



Corrigendum

Corrigendum to “PFOS-induced thyroid hormone system disrupted rats display organ-specific changes in their transcriptomes” [Environ. Pollut. 305 (2022) 119340]☆



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The published manuscript contained errors in labelling related to overall transcription patterns (across organs) and organ specific transcription patterns. The erroneous labelling relates to Fig. 2 of the main manuscript and Fig. S2 in supplementary materials. As a consequence, the Results sections related to Fig. 2 and Fig. S2 were incorrect. We apologize that these errors were not identified in the final review of the manuscript. The errors do not cause any changes to the Discussion or Conclusions of the manuscript, but they do complicate the interpretation of data as presented in the relevant Results section: *Transcriptomics revealed organ-specific effects of PFOS exposure*. We provide here the edited section in its entirety, which is meant to replace this section, and this section only, of the manuscript.

1. Results

1.1. Transcriptomics revealed organ-specific effects of PFOS exposure

The transcriptomic analysis identified 50 DEGs in the hypothalamus, 68 in the pituitary, 71 in the thyroid gland, and 181 in the liver (Fig. 1A-D) (S3_Table_RNA-seq_DEGs) which further clustered into 3, 3, 4, and 7 expression patterns in the distinct organs, respectively. Most of the genes were affected by PFOS exposure rather than by vancomycin treatment. A functional analysis was performed to determine whether some of those expression patterns are significantly associated with specific biological processes and pathways (S4_Table_Functional_analysis).

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In the hypothalamus, the 50 DEGs were partitioned into three broad expression patterns denoted Hy1-3 to accurately represent the trends in the data (Fig. 2A). Pattern Hy1 was generally upregulated in response to PFOS exposure, alone and in combination with vancomycin. Similarly, Hy2 was downregulated following exposure to PFOS. For pattern Hy3, there was a general downregulation in both PFOS treated groups, with the strongest downregulation occurring with PFOS alone. Of these, only pattern Hy3 was significantly associated with GO terms, of which there was 26 (Fig. S3A) (S2_Supplementary_Figures). The GO-terms with the highest representation were axon (GO:0030424, 12 genes, p value < $4.0e^{-7}$) and neuron projection (GO:0043005, 13, $3.2e^{-5}$), plasma membrane bounded cell projection (GO:0120025, 13, $1.5e^{-3}$), and cell projection (GO:0042995, 13, $1.6e^{-3}$). This suggests dysregulation of genes related to neuronal communication in the hypothalamus.

For the pituitary, the 68 DEGs were clustered into three expression patterns denoted Pi1-3 (Fig. 2B). In pattern Pi1, there were no clear effects in any treatment group. In pattern Pi2, all groups showed a slight upregulation. For pattern Pi3 there was a general downregulation in all groups, with the strongest effects occurring in the PFOS treated groups. However, no functional terms were significantly associated within these. To our knowledge, neither of the functional terms associated with the TH system. This was further evident by the fact that none of the 68 DEGs are key factors of the HPT axis.

In the thyroid, 71 DEGs were partitioned into four expression patterns denoted Ty1-4 (Fig. 2C). Pattern Ty1 was generally downregulated by PFOS treatment, but these effects seem to be counteracted by the presence of vancomycin. PFOS treatment caused a downregulation in pattern Ty2, both with and without vancomycin. In pattern Ty3 PFOS caused a strong upregulation in the PFOS treated group, and in pattern Ty4, there was a general downregulation following treatment with PFOS which was exacerbated with the presence of vancomycin. Among those four expression patterns, only patterns Ty2 and Ty3 were significantly associated with enriched functional terms. Pattern Ty2, with the GSEA term «SENESE_HDAC1_TARGETS_DN», had 5 DEGs: *Baz2B*, *Cdc42ep3*, *Igfbp5*, *Sesn3*, and *Utrn* (5, $5.8e^{-3}$). Pattern Ty3, with the GSEA term «PHONG_TNF_RESPONSE_NOT_VIA_P38», had 7 DEGs: *Jund*, *Larp6*, *Sox9*, *Tmem265*, *Wtap*, *Optn*, and *Plcb1* (7, $9.8e^{-3}$). None of the 71 DEGs

identified in the thyroid are, to our knowledge, regulated by TSHr or implicated in TH synthesis or the TH system in general.

