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LETTER TO THE EDITOR

Serotonergic modulation as a pharmacological modality in the treatment of Dravet syndrome

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Sir,

We read with great interest the article titled 'Clemizole and modulators of serotonin signalling suppress seizures in Dravet syndrome' (Griffin *et al.*, 2017). In their study, the authors report the antiepileptic activity of clemizole in zebrafish *scn1* mutants, reconfirming the pharmacological potential of the compound identified in a previously published screen (Baraban *et al.*, 2013). The *scn1* mutant fish were obtained by the group of Prof. H. Baier through an ENU-mutagenesis screen, and contain a mutation that disrupts the voltage-gated sodium channel scn1Lab ($Na_v1.lb$) (Schoonheim *et al.*, 2010). The mutants exhibit epilepsy-like behaviour and respond to treatment as typically observed in patients with Dravet syndrome (Baraban *et al.*, 2013). Hence, the *scn1* mutant model and its morphant antisense equivalent have been used to find potential new leads in the fight against Dravet syndrome (Baraban *et al.*, 2013; Dinday and Baraban, 2015; Zhang *et al.*, 2015; Sourbron *et al.*, 2016).

In their paper, the authors touch upon the difference in procedures used to test for antiepileptic compounds, and claim that the methodology used by our group was not validated for the identification of possible treatments for Dravet syndrome. We strongly disagree with this assertion. For drug discovery purposes, we typically immerse larvae in low micromolar concentrations for 22 h, whereas the Baraban group uses higher concentrations in combination with short incubations (typically ~30 min). Using our procedure, we have clearly demonstrated that fenfluramine significantly decreased epileptiform activity in the *scn1* mutant zebrafish (Sourbron *et al.*, 2016). As fenfluramine has shown effectiveness in patients with Dravet syndrome in an ongoing 27-year observational study in Belgium (Ceulemans *et al.*, 2012; and reviewed in Bialer *et al.*, 2017), we assert that our approach is scientifically sound, and represents a validated testing platform for the discovery of therapeutics in the field of Dravet research. Significantly, using the same model it was shown that fenfluramine is also active after short incubations at 10-fold higher concentrations (Dinday and Baraban, 2015). Obviously, the concentration level used to incubate the zebrafish larvae determines the time period needed to reach active concentrations in their organs and tissues. This may be predicted from Fick's first diffusion law that simply states that molecules move from high to low concentrations, with a speed proportional to the concentration gradient.

Of further interest, during a compound library screening the authors identified lorcaserin as a compound that exerts an inhibitory activity on the abnormal behavioural and electrographic seizure activity of the mutants. In fact, this activity of lorcaserin had already been discovered by our group using similar assays (Sourbron *et al.*, 2016), once more demonstrating that short or long incubations of larvae with test compounds do not make any substantial difference in outcome. Actually, in our previous work we provide strong experimental evidence that selective modulation of specific serotonergic receptors (e.g. $5\text{-}HT_{1D}$, $5\text{-}HT_{2c}$, $5\text{-}HT_{2A}$) effectively reduce seizures in the *Scn1Lab* mutant zebrafish larvae (Sourbron *et al.*, 2016). In that paper we further concluded that the findings open up new possibilities in the search for effective drugs to treat

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patients with Dravet syndrome. For this reason, but also because clinically tested fenfluramine is known to exert its action through a serotonergic mechanism (Bialer *et al.*, 2017), we believe that the conclusion drawn by Griffin *et al.* (2017) that 5-HT signalling represents a 'novel' therapeutic intervention for the treatment of patients with Dravet syndrome is invalid.

Finally, the results of the small non-placebo controlled clinical study with lorcaserin in five patients with Dravet syndrome are difficult to interpret as exact baseline and treatment-related seizure frequencies are not given. The effect size seems small as some patients only had temporary reduction. After 3 months of treatment, four of five patients returned to their 'baseline seizure frequency' and only two remained on the drug. It therefore seems too preliminary with this available dataset to attribute these minimal effects to lorcaserin.

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