

ABBREVIATIONS / DRUGS

5-HT	5-hydroxytryptamine
AGSz	Audiogenic seizures
ED ₅₀	Effective dose in 50%
Fenfluramine	Enhancer of 5-HT release
i.p.	Intraperitoneal
N	Number of animals
PID	Post-ictal depression
RA	Respiratory arrest
S-IRA	Seizure-induced respiratory arrest
SUDEP	Sudden Unexpected Death in Epilepsy

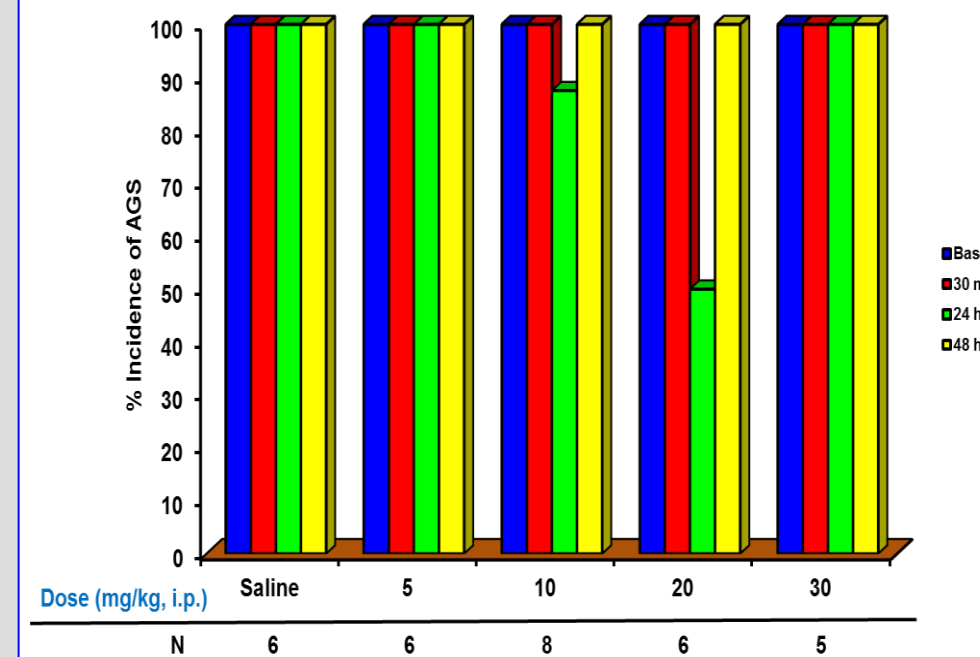
INTRODUCTION

Prevention of premature mortality due to sudden unexpected death in epilepsy (SUDEP) is a critical unmet need in neurology. The majority of witnessed clinical cases of SUDEP involved generalized seizures that led to respiratory and then cardiac failure during post-ictal behavioral depression (PID). Serotonin (5-hydroxytryptamine, 5-HT) is a critically important neurotransmitter released during PID that enhances respiration (Fisher and Schachter, 2000). Dravet Syndrome is an intractable form of epilepsy that shows an unusually high incidence of SUDEP. A recent report indicated that patients with Dravet Syndrome have an improved clinical course when treated with fenfluramine (Ceulemans et al., 2016), a drug that enhances 5-HT release. The DBA/1 mouse model of SUDEP reliably exhibits a highly elevated incidence of death due to seizure-induced respiratory arrest (S-IRA) during PID following audiogenic seizures (AGSz). Fluoxetine, which blocks 5-HT re-uptake, effectively suppresses SUDEP in DBA/1 mice (Faingold et al., 2011), but not all of the drugs that enhance the action of 5-HT are effective (Faingold et al., 2011a, 2014). In the present study we evaluated whether fenfluramine, a drug that enhances 5-HT release, could prevent S-IRA in the DBA/1 mouse model of SUDEP.

