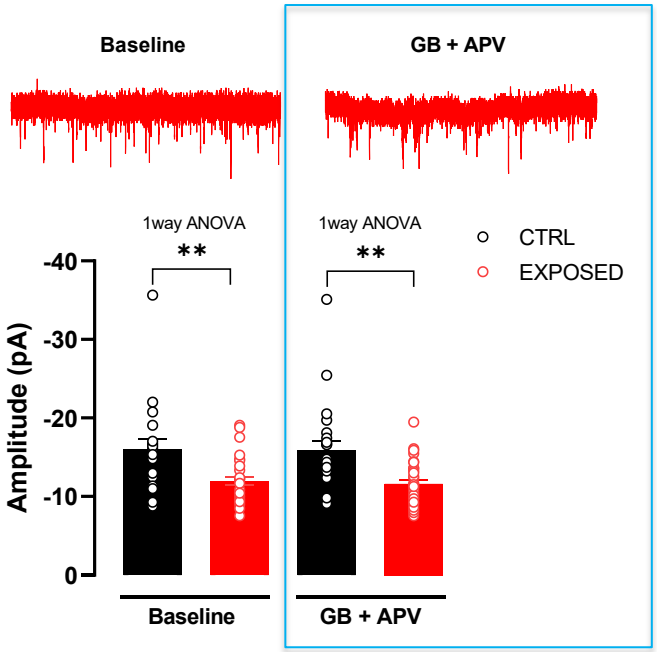
Risultati

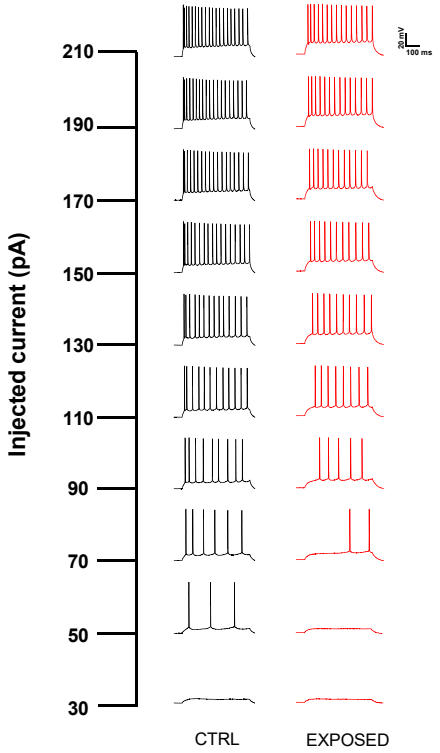
Attività sinaptica eccitatoria dei neuroni piramidali della CA1 dell'ippocampo di topi controllo e PAE

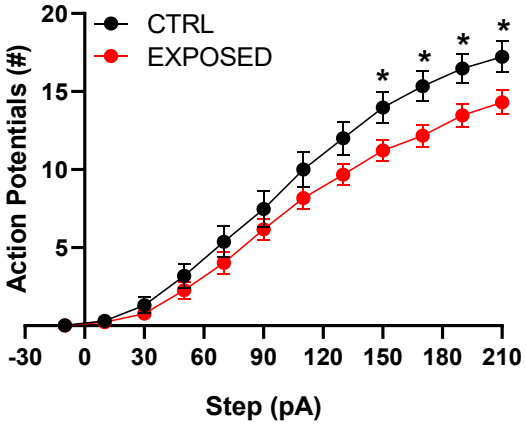


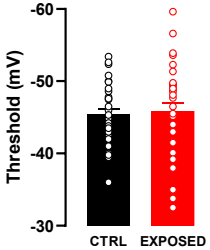


Risultati

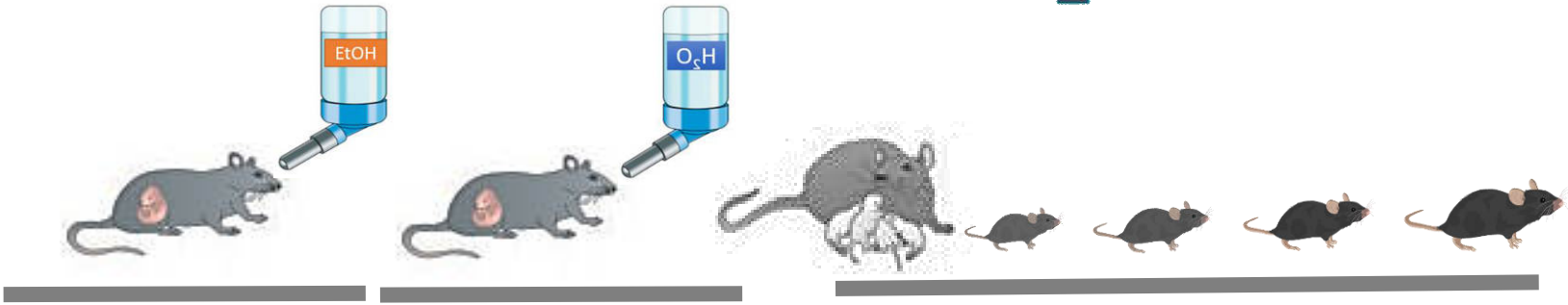
Eccitabilità intrinseca dei neuroni piramidali della CA1 dell'ippocampo di topi controllo e PAE



