STUDY OF BEHAVIOR, PLASTICITY-RELATED MARKERS AND NEUROINFLAMMATION IN A MOUSE MODEL OF DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY FOLLOWING A KETOGENIC DIET

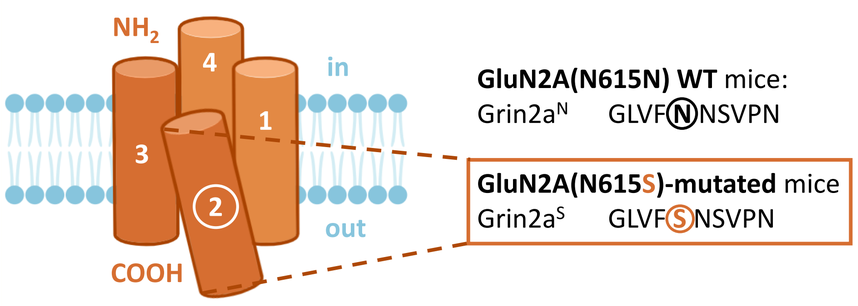
Lorenzo Cifarelli[1,2], Giacomo Einaudi[3], Alessandra Lodi[1,2], Alessandra Oberto[1,2,4], Carlo Cifani[3], Massimo Collino[2] and Ilaria Bertocchi[1,2,4]

[1] Neuroscience Institute Cavalieri Ottolenghi (NICO), University of Turin, Orbassano (Turin), Italy
[2] Department of Neuroscience «Rita Levi Montalcini», University of Turin, Turin, Italy
[3] Pharmacology Unit, School of Pharmacy, University of Camerino, Italy
[4] Neuroscience Institute of Turin, Orbassano (Turin), Italy

Developmental and epileptic encephalopathies (DEE) are early-life onset syndromes characterized by drug-resistant epilepsy and cognitive impairment. The **GluN2A(N615S)-mutated mice** carry a mutation in the Grin2a gene coding for the GluN2A subunit of the NMDA glutamate receptor and display symptoms similar to those described in human patients, representing a valuable murine model for GRIN-related DEEs. We investigated the effects of a **ketogenic diet** (KD) on the epileptic phenotype and behavior in the GluN2A(N615S) model. After behavioral and seizure testing, mice were sacrificed and several tissues were collected. Brains slices were stained for different markers such as WFA for perineuronal nets (PNNs), parvalbumin (PV) for PV+ interneurons (PV+ INs) and Iba1 for microglia

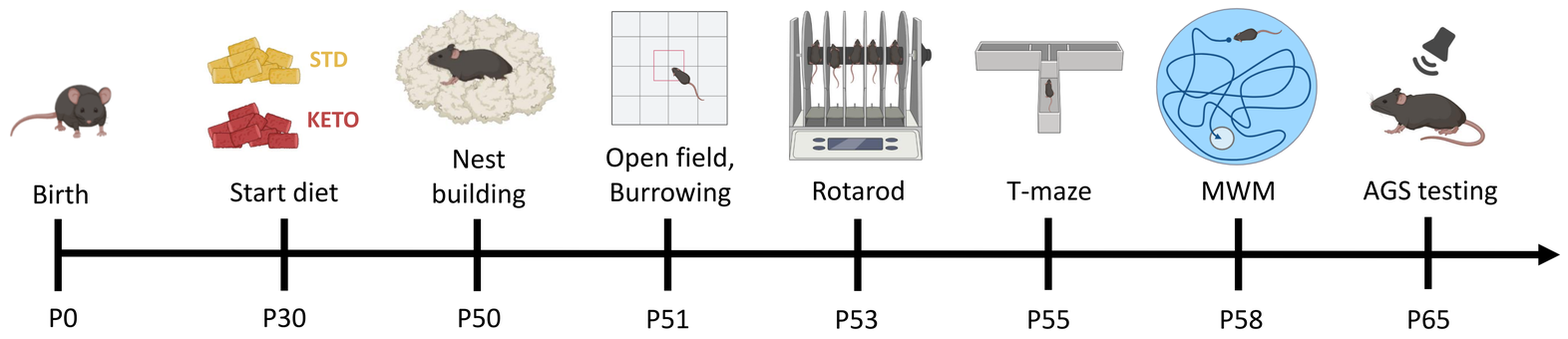
1 Mouse model of disease and behavioral tests

Point-mutated GluN2A subunit of NMDA receptor leading to DEE symptoms and audiogenic seizures (AGS)



