Analysis of neurobehavioural data by chemometric methods in ecotoxicological studies

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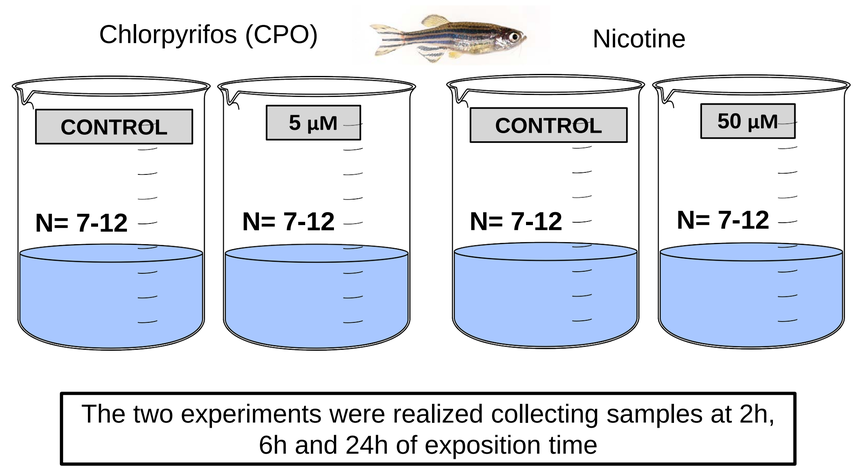
INTRODUCTION

Humans are routinely exposed to a wide range of environmental pollutants potentially inducing neurotoxicity [1]. Different animal models have been used in attempt to understand the neurobiological basis of the adverse effects induced by these neurotoxicants on the human central and peripheral nervous system. Zebrafish (*Danio rerio*) is a vertebrate model species increasingly used in biomedical research, drug discovery and safety pharmacology [2,3]. Low cost, well-established molecular genetic tools and high conservation of the main physiological processes involved in nervous system morphogenesis and maintenance all combine to make zebrafish a promising animal model for neuroscience research, including neurobehavioural toxicology [3,4]. Behavioural endpoints have been recently incorporated to the neurotoxicology screening protocols, and these functional endpoints are now used routinely to detect and characterize potential neurotoxicity of chemicals [1]. The recent development of different video-tracking software in neuroscience research has enabled standardize and automate behavioural endpoints, promoting reproducibility and allowing for multiple endpoints to be recorded at once [5]. Specific video-tracking systems to automate behavioural studies in zebrafish are commercially available from companies such as Noldus Information Technology (Ethovision® XT) and Viewpoint (ZebraLab). Once obtained all these data from the video-tracking systems, behavioural endpoints are usually compared between control and treated animals by using standard statistical analysis. This process, however, can become extremely time-consuming, as often hundreds of distributions need to be tested individually. Therefore, methodologies allowing the prioritization of those endpoints really relevant for a further statistical analysis and confirmation are urgently needed in the neurobehavioural toxicology field. Although multivariate data anlaysis, including Principal Component Analysis, PCA [6] and the Analysis of Variance and Simultaneous Component Analysis (ASCA) [7], could be extremely useful for this role, their use in zebrafish neurobehavioural research is still scarce. Thus, our hypothesis in this work is that chemometric tools are useful to predict the most relevant behavioural endpoints altered by neurotoxicants. As a proof of principle of this new approach, adult zebrafish have been exposed to chlorpyrifos (CPF) and nicotine, two well-known neurotoxic compounds modulating anxiety-like behaviour in mammalian models [8]. The open field test (OFT) and the video tracking system Ethovision XT 11.5 (Noldus Technologies) was selected for the behavioural analyses, as this experimental paradigm evaluates the natural neophobic response, providing information on both locomotor and anxiety-related behaviour [9]. The obtained data were first analysed with the most time-consuming statistical procedures and then, the behavioural profiles obtained with the two chemometric methods (PCA and ASCA) were compared.