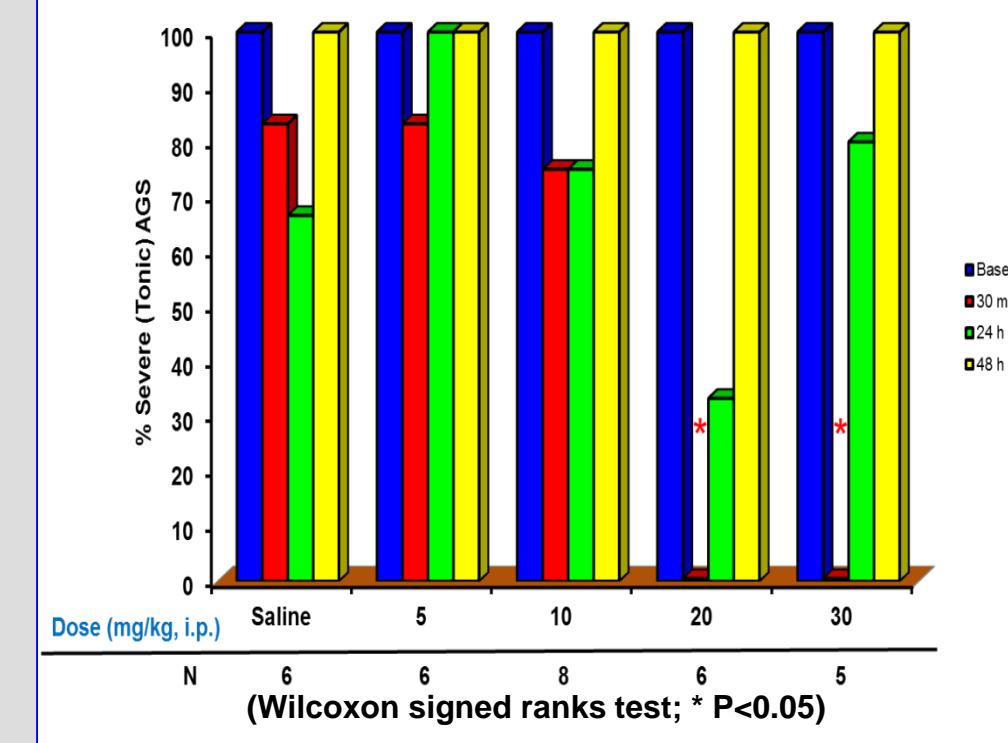
METHODS

1. DBA/1 male mice (21-30 day old) were utilized in this study.
2. The mice were tested for susceptibility to S-IRA with 3-4 seizures (priming) on succeeding days, using an electrical bell at 122 dB SPL (re: 0.0002 dyne/cm²) in a cylindrical chamber followed by resuscitation.
3. The acoustic stimulus was presented until severe (tonic extension) seizure occurred or for a maximum duration of 1 min.
4. Mice that exhibited S-IRA were resuscitated using a rodent respirator (operating at 180 strokes/min) by placing the inhalation tube over the nose.
5. At least 24 h after the final priming seizure DBA/1 mice that consistently exhibited S-IRA were given fenfluramine or vehicle (saline) intraperitoneally (i.p.).
6. Thirty min after administration of drug or saline, DBA/1 mice were tested to examine if seizure behaviors or susceptibility to S-IRA were affected.
7. The mice were acoustically stimulated at 24 h intervals until they displayed susceptibility to S-IRA again.
8. Behaviors were recorded on videotape, and seizure patterns were quantified and statistically compared offline (Wilcoxon signed ranks test).
9. The effective dose in 50% of mice (ED₅₀) of fenfluramine against S-IRA in DBA/1 mice was calculated using logarithmic regression analysis in Microsoft Excel.

