**INTRODUCTION**

**Epilepsy and Dravet syndrome**
- Epilepsy: common neurological disorder (up to 65 million people worldwide)
- 30%: not responding to current anti-epileptic drugs (AEDs) (i.e. drug-resistant)
- Etiology: genetic, structural-metabolic or unknown
- SCN1A mutation in 80% of Dravet syndrome patients
- Dravet syndrome: drug-resistant, rare and severe epilepsy syndrome; starting in 1st year of life, accompanied by cognitive and behavioral impairments

**Zebrafish (ZF) and drug discovery**
- Small vertebrate model → ZF larvae: validated for fast, cost-efficient AED screening
- Serotonin receptors (5-hydroxytryptamine, 5-HT-R): suggested, novel target of future AEDs
- Experimental serotonergic drug in clinical trial for Dravet syndrome patients (fenfluramine):
  - Anti-epileptiform activity in morpholine knockdown ZF model of Dravet syndrome
  - Severe, possible side effects due to stimulation of 5-HT1A receptor (valvulopathy, cardiotoxicity)
- Mechanism of action: involvement of 5-HT or other receptors?

**RATIONALE**

How do scn1Lab mutant zebrafish larvae recapitulate Dravet syndrome and aid innovative anti-epileptiform drug discovery?
- Abnormal behavior and brain activity in scn1Lab-/- mutants (vs. wildtype) 
- Drug screening of serotonergic analogs in locomotor assay leading to hits:
  - Confirmation of hits by measuring local field potentials and elucidating the mechanism of action

**METHODS**

**1) Locomotor behavior and brain activity**
- Locomotor behavior 4 - 8 dpf:
  - Distance travelled in large movements for 10 minutes (min) after 30 min habituation
  - High throughput (HT) with an automated tracking device (ZebraBox® apparatus; Viewpoint, Lyon, France)
- Brain activity 7 dpf:
  - Invasive forebrain open-field recordings for 10 min
  - Low throughput testing (LT), ZF larvae embedded in 2% low-melting-point agarose

**2) Neurotransmission**

**LC-EDC 7 dpf**
- Determination of the amount of neurotransmitters in heads of ZF larvae by Microprobe Chromatography with Electrical Chemical Detection (LC-EDC)

**3) Drug screening**
- Locomotor behavior 6 - 7 dpf:
  - Treatment of fenfluramine and serotonergic agonists at maximum tolerated concentration (MTC)
  - Combined treatment of fenfluramine and serotonergic antagonists at MTC
- Brain activity 7 dpf:
  - Confirmation of hits if a significant decrease in epileptiform brain activity

**RESULTS**

**1) Behavior and brain activity**
- Locomotor behavior 6 - 7 dpf:
  - Higher activity of scn1Lab-/- mutants, compared to wildtype scn1Lab+/+
  - May reflect tonic-clonic onset of seizures in Dravet syndrome patients
- Brain activity 7 dpf:
  - Decrease in epileptiform events in heads of scn1Lab-/- mutants (normalized against these amphibian matched controls and expressed in percentage (%))
  - 5-HT1D- and 5-HT2B-agonist reduced epileptiform locomotor and brain activity
  - 5-HT1D receptor not involved

**2) Neurotransmission**

- Decrease in serotonin in heads of scn1Lab-/- mutants.
- Comparison to wildtype scn1Lab+/+
- 5-HT1D- and 5-HT2B-agonist reduced epileptiform locomotor and brain activity
- 5-HT1D receptor not involved

**3) Drug screening**

- Combined treatment experiment. (10 larvae for each condition, experiment in triplicate)

**CONCLUSIONS**

- scn1Lab-/- mutant zebrafish mimic Dravet syndrome, in line with the findings of Baraban et al.
- Reduction of serotonin in DS ZF larvae highlights role of serotonin in animal and human studies of drug-resistant epilepsies
- Fenfluramine, the 5-HT1D, 5-HT2A, and 5-HT2C-agonist reduced epileptiform locomotor and brain activity
- 5-HT1D receptor not involved
- 5-HT2B-agonist counteracted the decrease by fenfluramine in locomotor assays

**REFERENCES**


**STATISTICS**

- Locomotor behavioral analysis: One-way ANOVA followed by Dunnett’s multiple comparison test
- Brain activity analysis: Mann Whitney U test
- Neurotransmission: Student’s t-test (p<0.05). (p<0.001) (p<0.0001) (p<0.00001)

Contributes to elucidation of the mechanism of fenfluramine

Highlights 3 serotonin receptor subtypes (5-HT1D, 5-HT2A, and 5-HT2C) as interesting targets for future innovative AEDs.