Autistic-like behavioral effects of prenatal stress in the FMR1-KO mouse model of fragile X syndrome

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AUTISTIC-LIKE BEHAVIORAL EFFECTS OF PRENATAL STRESS IN THE FMR1-KO MOUSE MODEL OF FRAGILE X SYNDROME

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BACKGROUND

Fragile X Syndrome (FXS) is the most common heritable form of mental retardation and the main monogenic cause of autism spectrum disorder (ASD). **FXS** is due to a mutation in the X-linked FMR1 human gene consisting in more than 200 CGG repetitions leading to the absence of FMRP protein and is characterized by motor, cognitive and social alterations, mostly overlapping with autism spectrum disorder behavioral phenotypes.

FXS behavioral phenotype can be critically modulated by environmental factors e.g., stress exposure, both in terms of its severity and of the timing of appearance

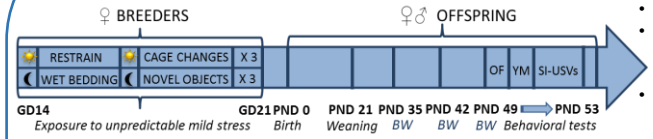
AIMS

Study 1: Evaluate the short-term effects of the prenatal exposure to unpredictable chronic stress on the behavioral phenotype of juveniles of both sexes in the Fmr1 knock-out (KO) mouse model of FXS, in order to asses whether the prenatal stress could advance and/or exacerbate the behavioral phenotype of Fmr1-KO offspring.

Study 2: Evaluate the long-term effects of prenatal exposure to unpredictable chronic stress during the last prenatal week on the FXS- and ASD-like behavioral phenotype of the Fmr1-KO model to asses the stability of the stress effects in Fmr1 mutant mice, extending their behavioral characterization during aging.

STUDY 1: SHORT-TERM EFFECTS - *JUVENILES*

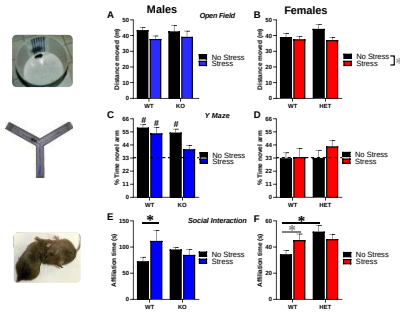
METHODS:



- **45 males** (n=9-15 for stress condition) and **48 females** (n=12 for stress condition).
- ***Unpredictable mild stress*** → restrain stress (3 sessions 30-min day 1) and overnight housing with wet bedding; sawdust and cage changes (3 sessions day 2), overnight housing with novel glass black beads.
- ***Behavioral tests (7-8 weeks):*** Open Field, Y-Maze, Social Interaction, Ultrasonic vocalizations

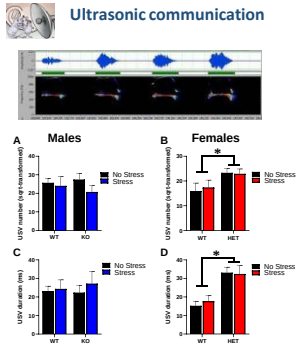
RESULTS:

Behavioral phenotype



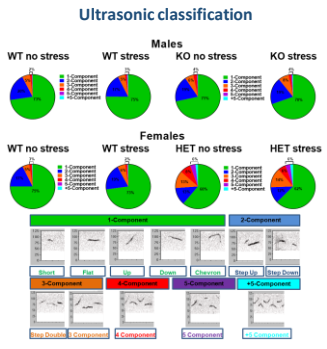
We observed:
- Mutants: ↑ HET females affiliation behavior
- Stress: 1. Cognitive deficit in Y maze only in **males**
  2. ↑ Affiliation behavior in **WT** males and females
  3. ↓ Locomotion in **females (HET and WT)**

Ultrasonic communication



We observed:
- Mutants: ↑ Number and duration of USVs in **HET females**
- Stress: **No** selective effect

Ultrasonic classification

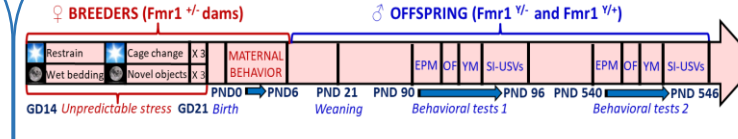


We observed:
- Mutants: Qualitative alterations of USVs (↑ complex calls) in **HET females**
- Stress: **No** selective effect