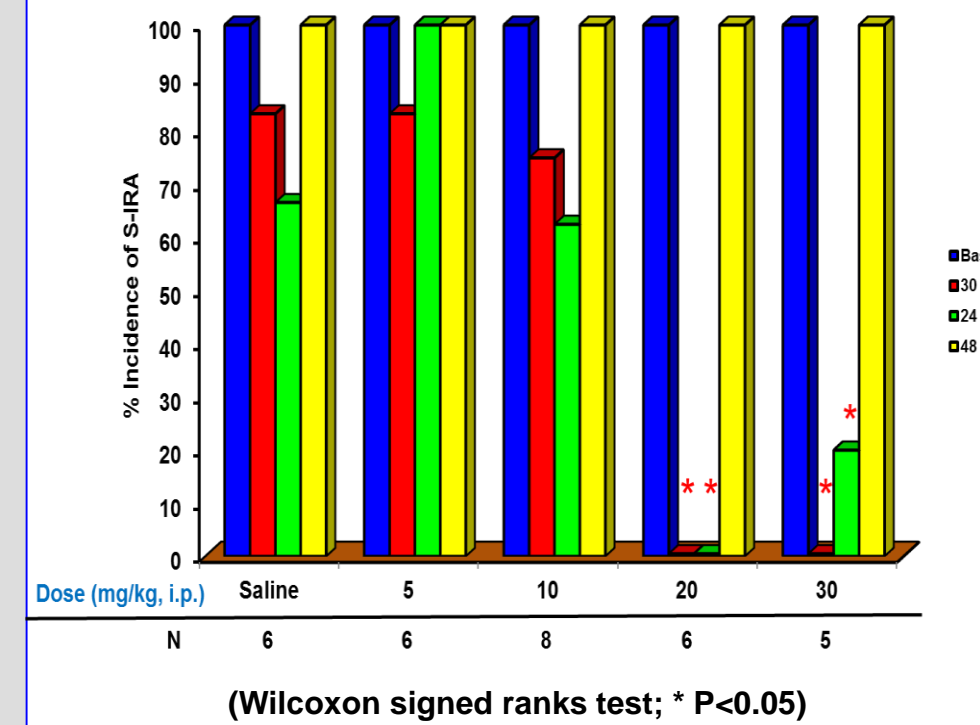
Fenfluramine at low doses does not significantly reduce AGSz in DBA/1 mice



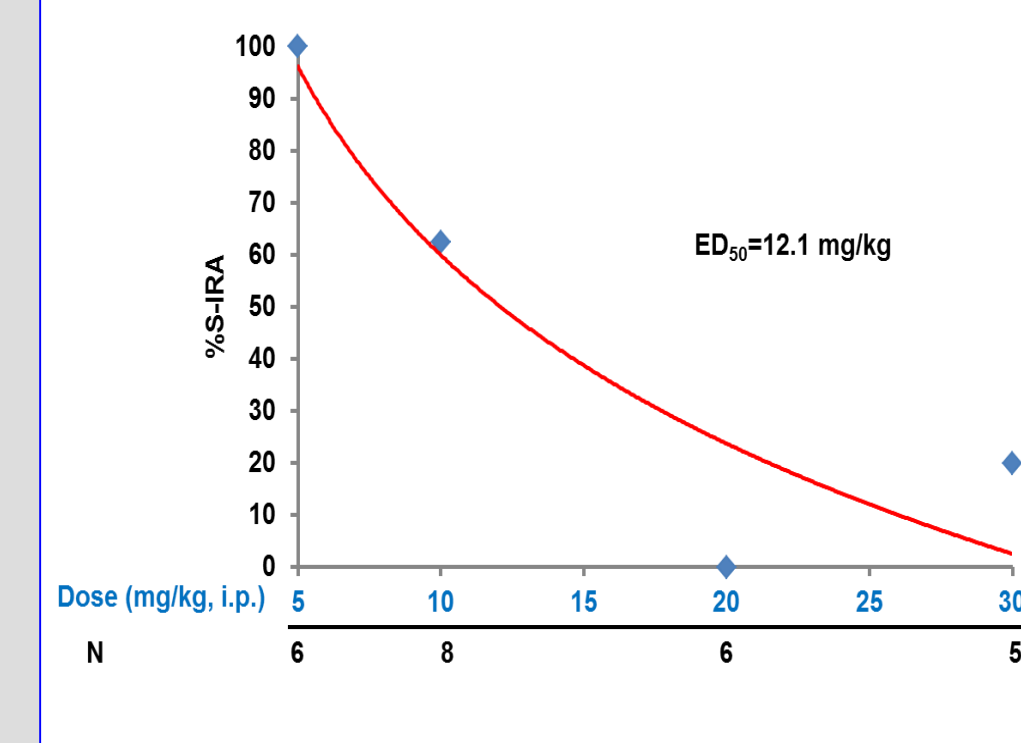
Fenfluramine reduces seizure severity in DBA/1 mice at 30 minutes