EXPOSITION + BEHAVIOURAL TESTS



CHEMOMETRIC ANALYSIS

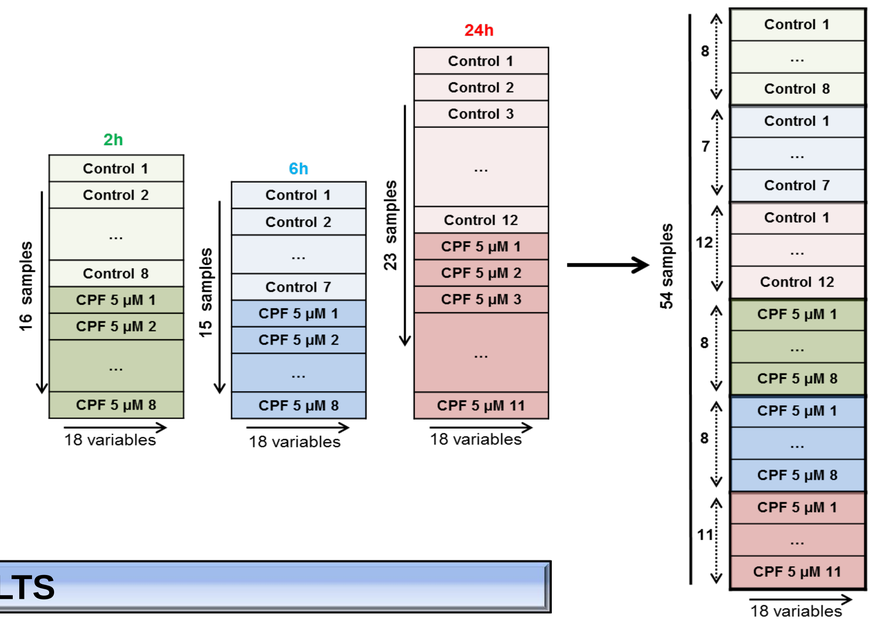


Figure 1. Structure of the experimental data sets arranged for PCA and ASCA analyses in the CPF experiment (similar data arrangements for nicotine).

*Each rectangle represents a zebrafish sample. Data sets at each exposure time are shown in different colours (green: 2h; blue: 6h and red: 24h). Data sets from control and treated zebrafish samples are further arranged into an augmented data matrix, as indicated in the right-hand side of the figure.

RESULTS

CHLORPYRIFOS

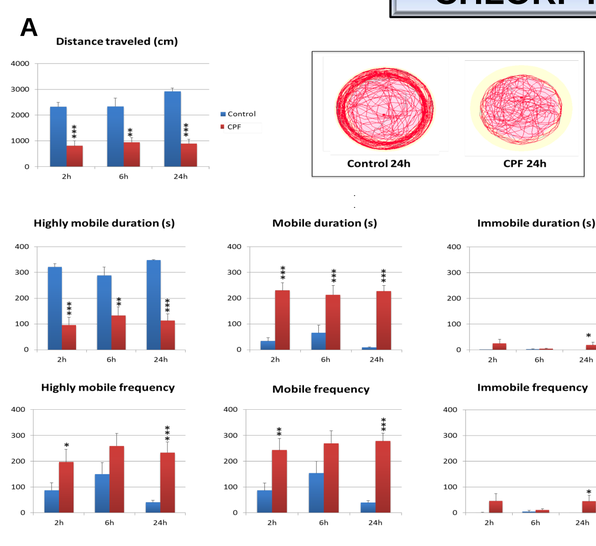


Figure 2. Time-course of behavioural effects induced by the acute exposure to 5 µM chlorpyrifos (CPF) in adult zebrafish. The 6-min open field test (OFT) was selected and different endpoints related with locomotion (A), anxiety (B) and exploratory (C) behaviours were analysed. Representative 2D traces of control- and CPF-treated fish 24 h after exposure generated by Ethovision XT11.5 are also shown. Data are reported as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.

NICOTINE

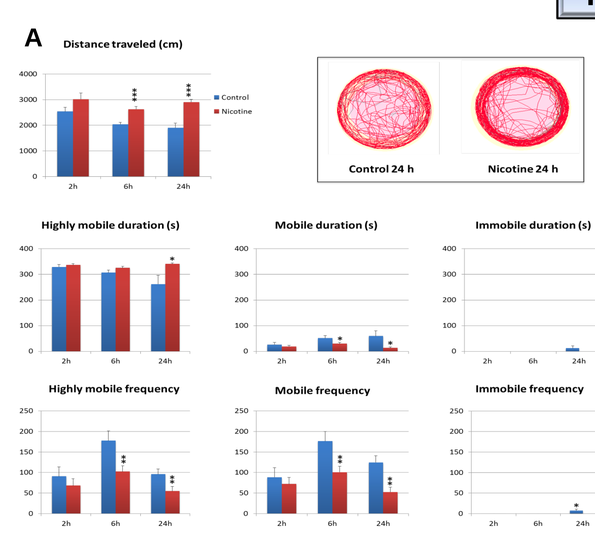


Figure 4. Time-course of behavioural effects induced by the acute exposure to 50 µM nicotine in adult zebrafish. The 6-min open field test (OFT) was selected and different endpoints related with locomotion (A), anxiety (B) and exploratory (C) behaviours were analysed. Representative 2D traces of control- and nicotine-treated fish 24 h after exposure generated by Ethovision XT11.5 are also shown. Data are reported as mean ± SEM. *p < 0.05, **p< 0.01, ***p < 0.001.

