Development and prevalidation of a zebrafish assay for identifying chemicals impairing the non-associative learning

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Habituation is a non-associative form of learning characterized by decreased probability of a behavioural output when the same stimulus is presented repeatedly **[1]**. In zebrafish, short-term habituation can be measured by assessing the acoustic startle response (ASR) in response to repetive acoustic/vibrational stimulati **[2]**. In this study, a new assay for identifying environmental pollutants and drugs able to impair non-associative leaning has been developed in zebrafish larvae. The assay is based in assessing the motor response evoked by repetitive acoustic/vibrational stimuli generated by tapping a 48-wells microplate. First of all, the different criteria to determine if the iterative reduction in motor response observed in the assay needed to be demonstrated. Indeed with our assay, we were able to fufill hallmark criterias of habituation: spontaneous recovery, more rapid reductions in startle to shorter intertrial intervals and dishabituation. We then explored pathways affecting this behaviour using human based neural chemical drugs for learning and memory which included those that targeted the cholinergic, serotonergic, gultaminergic and adenosine systems. In general and with in line of previous reports found in either zebrafish or rodents, the startle response was enhanced by cholinergic agonism and glutaminergic and adenosine antagonism drugs and was impaired by those that increase serotonin signaling and concentration. Futhermore, we tested ASR with different concentrations of two pesticides known to enhance the cholinergic signaling either by inhibiting acetylcholinesterase (AChE) activity or as an agonist of the nicotinic acetylcholine receptors, the organophosphate chlorpyrifos oxon (CPO) and the neonicotinoide imidacloprid, respectively. Both compounds affected ASR in zebrafish, however, the observed responses were divergent. CPO increased ASR in a dose dependent manner, while, Imidacloprid declined it with dose increase. The results are consistent with observed responses in zebrafish exposed to other AChE inhibitors **[1]** and with habituation impairment studied in honeybees after exposure to imidacloprid **[3]**. These results suggest that our new approach together with high-throughput screening advanteges of the zebrafish model has been proven to be a usefull tool to identify compounds that can affect learning and memory.

[1] Best, et al., 2008. Non-associative learning in larval zebrafish. *Neuropsychopharmacology*, *33*(5), p.1206.**[2]** Wolman, et al., 2011. Chemical modulation of memory formation in larval zebrafish. *Proceedings of the National Academy of Sciences*, *108*(37), pp.15468-15473.**[3]** Blacquiere,et al., 2012. Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. *Ecotoxicology*, *21*(4), pp.973-992

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