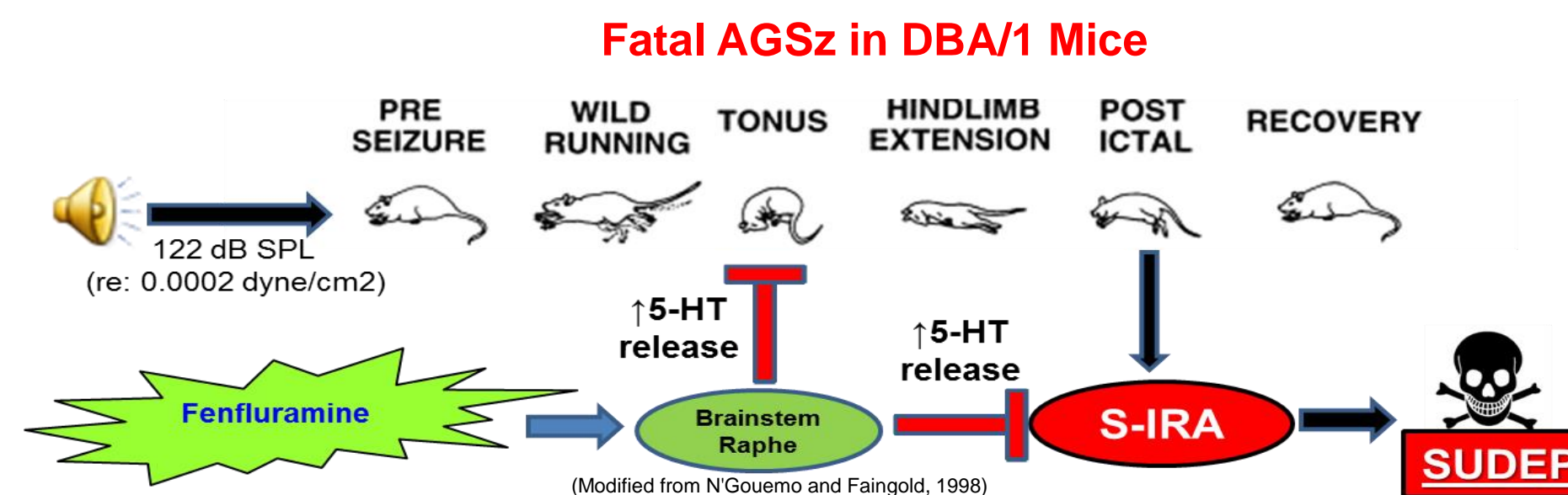
Fenfluramine induced a prolonged block (at 30 minutes & 24 hours) of S-IRA in DBA/1 mice



Dose-dependent S-IRA blocking effect of Fenfluramine at 24 hours in DBA/1 mice



Putative mechanism of S-IRA Blockade by Fenfluramine



SUMMARY & CONCLUSIONS

1. Fenfluramine, a drug that enhances the release of 5-HT, reduces S-IRA in DBA/1 mice.
2. Fenfluramine displayed both a dose- and time-dependence in blocking S-IRA along with an anticonvulsant effect.
3. The present study suggests a novel potential application of fenfluramine to enhance respiration after seizure, which may contribute to the improved clinical course in Dravet syndrome seen with this drug (Ceulemans et al., 2016).
4. These findings suggest that fenfluramine may be useful to better control seizures and is a potential therapeutic agent that deserves investigation for preventing SUDEP in epilepsy patients.

SIGNIFICANCE & FUTURE DIRECTIONS

1. These data are the first evidence of the prolonged S-IRA blocking effect of fenfluramine even at doses that do not affect seizure severity in the DBA/1 mouse model of SUDEP.
2. This study suggests that clinical studies should investigate seizure and SUDEP prevention with fenfluramine.
3. Further studies into fenfluramine's dose-response and prolonged time course of effects are needed.
4. Receptor mechanisms mediating the anticonvulsant and S-IRA blocking effect and sites of action within the brain of fenfluramine remain to be determined.

REFERENCES

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SUPPORT

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