Petroni et al. 2022, Scientific Reports. Autistic-like behavioral effects of prenatal stress in juvenile Fmr1 mice: the relevance of sex differences and gene-environment interactions

STUDY 2: LONG-TERM EFFECTS - *ADULTS AND OLD*

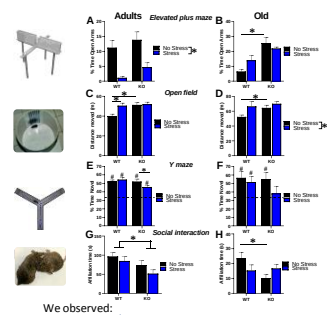
METHODS:



- **38** males (n=8-10 for stress condition) tested at **3** months (**Adults**).
- **31** surviving animals: n=8 for each stress condition) tested again at **18** months (**Old**).
- Same stress procedure as in study 1 → ***Unpredictable mild stress***
- ***Behavioral tests***: Elevated Plus Maze, Open Field, Y-Maze, Social Interaction, Ultrasonic vocalizations

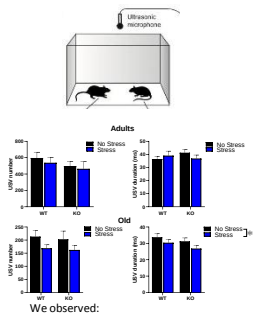
RESULTS:

Behavioral phenotype



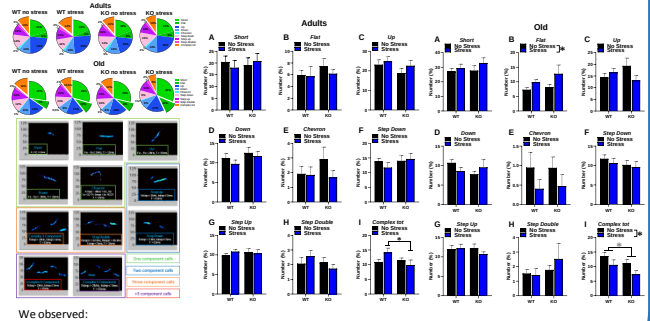
We observed:
- Mutants: 1. ↑ Locomotion at **both** ages
  2. ↓ Affiliative behavior at **both** ages
- Stress: 1. ↑ Anxiety and locomotion at **adulthood**
  2. Hyperactivity (KO and WT) at **aging**
  3. Cognitive deficit at **both** ages KO (↑ **old**)
  4. ↓ Affiliative behavior at **adulthood**

Ultrasonic communication



We observed:
- Mutants: **No** effects
- Stress: 1. ↓ Duration of ultrasonic calls in **old** mice

Ultrasonic classification



We observed:
- Mutants: Qualitative alterations of ultrasonic calls in **adults KO**(↓ complex calls)
- Stress: 1. ↑ The percentage of flat calls in **old** mice (KO and WT)
  2. ↓ The percentage of complex calls in **adults** and **old** mice (KO and WT)

Petroni et al., Frontiers -Under review. Long-term behavioral effects of prenatal stress in the Fmr1-KO mouse model for Fragile X syndrome

CONCLUSIONS-STUDY 1 (SHORT-TERM EFFECTS):

- Stress induced the **cognitive deficits only** in Fmr1-KO **males** (deficit in Y maze at juvenile age)
- In **females** stress attenuated the Fmr1-KO phenotype because of its effects on locomotion, SI and USVs in WT mice

CONCLUSIONS-STUDY 2 (LONG-TERM EFFECTS):

- Stress exacerbates the **cognitive deficits** of Fmr1-KO mice at **both ages**
- Stress affected anxiety, locomotion, social interaction and communication in mice of both genotypes, especially at **aging**

Stress effects on FXS-phenotype are present only in the **cognitive domain** and in **males**. These effects are **stable** (**adult** and **old** mice) - Stress behavioral effects on locomotion, SI and USVS appear only as **long-term effects** → **Gene-environment interactions play a major role in modulating the behavioral FXS-like phenotype of Fmr1-KO mice**