In the liver, the 181 DEGs were partitioned into seven expression patterns denoted Li1-7 (Fig. 2D). Pattern Li1 showed a slight upregulation in the PFOS treated groups, which was most prominent in the group treated with both PFOS and vancomycin. The expression trend in pattern Li2 suggests a downregulation with all treatments which is most prominent in the two PFOS treated groups. Pattern Li3 showed downregulation following vancomycin treatment which was counteracted by PFOS treatment. Pattern Li4 showed an upregulation in response to PFOS, both alone and in combination with vancomycin. For pattern Li5, there was an upregulation with PFOS treatment. Pattern Li6 showed slight upregulation in all treatment groups. In pattern Li7, there was a downregulation in the PFOS treated group. Of these, only two patterns had identifiable functional terms that were significant (Figs. S3B and C). Pattern Li4 showed 56 GO terms, including lipid metabolic process (GO:0006629, 18, $1.81e^{-9}$), small molecule metabolic process (GO:0044281, 18, $1.04e^{-7}$), carboxylic acid metabolic process (GO:0019752, 17, $2.53e^{-10}$), oxoacid metabolic process (GO:0043436, 17, $4.0e^{-10}$), and organic acid metabolic process (GO:0006082, 17, $4.26e^{-10}$). Pattern Li5 was associated with a total of 18 GO terms, including cytosol (GO:0005829, 11, $1.9e^{-2}$), translation (GO:0006412, 7, $1.5e^{-2}$), amide biosynthetic process (GO:0043604, 7, 0.032), peptide metabolic process (GO:0006518, 7, $3.3e^{-2}$), and peptide biosynthetic process (GO:0043043, 7, $1.5e^{-2}$). This suggests disruption to several pathways in the exposed livers, particularly those related to lipid and fatty acid metabolism, and xenobiotic response elements.

A comparison of DEGs between the four organs revealed that DEGs were foremost specific to each organ. However, there were also overlapping DEGs in the different patterns of the four organs, albeit very few (Fig. 2E). For example, the neuronal guidance factor *Sema3a* was downregulated in both hypothalamus and pituitary of PFOS-exposed rats, whereas the actin-regulatory protein *Tmod2* was downregulated in hypothalamus but upregulated in pituitary. There were DEGs shared between pituitary and liver, such as *Coa5*, *Mtmr1*, *Vcl* and *Tob1* that were all downregulated, but genes that do not immediately point towards any shared functional term. Other examples include the CYP family member

