Evaluation of Anxiolytic Potential of Amitriptyline, Fluoxetine, and TP003 in Zebrafish Larvae Using the Light-Dark Challenge Assay

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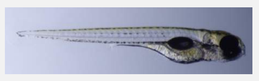
Introduction

The evaluation of the anxiolytic potential in preclinical models is crucial for developing effective treatments for anxiety disorders. Zebrafish larvae have emerged as a valuable tool in drug discovery due to their genetic similarity to humans and their rapid development, making them ideal candidates for the high-throughput screening.

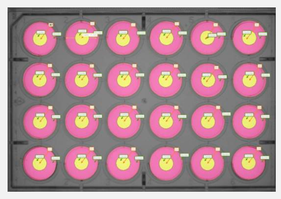
In this study, we aim to assess the anxiolytic effects of two commonly used antidepressant drugs: amitriptyline, fluoxetine, and one novel compound called TP003; non-selective benzodiazepine site agonist that induces anxiolysis via α2GABA$_A$ receptors.

The Light-Dark Challenge test (L-DC) has been utilized as an assay reflecting larval anxiety-like behaviour (response to light and dark). Additionally, thigmotaxis measurement has been conducted.

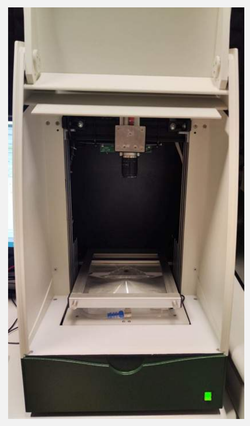
Experimental design



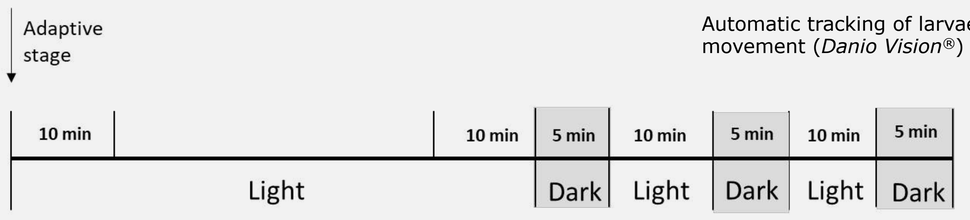
5 days post fertilization(dpf) zebrafish AB larvae



30 minutes of incubation with a drug on 24-well plate (arenas divided to inner and outer zone)



Automatic tracking of larvae movement (*Danio Vision®*)



Measured parameters

Distance moved [mm] – shows the average distance which larvae have travelled in 1-min time bins under changing: light and dark phase

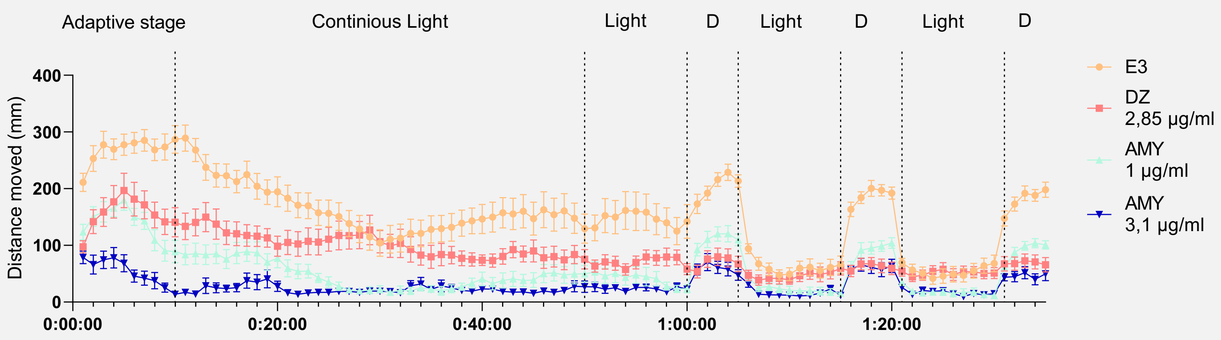
Anxiolytic activity [%] – shows the percentage of the time the larvae spent in the centre of the arena under the changing conditions.

