MONOACYLGLYCEROL LIPASE (MAGL) MEDIATES ANXIETY AND IRRITABILITY-LIKE BEHAVIORS IN A SEX-DEPENDENT MANNER IN MARCHIGIAN SARDINIAN ALCOHOL-PREFERRING (msP) RATS

Valentina Vozella[1], Hannah Feldman[2], Bryan Cruz[1], Luis A. Natividad[3], Eric P. Zorrilla[1], Roberto Ciccocioppo[4], Benjamin F. Cravatt[2], Marisa Roberto[1]

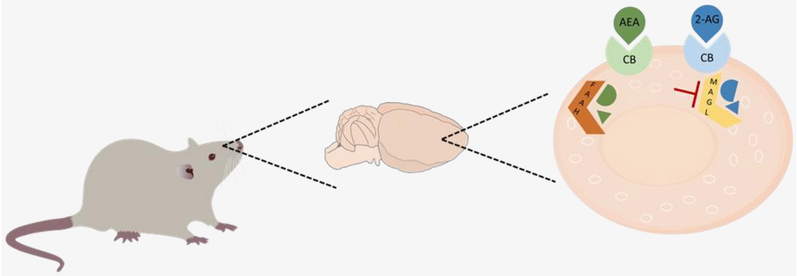
[1] The Scripps Research Institute, Department of Molecular Medicine, La Jolla, CA, USA, [2] The Scripps Research Institute, Department of Chemistry, La Jolla, CA, USA, [3] College of Pharmacy, The University of Texas at Austin, Austin, Texas, USA, [4] School of Pharmacy, Pharmacology Unit, University of Camerino, Camerino, Italy

1. BACKGROUND

- Genetically selected Marchigian Sardinian alcohol-preferring (**msP**) rats are characterized by an increased alcohol preference compared to non-selected Wistar controls[1].
- msP rats are highly sensitive to **stress** and show an anxious phenotype that is reduced by alcohol drinking.
- The heightened **anxiety** phenotype is driven by genetic polymorphism of the corticotropin-releasing factor type 1 receptor (**CRF1**) in limbic brain regions, as well as disruption of other stress-regulatory neurochemical mechanisms including endocannabinoid (**eCB**) system[2].
- Acute stress events activate the enzymatic clearance of AEA via fatty acid amide hydrolase (FAAH) in the amygdala[3].
- Glutamatergic synapses display **sex** and **strain**-specific differences in cannabinoid effects in msP but not in Wistar[4].

2. QUESTIONS

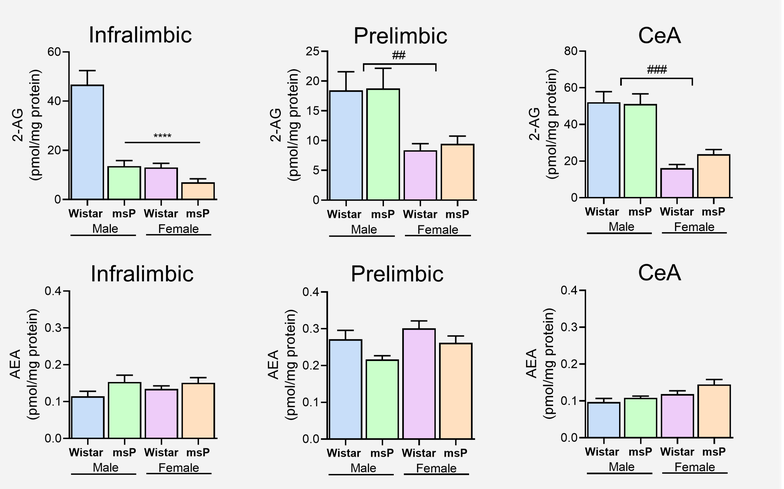
1. Is eCB signaling dysregulated in the limbic areas of an innate high anxiety rodent model such as the msPs?
2. Which are the affected brain regions?
3. Does sexual dimorphism play a role?
4. Is pharmacological modulation of eCB system through MAGL inhibition able to ameliorate anxiety and irritability-like behaviors?



