INTRODUCTION
Fenfluramine (FFA) provides unique, unexpected antiepileptic activity in Dravet syndrome (DS)\(^1\) –
• High degrees of seizure reduction and freedom
• Prolonged duration of efficacy
FFA antiepileptic activity is thought to arise from agonistic activity of neuronally-released serotonin (5-HT) or via a direct agonist effect on 5-HT receptors
Recent data suggest that FFA interaction with sigma-1 receptors (S1Rs) may also be responsible for its ability to reduce seizure activity
Studies utilizing the zebrafish model of DS have shown that:
• FFA’s antiseizure activity was due, at least in part, to agonism on several 5-HT receptors
• The S1R antagonist NE-100 also reduced seizure activity, while the S1R agonist PRE-084 reversed the efficacy of FFA, suggesting that FFA alters seizure activity by acting on S1Rs
• FFA behaves as a positive modulator of S1Rs in an in vitro S1R(5HSP70) chaperone (BP) dissociation assay\(^2\)
• The spontaneous alternation test and the passive avoidance test are well known tests used to study the mechanism of sigma-1 activity\(^3\)
We used the spontaneous alternation and passive avoidance tests to investigate the possibility that FFA is a positive modulator of S1Rs in vivo to corroborate earlier in vitro data\(^4\)

METHODS
A study employed male Swiss OF-1 mice, aged 7-9 weeks and weighing 32±2 g
• The dopamine-induced learning impairment mouse models of spontaneous alternation in Y-maze and step-through passive avoidance were used to assess any positive modulation of S1Rs by FFA (dopamine-induced learning impairment shown to be modulated by sigma ligands)\(^5\)
• Spontaneous alternation in the Y-maze
• An index of spatial working memory\(^6\)
• Alternation: alternation defined as successive entries into all three arms of the Y and
• Step-through passive avoidance test
• Measures nonspatial/contextual long-term memory\(^7\)
• Statistical analysis
Data were analyzed using a one-way analysis of variance (ANOVA, F-values), followed by a post-hoc Dunnett’s test or a Kruskal-Wallis nonparametric ANOVA (H value), followed by a Dunn’s multiple comparison test, for passive avoidance performance and by the Wilcoxon matched-pairs signed-rank test, for escape latency of the passive avoidance test. Data is presented as mean±SEM Median and interquartile range

RESULTS
Spontaneous alternation and passive avoidance tests demonstrated positive modulation of FFA on S1Rs
• Dose-dependent (limping impairment agent) produced substantial reductions in spontaneous alternation (Figure 1) and passive avoidance learning (Figure 2)
• FFA significantly attenuated both deficits; the most effective doses were 0.3 and 1 mg/kg
• PRE-084 (selective S1R agonist) attenuated dopamine-induced deficits at 0.3 but not 0.1 mg/kg

DISCUSSION
• FFA prevented the inhibition in step-through latency (STL) and reduced the escape latency induced by dopamine (panels a and b)
• The selective S1R agonist PRE-084 also partially prevented the inhibition in STL induced by dopamine (panel c, gray bar)
• FFA prevented STL induced by dizocilpine was mildly enhanced by a non-active dose of PRE-084 (panel b, dark green bar)
• FFA prevention of STL and enhancement of FFA by PRE-084 was blocked by the selective S1R antagonist NE-100 (panel c, dark green bar)
• Examination of CI values in both tests showed that a non-effective dose of PRE-084 (0.1 mg/kg) resulted in either a synergistic or additive effect when administered in combination with different doses of FFA (CI values ranging from 0.36 to 0.99; no evidence of antagonism was observed

CONCLUSIONS
• These data provide in vivo confirmation of earlier in vitro results suggesting that FFA’s antiepileptic activity may not be solely due to anticonvulsant effects, but could involve a synergistic effect on the sigma-1 receptor

REFERENCES

ACKNOWLEDGEMENTS
The study was funded by Zogenix, Inc. (Emeryville, CA), Medicated Writing and Editing (Princeton, NJ) and Brooks Boyd, PhD, of PharmaWrite, LLC (Princeton, NJ) was funded by Zogenix, Inc.