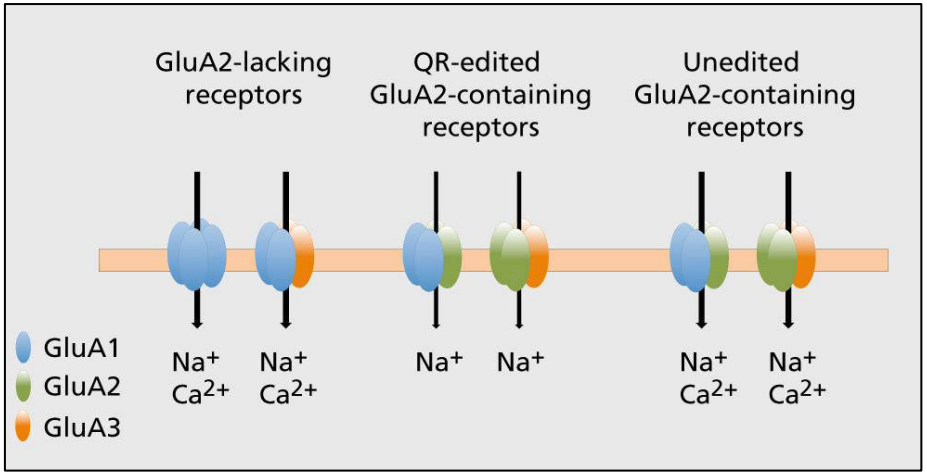


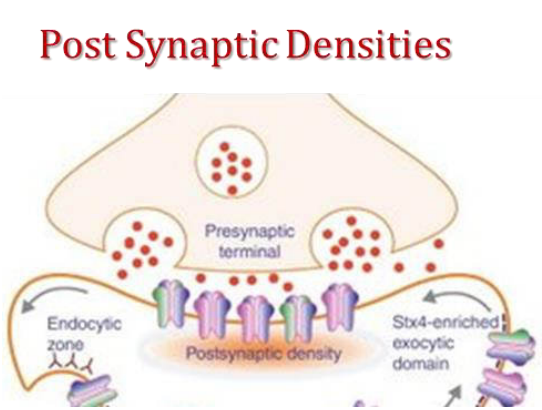


Piano sperimentale



Livelli di espressione dei recettori AMPA nell'ippocampo di topi controllo e PAE





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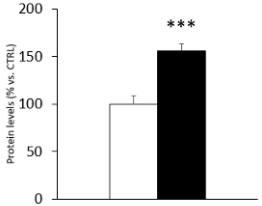
Beatrice Rizzi

Risultati

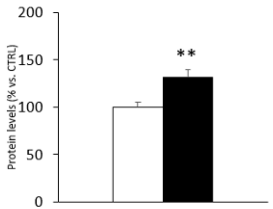
Composizione dei recettori AMPA nell'ippocampo di topi controllo e PAE