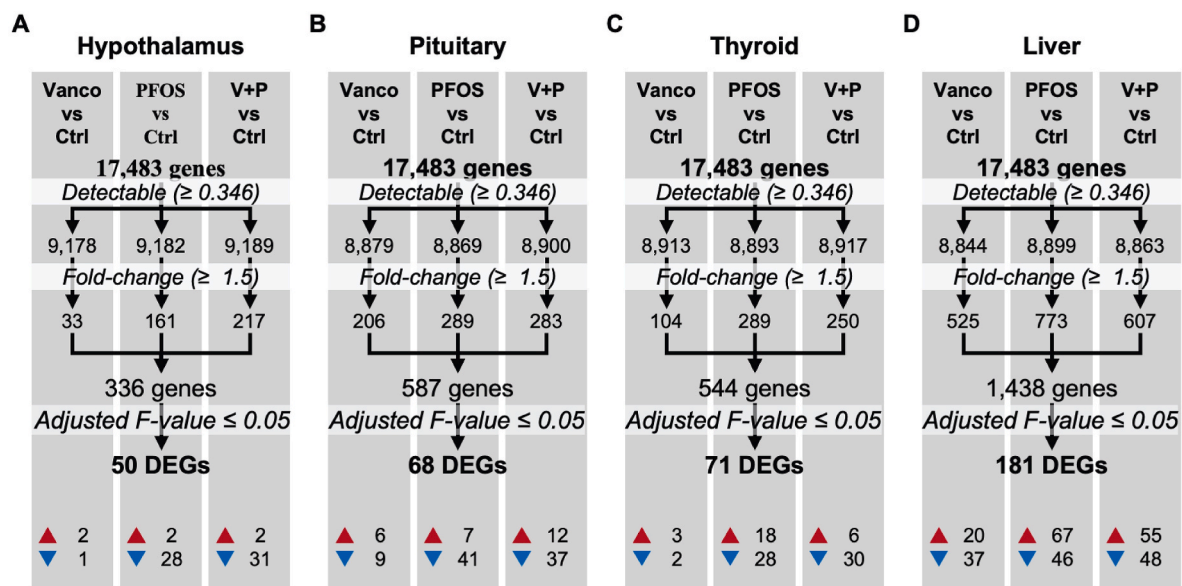


Fig. 1. RNA-sequencing results showed that the liver had the most differentially expressed genes (DEGs). RNA collected from hypothalamus, pituitary, thyroid, and liver was sequenced with BRB-seq. RNA was collected from organ samples collected following 7 days exposure to either corn oil (control), 8 mg/kg/day vancomycin, 3 mg/kg/day PFOS, or 8 mg/kg/day vancomycin and 3 mg/kg/day PFOS. DEGs were identified by comparing treatment groups to the control group using limma. Threshold for DEGs was set to ≥ 1.5 fold-change and significance was determined using adjusted F -value ≤ 0.05 . The final number of DEGs per group correspond to unique genes within each organ, with the discrepancy in total number of individual up- or down-regulated DEGs corresponding to genes shared between two or more groups (i.e. for hypothalamus the 50 DEGs represents 50 unique genes amongst the 66 DEGs observed between the three exposure groups).

Cyp2u1, which was downregulated in pituitary but upregulated in thyroid of PFOS-exposed rats. Genes that were downregulated in liver, but upregulated in thyroid, such as *Crebbp* and *Jund*, and several genes that were downregulated in both hypothalamus and thyroid, including *Slc38a1*, *Sptbn1*, *Trmtc4* and *Plcb1*. Again, these factors may point to important functions individually, but do not immediately enrich specific functional terms.

PFOSs are known to affect Wnt signaling (Yang et al., 2020), so we used the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway resource implemented into the pathview tool (Luo and Brouwer, 2013) to display potential DEGs relevant to Wnt signaling. Irrespective of GO functional terms, it revealed that some genes of the Wnt signaling pathway was affected by PFOS exposure when combining DEGs from all organs, for instance the G protein-coupled receptor *Frizzled* (Fig. 3).

