DISTRESS FOR COMMON CARP (*CYPRINUS CARPIO*)

Paulina Pawlak, Jonathan Konrad, Constanze Pietsch

University of Applied Sciences Bern (BFH), Switzerland

Introduction

Stress can have diverse effects on fish, but the signs of stress in fish differ depending on the fish species, the type of stressor, the duration and the intensity of the stress. In addition, it is important to investigate the effects of stress on gene expression patterns at the brain level separately for different brain parts to respect their differences in functionality. The present study reveals that negative stressors have different effects on the gene expression patterns in four brain parts of common carp. Distress was used for 1 min and the fish have been sampled 30 to 90 min after that to investigate the time course of stress responses. In addition, neurotransmitter levels in the different brain parts have been determined.

Materials & Methods

Following an acclimatization period, four control fish were taken directly from a rearing tank, whereas the remaining fish were exposed to air, chased or confined for 1 min and returned to their rearing tank for further 30, 60, or 90 minutes. Anesthesia was performed with MS222. The brains were sampled and divided into the 4 brain regions (telencephalon, hypothalamus, optic tectum, rhombencephalon). After RNA extraction and cDNA synthesis, real-time PCRs were performed with the LightCycler® SYBR® Green I Master mix (Roche, CH). Normalized fold change in expression were calculated according to Taylor et al. [52]. Principal component analysis (PCA) was performed on the log-transformed normalized expression data to describe genes with the most contribution towards the common variance within the gene expression patterns in the different brain regions. The representation of the variables for the principal components is calculated as a cos2 value. For a given variable, the sum of the cos2 on all the principal components is equal to one. The gene expression studies comprised investigations of immediate early genes (*c-fos, palld, eif4A, egr, erk2, neurod*), metabolic genes (*gapdh, succdh, pyrkin*) and genes related to the stress axis (*crf1, crf2, pomc1, pomc2, crf-r1, crf-r2, gr1, gr2, mr*). In parallel, neurotransmitter levels including serotonin (5-ht) and dopamine (DA) and their main metabolites HIAA and DOPAC have been measured in the respective brain parts using commercial ELISA kits.

