

**Title:** Optimized administration of the M<sub>4</sub> PAM VU0467154 demonstrates broad efficacy, but limited effective concentrations in *Mecp2*<sup>+/-</sup> mice

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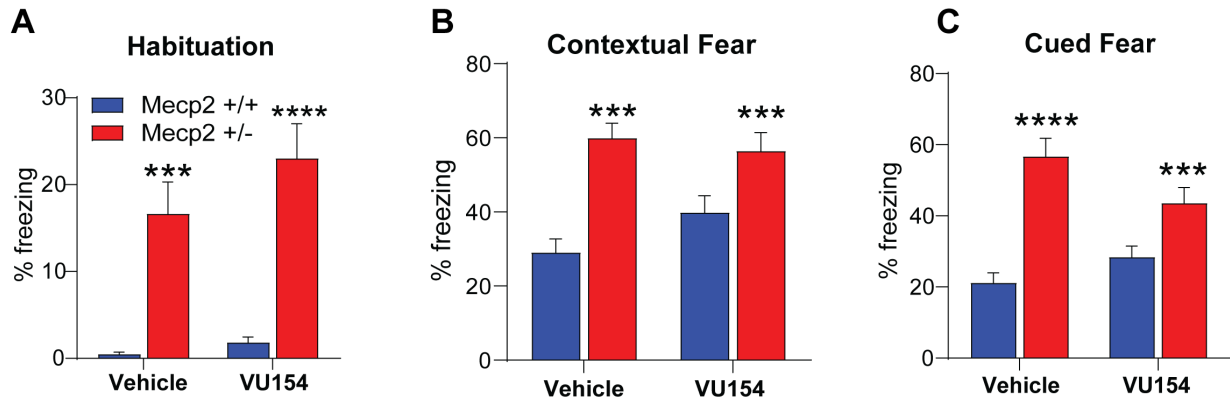
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**Figure S1. Chronic vehicle and VU154 administration decrease freezing responses in *Mecp2*<sup>+/-</sup> control mice.** Fear conditioning. N=13-15 / genotype / treatment. **A)** Similar to acute studies, VU154 administration increased immobility during the habituation phase of the fear conditioning assay in *Mecp2*<sup>+/-</sup> mice. **B-C)** Independent of treatment, contextual freezing behavior in *Mecp2*<sup>+/-</sup> mice presented at only 30%. This stands in stark contrast to acute studies (Fig 2F), where ~80% freezing was observed under identical assay conditions. By comparison, *Mecp2*<sup>+/-</sup> mice froze ~60% of the time with both acute and chronic treatment. Similar results were also observed in the cued conditioning assay. The change in baseline freezing of controls complicates the analysis of this data set. Two-way ANOVA with Tukey post hoc analysis \*\*\*p<0.001, \*\*\*\*p<0.0001. Data are expressed as mean ± SEM.