$$\textit{Anxiolytic activity}\ [\%] = \frac{\textit{Time in central arena}}{\textit{Time in outer zone} + \textit{Time in inner zone}}\ x\ 100\%$$

Results

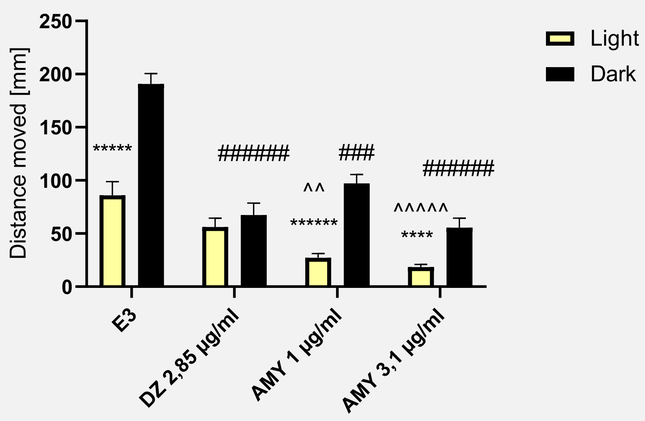
Anxiolytics effect of amitriptyline (AMY)

Timeline



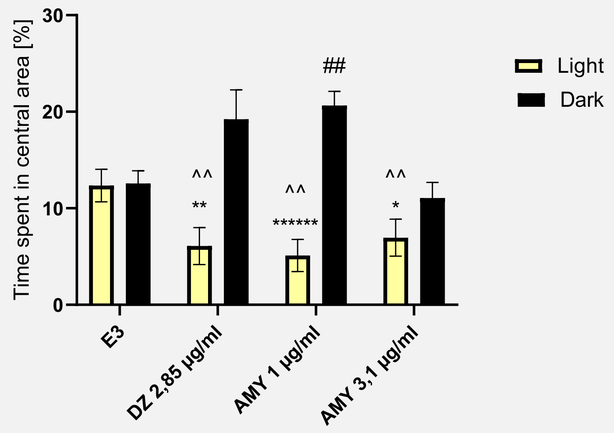
Combined results from 4 independent repetitions, n=24

Distance moved in L-DC



Average distances moved by 5dpf zebrafish under light and dark conditions. Data presented as mean ± SEM, n=24 animals per group. *****p<0.00001, ******p<0.000001 – light vs dark conditions (T-student for parametric or Mann–Whitney U test for nonparametric data). ###p<0.001, ######p<0.000001 dark treatment groups vs dark control group (E3), ^^p<0.01, ^^^^^p<0.00001 – light treatment groups vs light control group (E3) (One way ANOVA, post hoc: Dunnett test for parametric and Kruskal-Wallis test for nonparametric data)

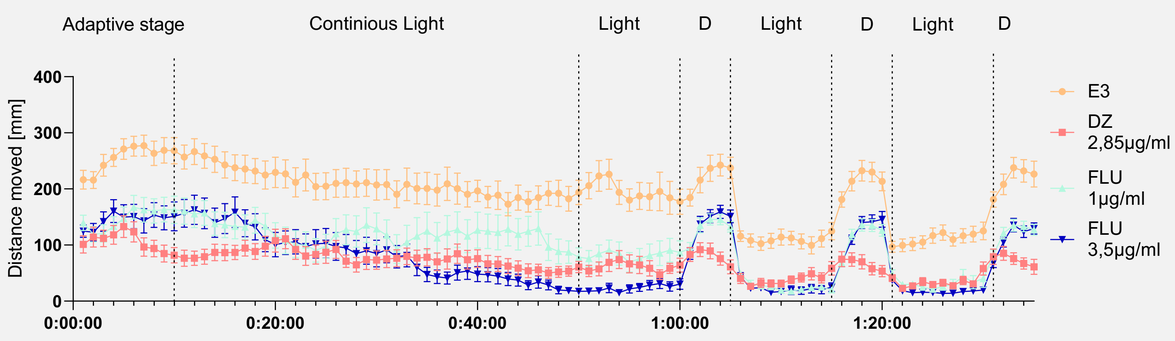
Anxiolytic Activity (Thigmotaxis)