GluN2A(N615N) WT mice:
Grin2a^{N} GLVF(N)NSVPN

GluN2A(N615S)-mutated mice
Grin2a^{S} GLVF(S)NSVPN



2 Results of behavioral tests: KD influences activities of daily living, reduces hyperactivity, improves spatial learning and memory (in male mice) and protects against AGS in mutated mice

1 ACTIVITY OF DAILY LIVING: NESTING and BURROWING

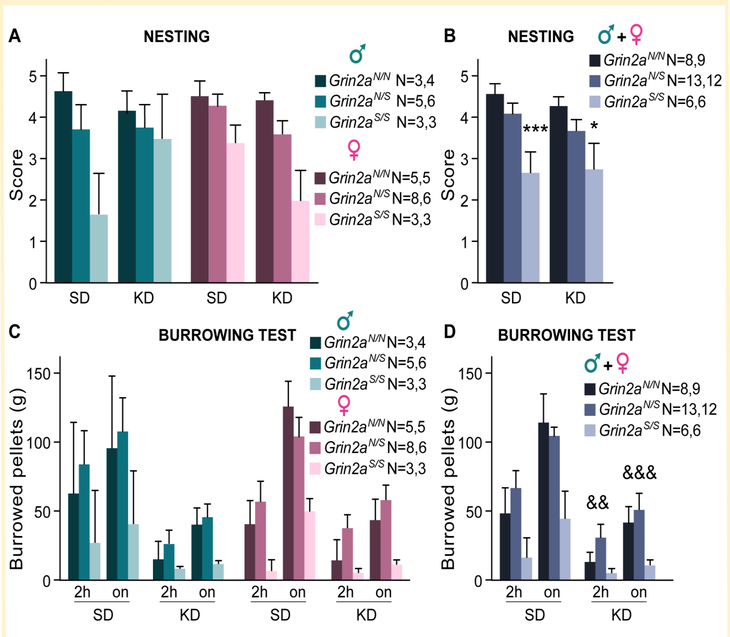


Fig. 1: NESTING A) 3-way ANOVA shows effect on genotype (F 1.647,17.30=17.24,P=0.0001) and interaction between genotype, sex and diet (F(2,21)=4.370,P=0.0259) **B)** Effect of the genotype (2-way ANOVA: F(2,47)=12.98,P<0.0001). * P<0.05, ** P<0.01 and *** P<0.001 for Grin2a$^{S/S}$ vs Grin2a$^{N/N}$ and Grin2a$^{N/S}$ after the Bonferroni post-test. **BURROWING C)** Effect on diet and genotype (3-way ANOVA) **D)** Effect of the genotype (2-way ANOVA: F(2,47)=12.98,P<0.0001). && P<0.01, &&& P<0.0001 indicate effect of the diet

2 OPEN FIELD and MORRIS WATER MAZE

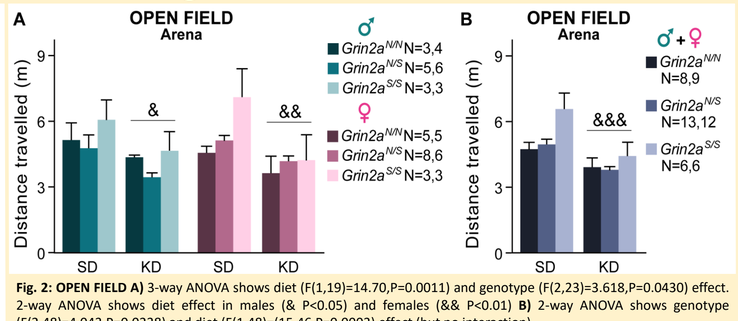


Fig. 2: OPEN FIELD A) 3-way ANOVA shows diet (F(1,19)=14.70,P=0.0011) and genotype (F(2,23)=3.618,P=0.0430) effect. 2-way ANOVA shows diet effect in males (& P<0.05) and females (&& P<0.01) **B)** 2-way ANOVA shows genotype (F(2,48)=4.043,P=0.0238) and diet (F(1,48)=(15.46,P=0.0003) effect (but no interaction)