3. MATERIALS AND METHODS

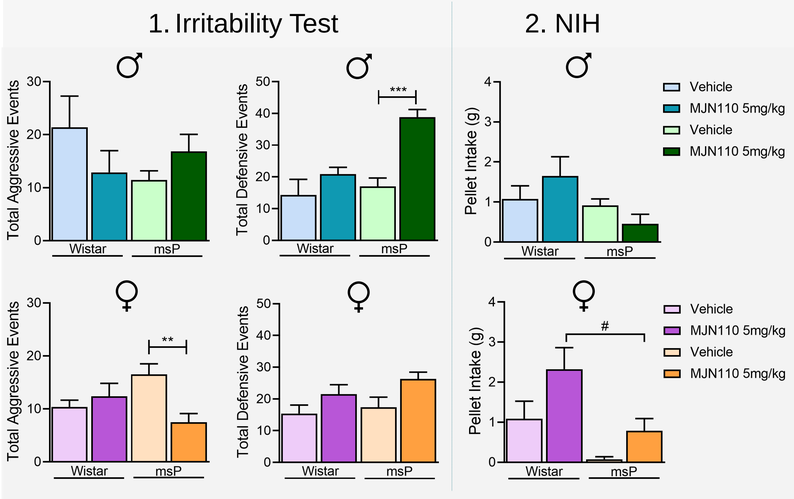
- **Animals**: adult male (~450-500 g) and female (~250-300 g) Wistar and msP rats. A total of 56 rats were used (n = 6-8/group).
- **Tissues**: micropunches from infralimbic cortex (IL), prelimbic cortex (PrL), and central amygdala (CeA).
- **Lipid Extraction**: neutral extraction in cold solvent mixture 2:1:1 = $CHCl_3$:MeOH:DPBS.
- **Analytical Method**: LC-MS/MS.
- **Treatment**: MAGL inhibitor MJN110[5], single intraperitoneal injection, 5 mg/kg, 3 hours prior to each test.
- **Behavioral tests**: **1. irritability-like behavior** using bottle-brush test. 10 trials/rat. Scored behaviors: - aggressive (biting, boxing, siding, following, mounting, rattling); - defensive (avoiding, digging, freezing, jumping, startling, vocalizing); - other (grooming, rearing, exploring). **2. novelty induced hypophagia (NIH)** using 10 min exposure to palatable chocolate pellets in novel cage, light, noise.
- **Statistics**: 2-way ANOVA followed by LSD post-hoc test when interactions occur. # P < 0.05, * P < 0.05.

4. LIPIDOMIC: 2-AG, but not AEA, is decreased in infralimbic cortex of msP and female Wistar rats



Data are expressed as mean ± SEM. Two-way ANOVA followed by LSD post-hoc test when strain*sex interaction occurs, # P < 0.05, sex effect, *P < 0.05, strain*sex interaction, n = 6/group

5. BEHAVIORAL RESULTS: MJN110 ameliorates anxiety-related behaviors in msP rats



Data are expressed as mean ± SEM. Two-way ANOVA followed by LSD post-hoc test when strain*drug interaction occurs, # P < 0.05, drug effect, * P < 0.05, strain*drug, n = 8/group

6. CONCLUSIONS

1. 2-AG is significantly decreased in the infralimbic cortex of male msP rats and female rats compared to male Wistar rats.
2. 2-AG infralimbic level is affected by sex and strain while a sex effect is observed in prelimbic cortex and CeA.
3. No sex by strain-dependent changes are observed in AEA levels.
4. Pharmacological inhibition of MAGL has strain- and sex-dependent effects on the expression of anxiety and irritability-like behaviors, yielding differential stress coping strategies in male and female rats.
5. Overall, our data support that anxiety-related behaviors in msP rats are driven by dysregulated eCB signaling mechanisms.
6. Future work will investigate the sex-dependent neurobiological effects of MAGL inhibition.

REFERENCES

[1]Ciccocioppo R. et al., *Addict Biol*, (2006), **11**(3-4):339-55. [2]Stopponi S. et al., *Addict Biol*, (2018), **23**(6):1223-1232. [3]Natividad L.A. et al., *Biol Psychiatry*, (2017), **82**(7):500-510. [4]Kirson D. et al., *Addict Biol*, (2018), **23**(2):676-688. [5]Niphakis M.J. et al., *ACS Chem Neurosci*, (2013), **4**(9):1322-32.

FUNDINGS

NIH/NIAAA grants AA017447, AA015566, AA021491, AA006420, AA013498, AA027700, T32 AA007456, and The Pearson Center for Alcoholism and Addiction Research

CONTACT INFORMATION

Valentina Vozella: vvozella@scripps.edu

Marisa Roberto: mroberto@scripps.edu