homogenate

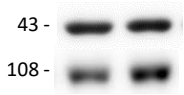
GluA1

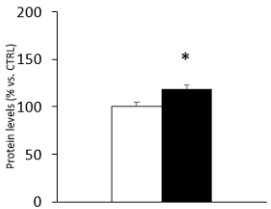


GluA2



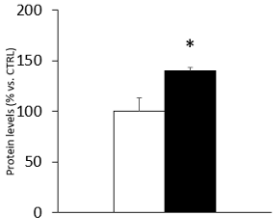
GluA3



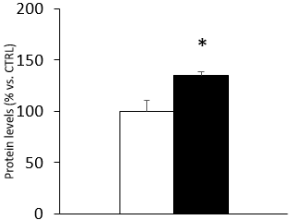


Post-synaptic density

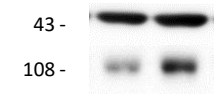
GluA1

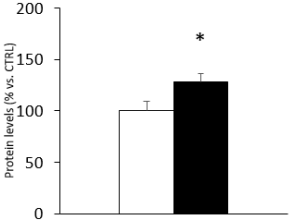


GluA2



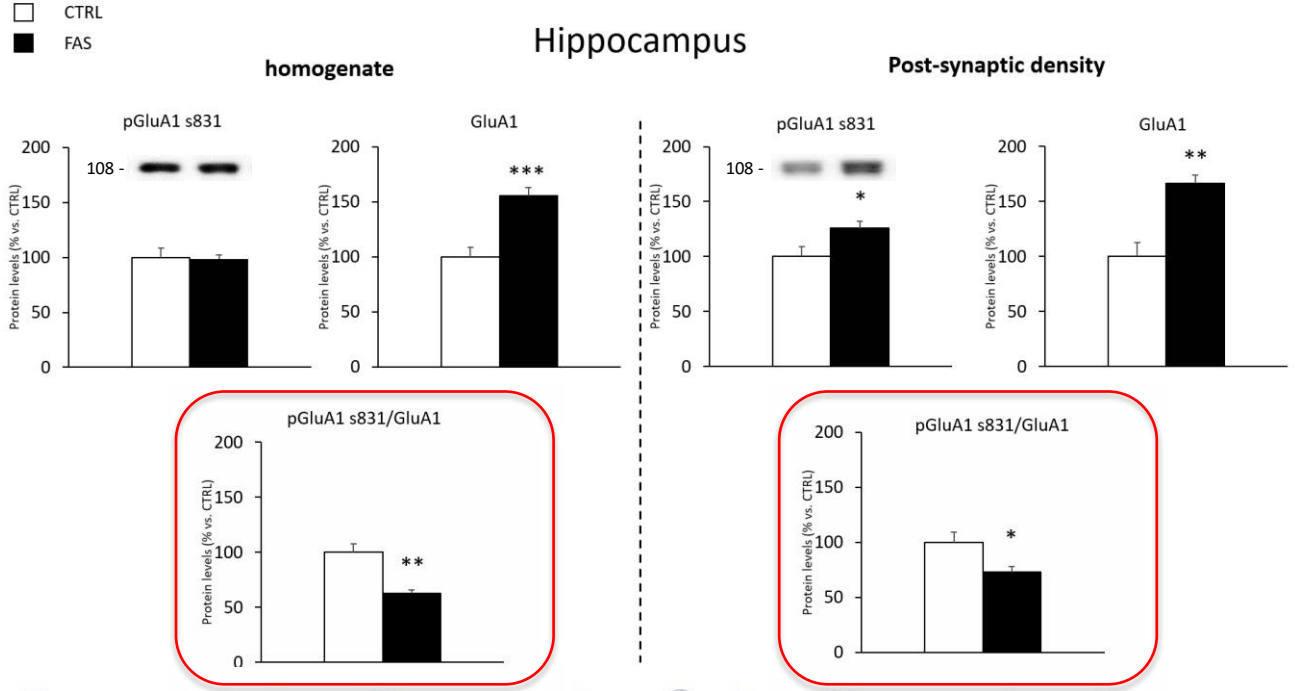
GluA3





Risultati

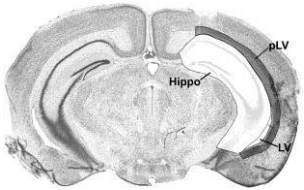
Livelli di fosforilazione (attivazione) della subunità AMPA GluA1 nell'ippocampo di topi controllo e PAE



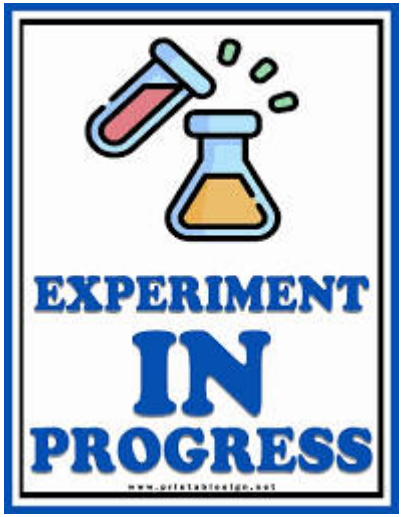
Risultati

Livelli di miR-137 e miR-501-3p nel plasma e negli ippocampi di topi controllo e PAE