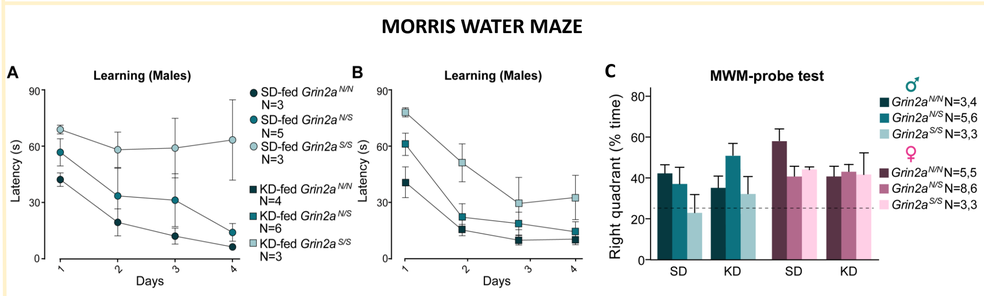


Fig. 3: MWM A) 2-way RM ANOVA analysis in male SD-fed mice shows a day effect (F(2.695,21.56)=7.865,P=0.0013). **B)** 2-way RM ANOVA analysis indicates a significant day (F(1.338,13.38)=32.22,P<0.0001) and genotype (F(2,10)=6.828,P=0.0135) effects only in KD-fed male mice **C)** 3-way ANOVA analysis shows a significant sex effect (F(1,42)= 4.508,P=0.04)

3 Audiogenic seizures testing

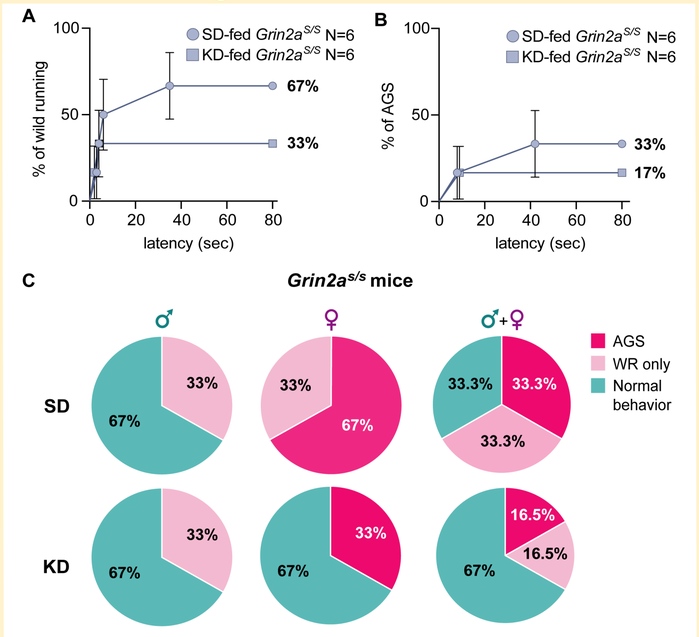


Fig. 4: AGS TESTING A) Incidence of **WR** in Grin2a$^{S/S}$ mice decreases from 67% in Grin2aS/S SD-fed mice to 33% in KD-fed mice (no statistical significance) **B)** Incidence of **AGS** in Grin2a$^{S/S}$ mice decreases from 33% in SD-fed mice to 17% in KD-fed mice (no statistical significance) **C)** Female mice show greater susceptibility to AGS than males (no statistical significance)

3 Results of IHC analysis: preliminary data show a reduced neuroinflammation (Iba1^{+} microglial cells) and an increase in PV+ INs and PNNs in the hippocampus of KD-fed Grin2a$^{S/S}$ mice

IHC ANALYSIS OF HIPPOCAMPUS IN FEMALE MICE

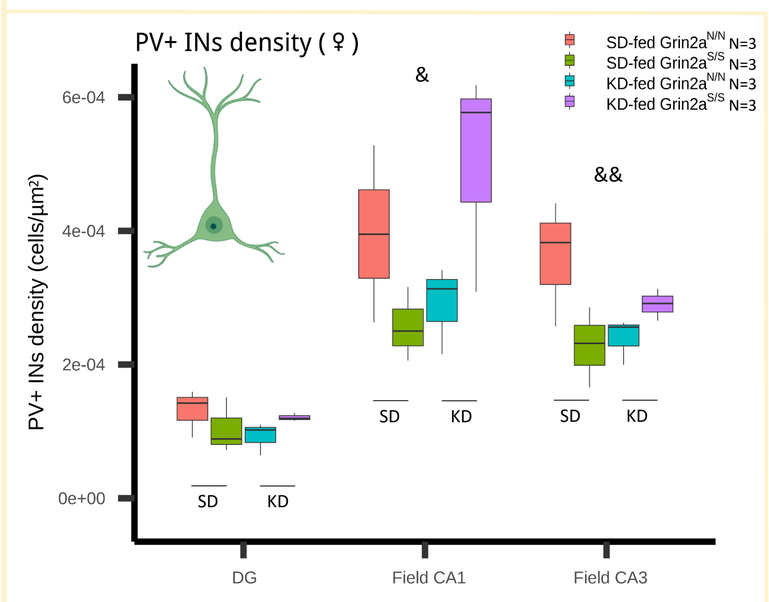


Fig. 5: PV^{+} INs density 2-way ANOVA analysis revealed a significant interaction between genotype and diet in CA1 (& indicates P=0.0305) and CA3 (&& indicates P=0.0287) regions of the hippocampus