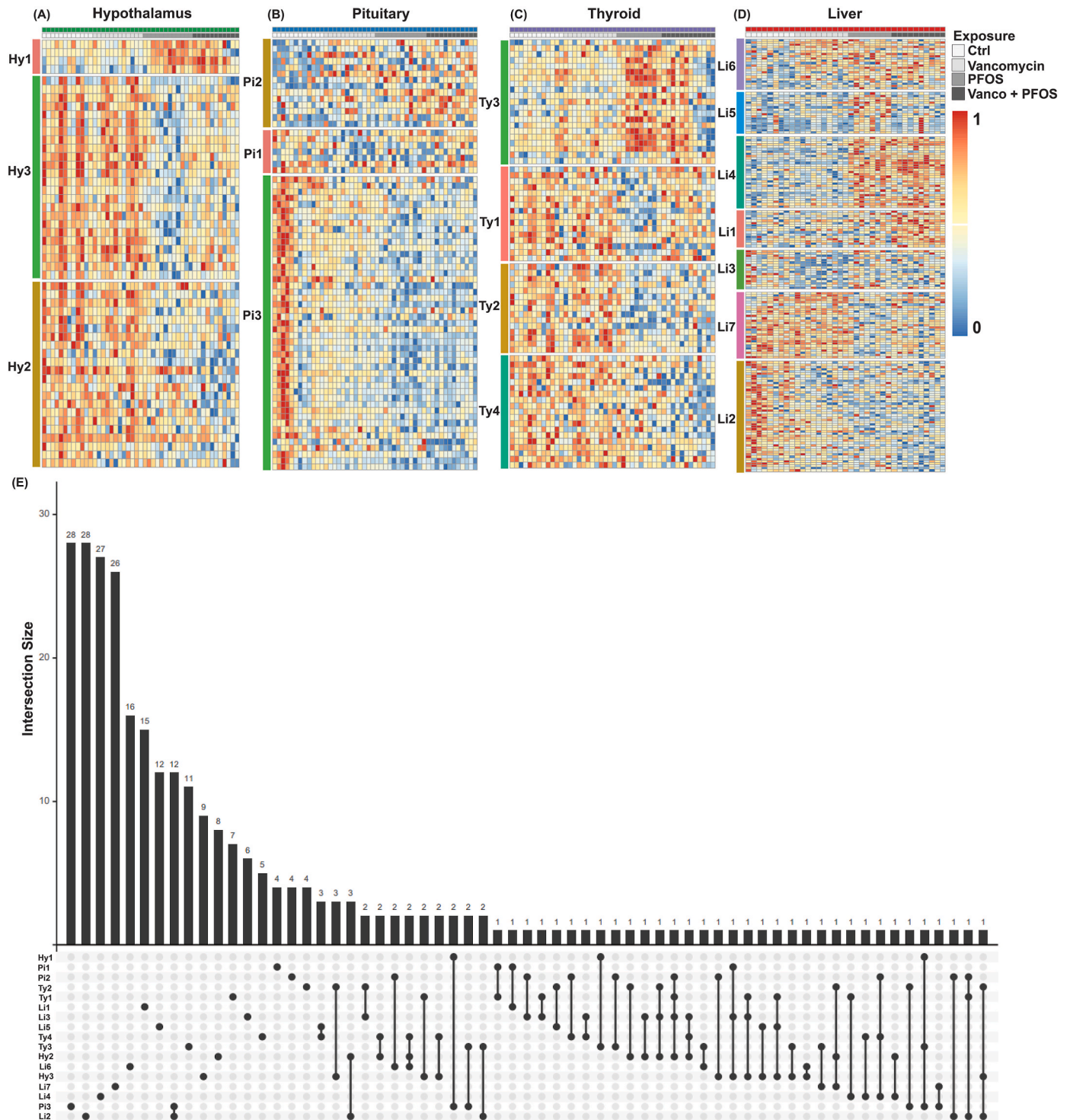


Fig. 2. Clustering analysis shown as heatmaps, the hypothalamus and liver had most GO terms. RNA collected following 7 days of exposure to either control, 8 mg/kg/day vancomycin, 3 mg/kg/day PFOS, or 8 mg/kg/day vancomycin +3 mg/kg/day PFOS was sequenced using BRB-seq. DEGs from the tissues hypothalamus (A), pituitary (B), thyroid (C), and liver (D) were clustered after filtering and testing for significance. Shared DEGs between the different patterns are shown in the intersection plot (E).