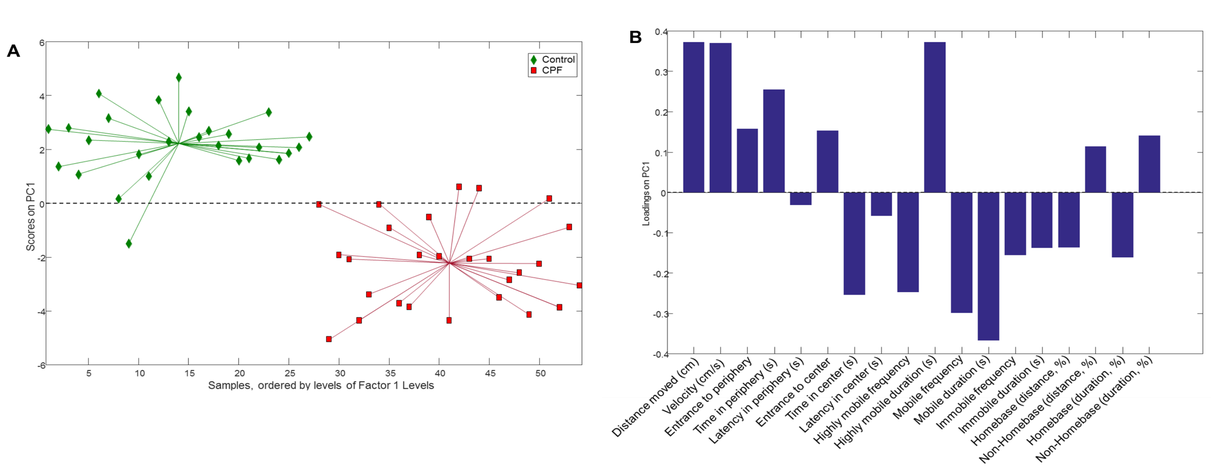


Figure 3. ASCA results of OFT data from control and CPF-treated adult zebrafish samples. (A) SCA scores plot for the "dose" factor matrix. Color symbols indicate the different samples studied: green diamonds are controls and red squares are the zebrafish samples at 5 µM CPF and (B) SCA PC1 loadings plot for the dose sample factor matrix with their variables in the x-axis.

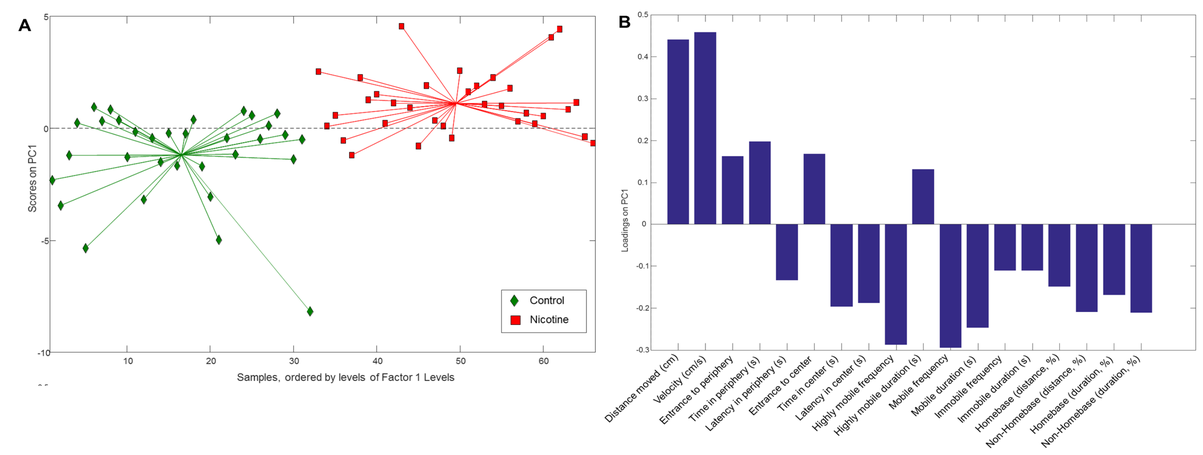


Figure 5. ASCA results of the OFT data from s in control and nicotine-treated adult zebrafish samples. (A) SCA scores plot for the "dose" factor matrix. Color symbols indicate the different samples studied: green diamonds are controls and red squares are the zebrafish samples at 50 µM of nicotine and (B) SCA PC1 loadings plot of dose sample factor matrix with their variables in the x-axis.

CONCLUSIONS

✓ The application of chemometric multivariate data analysis steps in behavioural data management workflow allows for an early fast identification of the most relevant endpoints altered by the different treatments.

✓ The most relevant behavioural effects induced by CPF (decreased locomotor activity, anxiolytic effect, altered exploratory behaviour) and nicotine (increased locomotor activity and anxiogenic effect) identified by a time-consuming statistical analyses were also successfully identified by using ASCA.

✓ The results presented in this manuscript support that the incorporation of the proposed chemometric methods in neurobehavioural assessment studies of neurotoxic effects of chemicals and drugs can be very useful as a preliminary fast screening step.

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