Plasma

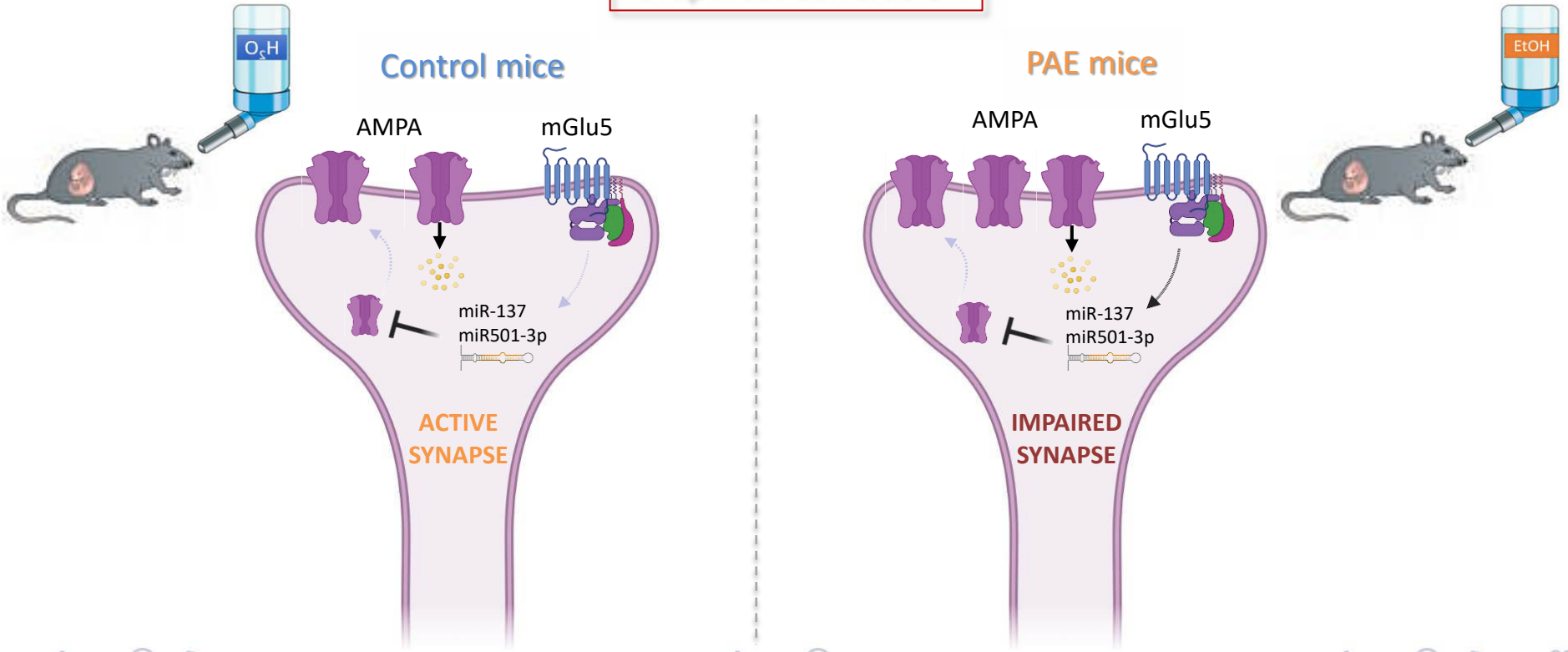


Ippocampo



PART #2

Graphical conclusion



Significato e Implicazioni

Grazie a ….

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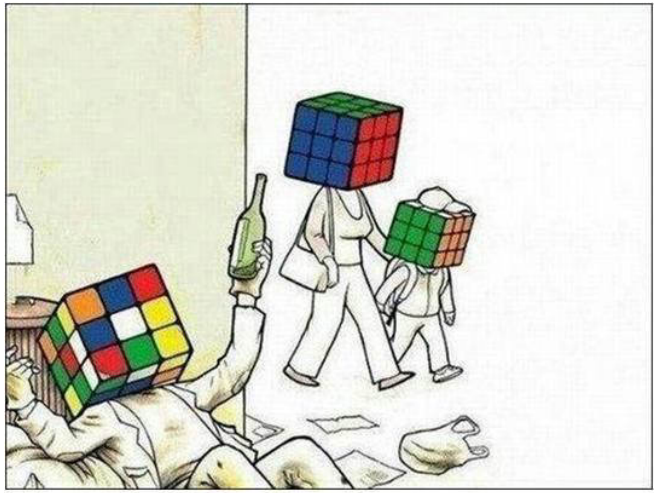
ENTE CASSA DI RISPARMIO DI FIRENZE

Italian Zardi-Gori Foundation for Addiction Research
FONDAZIONE ZARDI-GORI



Grazie a voi per il vostro tempo e per la vostra attenzione