The percentage of time spent by 5dpf zebrafish larvae in the central area. Data presented as mean ± SEM, n=24. *p<0.05, **p<0.01, ******p<0.000001 – light vs dark conditions (T-student for parametric or Mann–Whitney U test for nonparametric data. ##p<0.01 dark treatment groups vs dark control group (E3), ^^p<0.01, – light treatment groups vs light control group (E3) (One way ANOVA, post hoc: Dunnett test for parametric and Kruskal-Wallis test for nonparametric data)

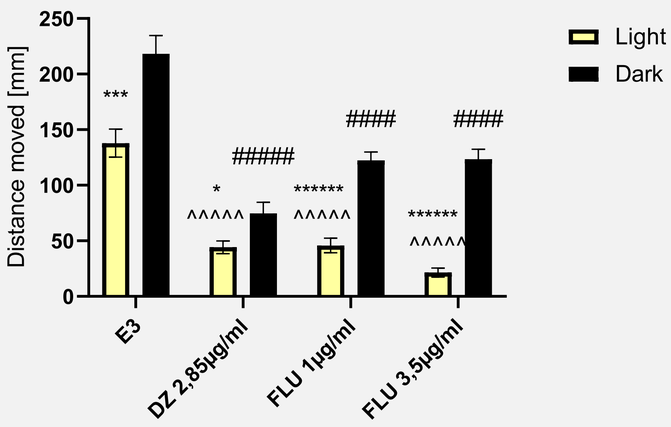
Anxiolytics effect of fluoxetine (FLU)

Timeline



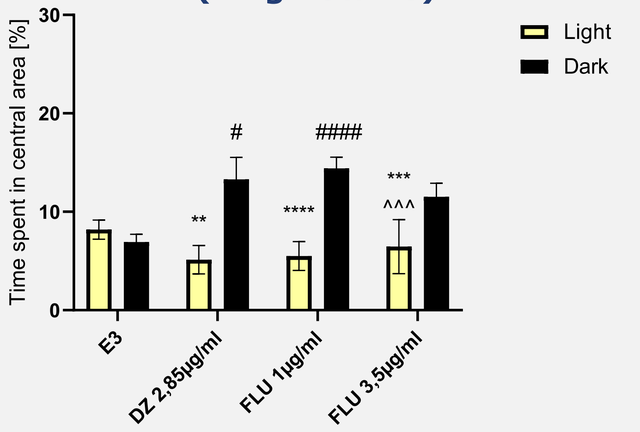
Combined results from 4 independent repetitions, n=24

Distance moved in L-DC



Average distances moved by 5dpf zebrafish under light or dark conditions. Data presented as mean ± SEM, n=24 animals per group. *p<0.05, ***p<0.001, ******p<0.000001 – light vs dark conditions (T-student for parametric or Mann–Whitney U test for nonparametric data). ####p<0.0001, #####p<0.00001 dark treatment groups vs dark control group (E3), ^^^^^p<0.00001 – light treatment groups vs light control group (E3) (One way ANOVA, post hoc: Dunnett test for parametric and Kruskal-Wallis test for nonparametric data)

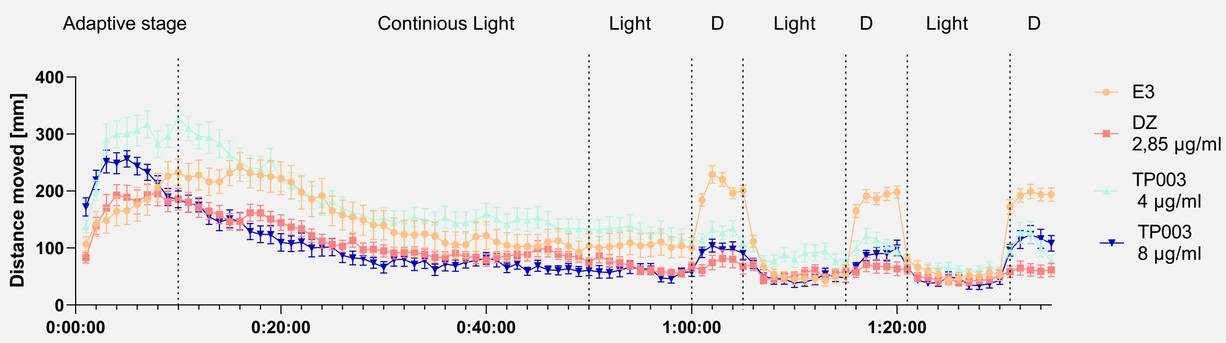
Anxiolytic Activity (Thigmotaxis)