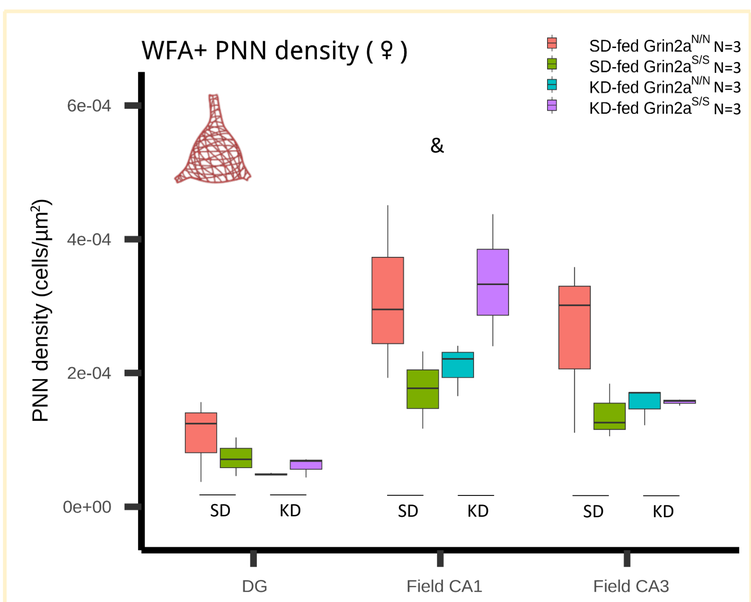


Fig. 6: WFA^{+} PNN density 2-way ANOVA analysis revealed a significant diet-genotype interaction (P=0.0323) in CA1 region of the hippocampus

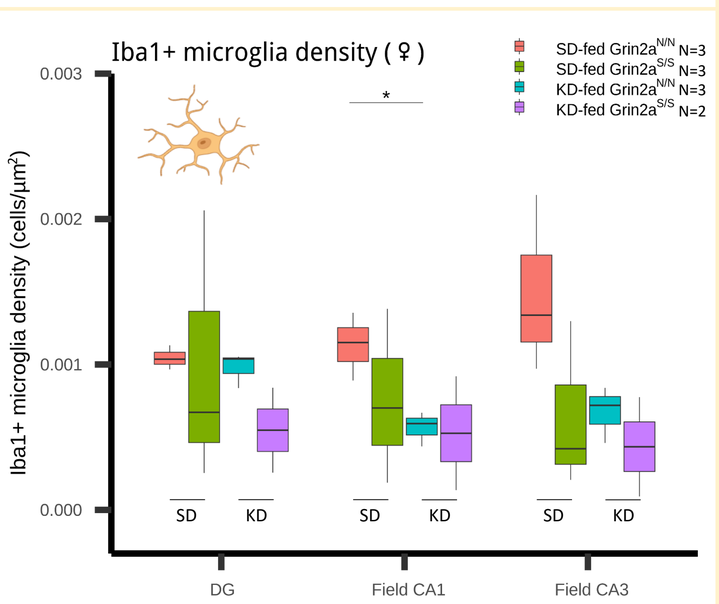


Fig. 7: Iba1^{+} microglia density student t-test analysis revealed a statistically significant difference (* indicates P=0.034) in CA1 region of the hippocampus between SD-fed and KD-fed Grin2a$^{N/N}$ mice

4 Conclusions: we confirmed previous data indicating several deficits and impairments in Grin2a$^{S/S}$ mice – consistent with DEE phenotypes in patients – and proved here that some of them overall improve with KD, such as nest building performance and hyperactivity, whereas memory and learning ameliorate in a sex-based manner (males). We demonstrated for the first time in this DEE model that KD is effective in reducing susceptibility to AGS: preliminary IHC data show that this achievement could be mediated by an increase in inhibitory activity through PV+ INs and PNNs, and by a reduced neuroinflammation

References: Bertocchi I. et al., *Comm Biol*, 2021; Bertocchi I., **Cifarelli L.** et al., *Br J Pharmacol*, 2024