A CONDITIONED PLACE PREFERENCE ASSAY FOR MODELLING ADDICTION IN ZEBRAFISH

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Previously used extensively in vertebrate development studies, the zebrafish is becoming a valuable model system for the study of disease (Shin *et al*, 2002, Berghman *et al*, 2005). Zebrafish have been used to study the acute effects of alcohol (Lockwood *et al*, 2004), and the genetic basis of cocaine-associated reward, using conditioned place preference (CPP) analysis (Darland *et al*, 2002). The latter study identified 4 mutants with altered CPP on exposure to cocaine, and their phenotypes were consistent with defects in dopaminergic pathways. This supports the conservation of reward pathways in zebrafish and confirms its suitability for the genetic analysis of addiction. In rats, CPP after an abstinence period or despite an adverse stimulus is taken as a model for addiction (Rimondi *et al*, 2002). Using this model, in accordance with Home Office licence, we have established a CPP assay in response to nicotine or ethanol in zebrafish, as a first step in the use of this system for addiction studies. The conditioning apparatus consisted of a 2 litre tank with distinct visual cues at either end (Darland *et al* 2002). Prior to drug treatment, individual adult fish (n= 33-35, sex and age matched, 4 month old *Danio rerio* for each treatment group) were assessed for their basal place preference by determining the time spent in each half of the tank over a 2 minute period. Then they were restricted to the least preferred side and exposed to nicotine (0.5 to 50 mg/L) or ethanol (0.5 to 1.5% v/v) for 20 min, before removal to a recovery tank . The next day, their place preference was assessed for 2 min, to assess the acute reward response. 5mg/L nicotine or 1% v/v ethanol elicited a significant reward response ($p<0.05$) and were subsequently used for chronic treatments. Treatments were repeated daily for 4 weeks (nicotine) or 5 weeks (ethanol), after which time CPP was assessed again for 2 min. We also tested the preference in the presence of an adverse stimulus: 30-60 min after the CPP assessment, fish were placed in the tank, and return to the drug exposure site was punished by a 3 sec removal from the tank. The number of returns was counted over 10 min. Data was expressed as means ± S.E.M. and analysed with ANOVA followed by Tukey's comparisons. After 4 weeks (nicotine) or 5 weeks (ethanol), the treated adults showed significant increases ($p<0.05$) in time spent in the site of drug exposure: 38 ± 5 % and 61 ± 7 % for nicotine and ethanol respectively, vs. 3 ± 5% for controls. This preference was maintained 3 weeks after the last drug exposure. Furthermore, treated individuals showed a significant preference ($p<0.05$) for the site of drug exposure, despite the adverse stimulus. Thus, 24 hours after the last drug treatment nicotine-treated fish returned to the exposure site 9 ± 1 times compared to 4 ± 1 times for controls, and ethanol-treated fish returned 4 ± 1 times compared to 2 ± 1 times for controls. This study establishes a chronic treatment regime and a CPP assay in zebrafish that can now be used as a basis of a screen for factors that contribute to addiction, and to search for compounds that prevent addiction.

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