**Validation of the Zebrafish Model to Screen Novel Pro-Sleep Compounds**

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**Background and aims.** With the high prevalence of chronic insomnia around the world, there is great need for the ideal pro-sleep drug. The similarity between zebrafish sleep and mammalian sleep makes them a valuable translational model for sleep research. This study aims to establish a reliable high-throughput platform to screen novel pro-sleep agents using larval zebrafish.

**Methods.** To validate the zebrafish sleep model, commonly available sleep drugs were tested in wildtype (AB) larval 5/6 dpf zebrafish over 24 hours. Using a 6:12:6 light:dark:light cycle, behaviour was measured to characterise the effects of multiple drug classes on zebrafish sleep to establish the model for future, reliable novel drug testing. Various concentrations of the positive controls, Diazepam and Zolpidem were tested (3 µM, 10 µM and 30 µM), to visualise the dose-dependent effects on sleep.

Individual zebrafish larvae were placed into separate wells of a 96-well plate, each containing 100 μL of fresh E3 medium. The first solution was added with treatment groups receiving 100 μL of the test compound to achieve final concentrations in 1% DMSO (w/v) in a total volume of 200 μL. The negative control group received 100 μL of 2% DMSO, also resulting in a final DMSO concentration of 1% (w/v) in a total volume of 200 μL. The plate was then placed into the Zantiks MWP System for a 24-hour recording at 28 °C, with a light: dark: light cycle (6:12:6 hours).

**Results.** Strong dose-dependent pro-sleep effects were observed with the treatment of Diazepam and Zolpidem at concentrations of 3 µM, 10 µM and 30 µM. This is a promising foundation for the testing of novel pro-sleep compounds.

**Conclusion/Discussion.** To address the lack of effective pharmacological treatment of insomnia and related sleep disorders, our study employs a practical and reliable drug screening method using larval zebrafish. The zebrafish sleep model is a high-throughput and cost-effective platform for the screening of novel pro-sleep agents, enabling accelerated drug discovery.