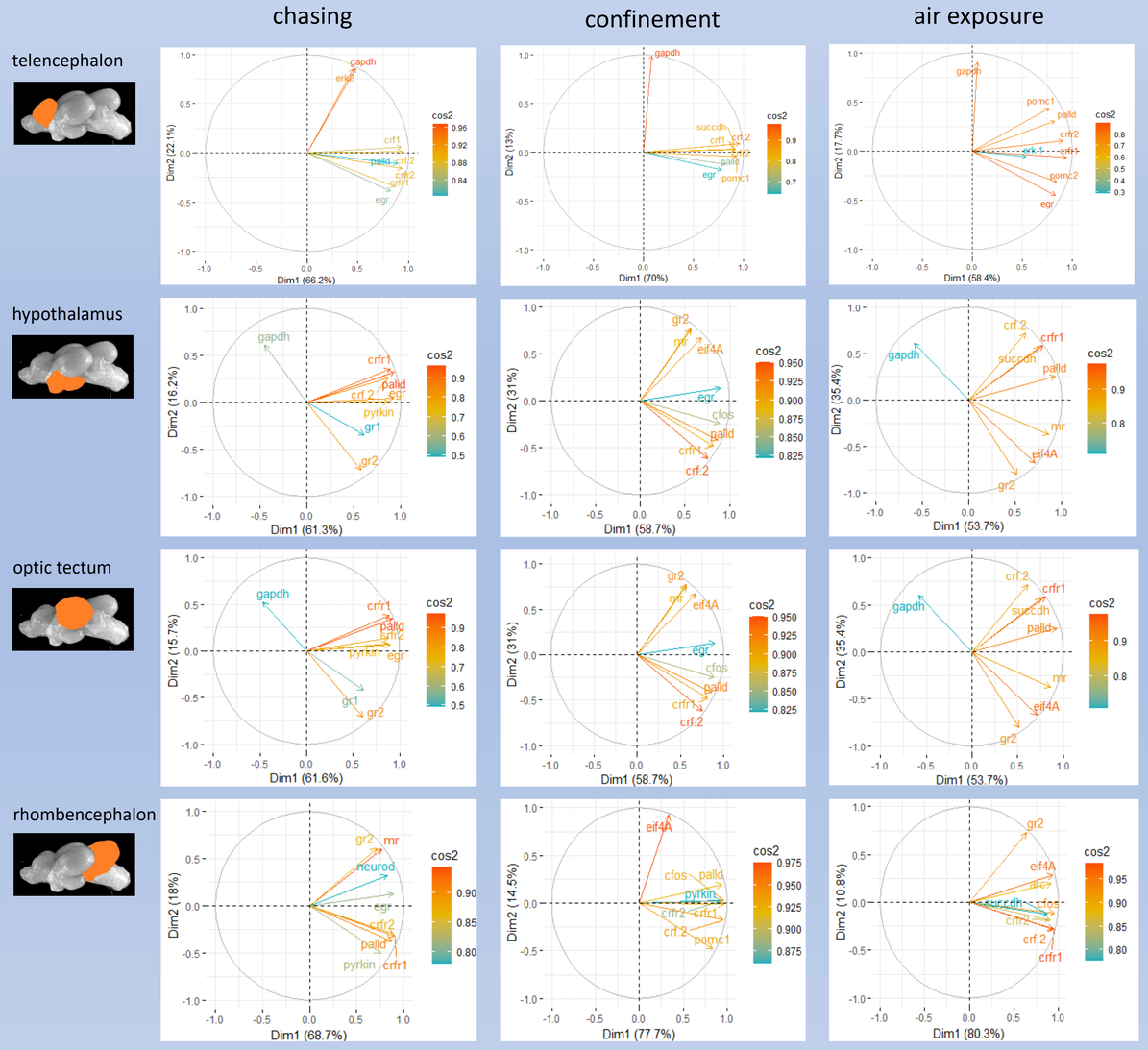

Figure 1: Gene expression profile analyzed with PCA in the four brain parts of fish 0, 30, 60, and 90 min after treatment, n = 6 per treatment.

Results & Discussion

The PCA for the mRNA levels of the genes in the telencephalon explained on average 64.9 % of the variance in the data set (Fig. 1) whereas for the hypothalamus, the optic tectum and the rhombencephalon 57.9 %, 58 %, and 75.6 % were explained. The high cos^2 values of genes in these brain parts (e.g., *gapdh* expression in the telencephalon) indicates their high congruency with the experimental treatments that were used for the fish. Moreover, the study aimed at identifying genes that are uniquely contributing to the gene expression patterns in the brain parts after certain stressors have been applied. Accordingly, the *pomc* genes did not belong to the strong influencing genes in fish after chasing compared with the confined or air-exposed fish. In addition, the metabolic gene *succdh* was only observed among the most contributing genes in the hypothalamus, optic tectum and rhombencephalon of air-exposed fish. Consequently, a number of genes specific for the stress responses in each brain part and for each stressor was identified which will improve the analyses of stress effects in fish in the future.

For neurotransmitters, often the ratio of inactive compounds and the active neurotransmitter is analyzed. The neurotransmitter analyses in the different brain parts showed differences after treatment with the stressors. As an example, Figure 2 shows the neurotransmitter ratios in the optic tectum.

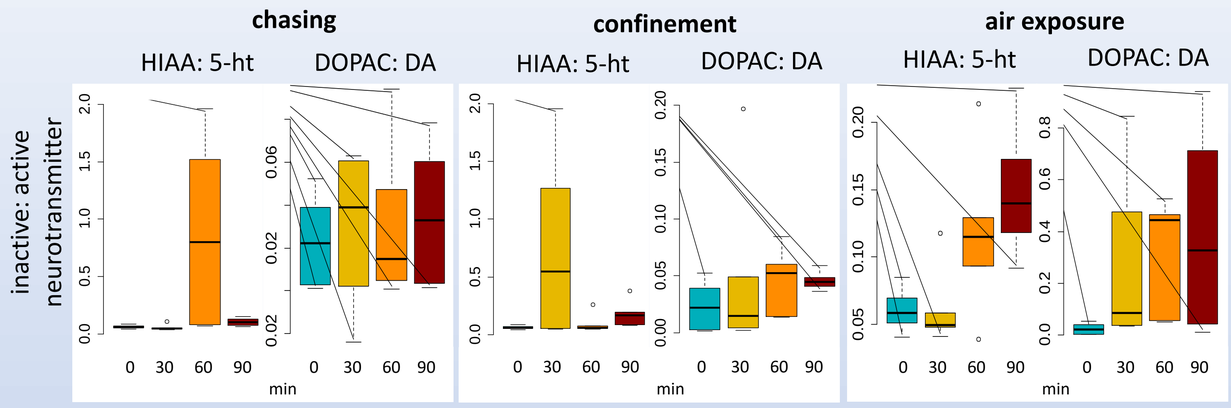

Figure 2. Ratio of the inactive metabolites HIAA and DOPAC to their parent compound serotonin and dopamine in the optic tectum of fish 0, 30, 60 and 90 min after chasing, confinement and air exposure; n = 6 per treatment.

Chasing did not change the ratio of DOPAC to DA, whereas for the treatments confinement and air exposure a tendency for higher ratios with increasing sampling time after stressor application was observed (Figure 2). Moreover, an increased ratio of 5-th to HIAA was also observed for air-exposed fish 60 min and 90 min after treatment. Thus, stressors appear also to influence neurotransmitter ratios in the carp brain, although high variability of the responses was observed in some treatment groups (i.e., the serotonin measurements in the fish 60 min after chasing or the fish 30 min after confinement). This makes neurotransmitter levels less reliable as stress markers.

Conclusion

This is the best description to date of the previously generally unknown effects of stress in different brain regions in carp. Principal component analyses were performed to reveal possible regulation patterns in the different regions of the fish brain. These revealed genes that may be used as stress markers in future experiment. In contrast, the neurotransmitter analyses were less reliable as markers of stress since the fish in some treatment groups showed variable levels of the neurotransmitters after stress application.

Contact

Dr Constanze Pietsch, Manager of Fish Welfare Projects, University of Applied Sciences Bern, Switzerland, e-mail constanze.pietsch@bfh.ch

Reference: Taylor et al. 2019. Trends Biotechnol. 2019, 37, 761–774.