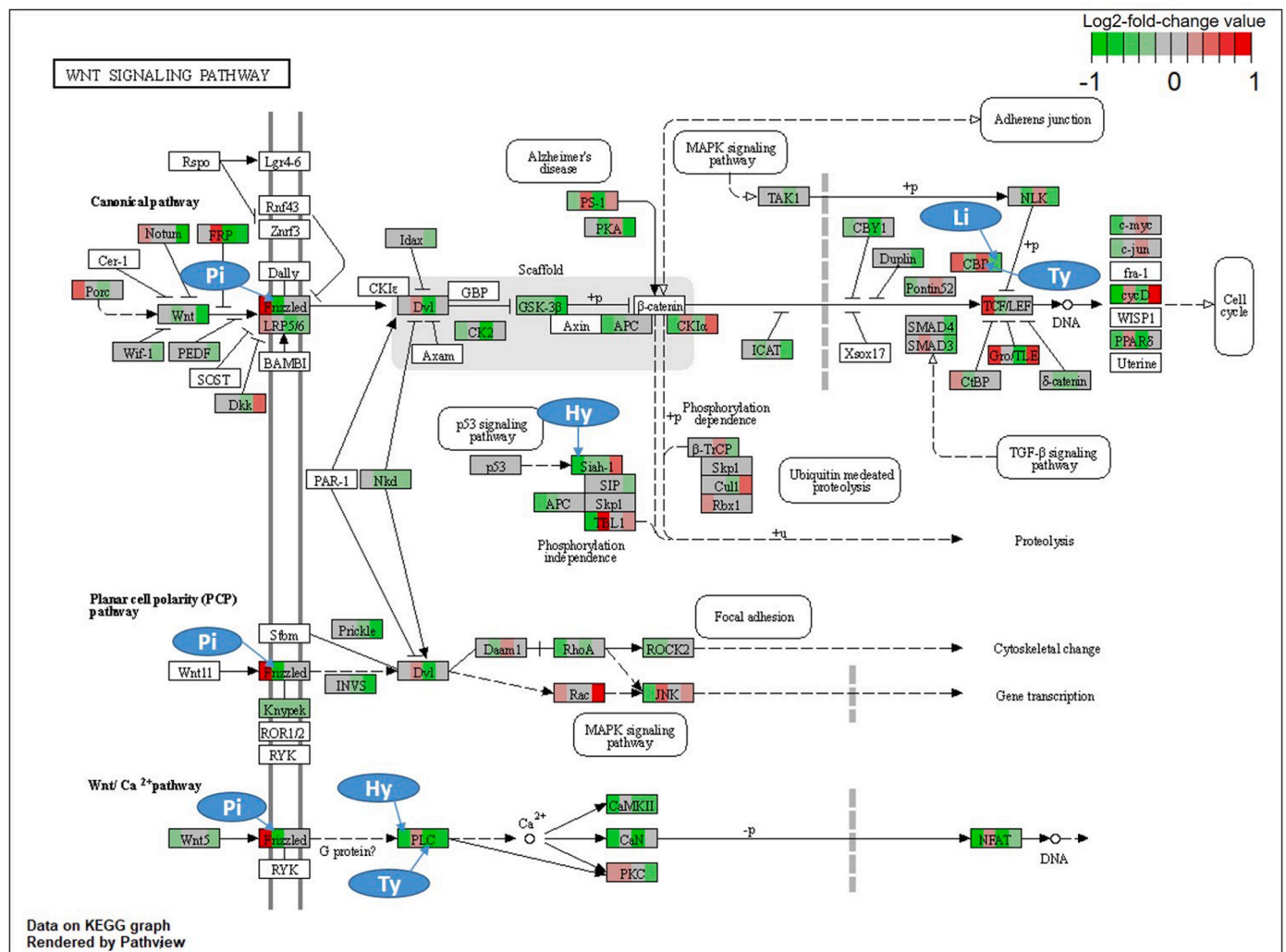


Fig. 3. Genes of the WNT signaling pathway affected by PFOS exposure in different organs. When compared to controls, adult rats exposed to PFOS display some dysregulated genes in different organs. Each gene is represented in a box with four sub-boxes color-coded (green = downregulated; red = upregulated) according to its log2-fold change expression (PFOS vs Ctrl) in the order, left to right: hypothalamus (Hy), pituitary (Pi), thyroid (Ty) and liver (Li). In the pituitary, *Frizzled (Fzd1)* was significantly downregulated. Both *siah E3 ubiquitin protein ligase 1 (Siah1)* and *phospholipase C β 1 (Plc)* were downregulated in the hypothalamus (Hy). In the thyroid (Ty), *Plc* was also downregulated, whereas *CREB binding protein (Cbp)* was upregulated. Finally, in the liver (Li), *Cbp* was downregulated. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Similarly, some of the DEGs identified in the animals exposed to PFOS are known to be involved in MAPK, calcium, or PPAR signaling pathways (Figs. S4–S6, S2_Supplementary_Figures). Notably, none of the identified DEGs aligned with thyroid hormone signaling pathway.

Author statements

Nichlas Davidsen: Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Louise Ramhøj:** Conceptualization, Data curation, Formal analysis, Investigation, Supervision, Writing- original draft, Writing – review & editing. **Claus Asger Lykkebo:** Conceptualization, Formal analysis, Investigation, Writing – review & editing. **Indusha Kugathas:** Formal analysis, Writing – review & editing. **Rikke Poulsen:** Data curation, Writing – review & editing. **Anna Kjerstine Rosenmai:** Formal analysis, Writing – review & editing. **Bertrand Evrard:** Data

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The relevant data for this manuscript can be found in the original manuscript: <https://doi.org/10.1016/j.envpol.2022.119340>

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2022.120709>.

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