The percentage of time spent by 5dpf zebrafish larvae in the central area. Data presented as mean ± SEM, n=24. p<0.01, ***p<0.001 ****p<0.0001 – light vs dark conditions (T-student for parametric or Mann–Whitney U test for nonparametric data. #p<0.05, ####p<0.0001 dark treatment groups vs dark control group (E3), ^^^p<0.001, – light treatment groups vs light control group (E3) (One way ANOVA, post hoc: Dunnett test for parametric and Kruskal-Wallis test for nonparametric data)

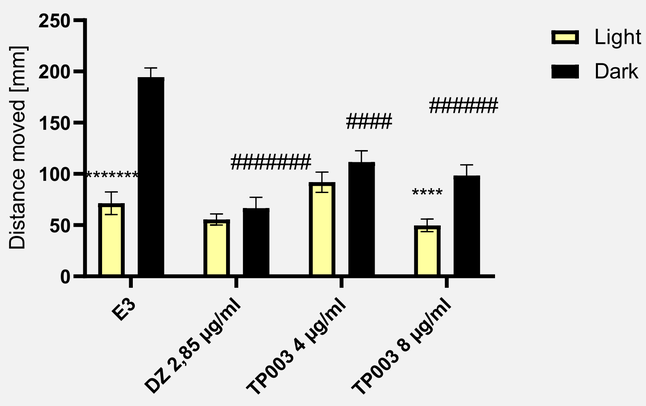
Anxiolytics effect of TP003

Timeline



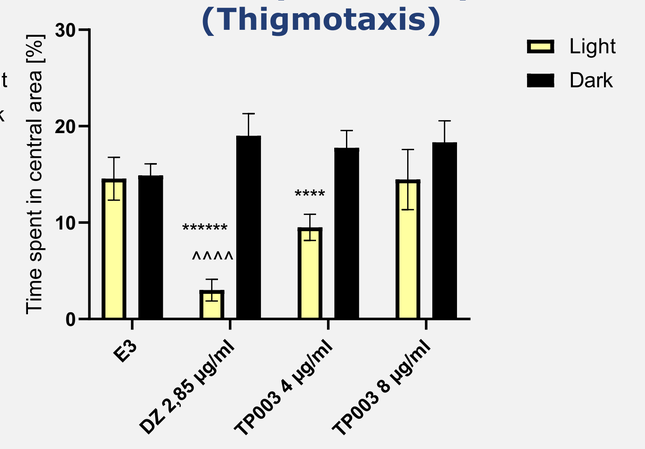
Combined results from 4 independent repetitions, n=24

Distance moved in L-DC



Average distances moved by 5dpf zebrafish larvae under light and dark conditions. Data presented as mean ± SEM, n=24 animals per group. ****p<0.0001, *******p<0.0000001 – light vs dark (T-student for parametric or Mann–Whitney U test for nonparametric data). ####p<0.0001, ######p<0.000001, ########p<0.0000001– dark treatment groups vs dark control group (E3) (One way ANOVA, post hoc: Dunnett test for parametric and Kruskal-Wallis test for nonparametric data)

Anxiolytic Activity (Thigmotaxis)



The percentage of time spent by 5dpf zebrafish larvae in the central area. Data presented as mean ± SEM, n=24, ****p<0.0001, ******p<0.000001 – light vs dark conditions (T-student for parametric or Mann–Whitney U test for nonparametric data. ^^^^p<0.0001– light treatment groups vs light control group (E3) (One way ANOVA, post hoc: Dunnett test for parametric and Kruskal-Wallis test for nonparametric data)

Conclusions

- All tested substances exhibit an anxiolytic effect, albeit with varying levels of efficacy contingent upon the administered dosage.
- Our results provide further evidence supporting the use of TP003 as a potential therapeutic option for anxiety-related disorders.
- Collected data contribute to our understanding of the mechanisms underlying anxiety and provide a foundation for future research on the development of novel treatments.
- The Light-Dark Challenge assay is a reliable tool for anxiolytic efficacy testing in zebrafish larvae model.