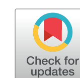
Science of the Total Environment 756 (2021) 143745

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Alteration of neuro-dopamine and steroid hormone homeostasis in wild Bank voles in relation to tissue concentrations of PFAS at a Nordic skiing area



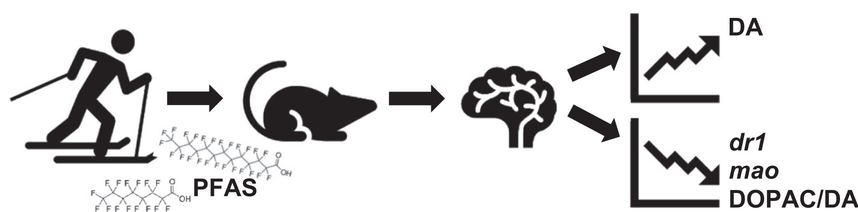
Randi Grønnestad [a,*], Daniel Schlenk [b], Åse Krøkje [a], Veerle L.B. Jaspers [a], Bjørn Munro Jenssen [a,c], Scott Coffin [b], Luísa Becker Bertotto [b], Marissa Giroux [b], Jan L. Lyche [d], Augustine Arukwe [a]

[a] Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway
[b] Department of Environmental Sciences, University of California, Riverside, CA, USA
[c] Department of Biosciences, Aarhus University, Roskilde, Denmark
[d] Department of Food Safety and Infection Biology, Norwegian University of Life Sciences, Oslo, Norway

HIGHLIGHTS

- Elevation of DA levels in Bank vole brains from a skiing area parallel PFAS contamination
- A negative association between dopamine receptor 1 (*dr1*) mRNA and several PFAS
- DOPAC/DA ratios and monoamine oxidase (*mao*) mRNA is negatively associated with PFAS.
- Sex-specific negative relationship between some PFAS and T concentrations

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 September 2020
Received in revised form 30 October 2020
Accepted 31 October 2020
Available online 24 November 2020

Editor: Henner Hollert

Keywords:
Rodents
Terrestrial pollution
PFOS
Ski wax
Toxicological effects

ABSTRACT

Perfluoroalkyl substances (PFAS) are contaminants that are applied in a wide range of consumer products, including ski products. The present study investigated the neuro-dopamine (DA) and cellular steroid hormone homeostasis of wild Bank voles (*Myodes glareolus*) from a skiing area in Norway (Trondheim), in relation to tissue concentrations of PFAS. We found a positive association between brain DA concentrations and the concentration of several PFAS, while there was a negative association between PFAS and dopamine receptor 1 (*dr1*) mRNA. The ratio between DA and its metabolites (3,4-dihydroxyphenylacetic acid: DOPAC and homovanillic acid: HVA) showed a negative association between DOPAC/DA and several PFAS, suggesting that PFAS altered the metabolism of DA via monoamine oxidase (Mao). This assumption is supported by an observed negative association between *mao* mRNA and PFAS. Previous studies have shown that DA homeostasis can indirectly regulate cellular estrogen (E2) and testosterone (T) biosynthesis. We found no association between DA and steroid hormone levels, while there was a negative association between some PFAS and T concentrations, suggesting that PFAS might affect T through other mechanisms. The results from the current study indicate that PFAS may alter neuro-DA and steroid hormone homeostasis in Bank voles, with potential consequences on reproduction and general health.

© 2020 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are anthropogenic, persistent chemicals that are widespread in the environment (Houde

* Corresponding author at: Department of Biology, Norwegian University of Science and Technology (NTNU), N-7491 Trondheim, Norway.
E-mail address: randi.gronnestad@ntnu.no (R. Grønnestad).

https://doi.org/10.1016/j.scitotenv.2020.143745
0048-9697/© 2020 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

et al., 2006; Glüge et al., 2020). They are a group of surface-active compounds that are applied in a wide range of consumer products, such as textiles, carpets, impregnating agents and in some types of ski products, such as ski waxes, gliders and powders (Kissa, 2001). Among the mentioned consumer products, ski products show the highest PFAS concentrations (Kotthoff et al., 2015). Abrasion of waxes from the ski sole results in the deposition of PFAS to nearby environments (Plassmann and Berger, 2013). Since PFAS are very persistent, they can remain in the environment for decades, thus creating PFAS-hotspots at skiing areas (Kissa, 2001; Grønnestad et al., 2019).

Previous studies have shown that some PFAS can cross the blood-brain barrier and accumulate in the brain (Maestri et al., 2006). Studies of large mammals suggest that PFAS can potentially be neurotoxic to exposed individuals. In polar bears (*Ursus maritimus*), brain PFAS levels were found to correlate with neurotransmitter alterations (Pedersen et al., 2015). Further, PFAS in North Atlantic pilot whales (*Globicephala melas*) were found to accumulate in brain, with higher levels only detected in the liver (Dassuncao et al., 2019). Neurotoxicity studies in rodents have shown that PFAS produced neurobehavioral alterations (Johansson et al., 2009; Lee and Viberg, 2013), and developmental and motor deficits (Onishchenko et al., 2011). Due to its important role in both motoric and cognitive functions, the central cholinergic system has received most attention in neurotoxicity studies (Eriksson and Viberg, 2005; Johansson et al., 2009). However, the dopamine (DA) neurotransmitter system plays an equally important role in behavior and cognitive functions, but has received less attention in these types of toxicity studies.

DA is derived from tyrosine, an amino acid which is converted to L-3,4-dihydroxyphenylalanine (L-DOPA) by tyrosine hydroxylase (Th). L-DOPA is further metabolized to DA by DOPA decarboxylase (DDC). Dopamine catabolism occurs through the breakdown of 3,4-dihydroxyphenylacetic acid (DOPAC) by monoamine oxidase (Mao), and then to homovanillic acid (HVA) by catechol-O methyltransferase (Comt) (Ashcroft, 1969). DA controls several brain conditions such as cognition, mood, fear, anxiety, as well as vascular and reproductive functions (Nakajima et al., 2013; Goschke and Bolte, 2014).

Studies have shown that alterations of the DA system can affect a number of signaling cascades in the body (Zohar et al., 2010). The DA neurons can, among others, indirectly regulate estrogen (E2) and testosterone (T) biosynthesis. Biosynthesis of E2 and T is regulated through the hypothalamus–pituitary–gonadal (HPG) axis (Zohar et al., 2010). The hypothalamus produces gonadotropin-releasing hormone (GnRH), and hypothalamic secretion of GnRH stimulates the release of gonadotropins (GtHs): luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into the blood. Both LH and FSH controls egg and sperm development, maturation and release, and they induce gonadal hormone synthesis, including E2 and T. DA can block the synthesis and release of GnRH (Yu et al., 1991), modulate gonadotropin levels by increasing and decreasing these hormones based on receptor subtype (Chang et al., 1990), and subsequently lead to decreased levels of E2 and T. Furthermore, DA can also control E2 levels by regulating brain aromatase, the enzyme that converts androgens to estrogens in the brain (Xing et al., 2016).

Neurobehavioral alterations in vertebrates have been examined in several developmental and adult exposure studies, with some behavioral endpoints suggesting that the DA-system is a potential target for environmental contaminants (Hallgren and Viberg, 2016). Northern leopard frogs (*Lithobates pipiens*) exposed to perfluorooctane sulfonate (PFOS) and perfuorooctanoic acid (PFOA) during developmental life-stages showed decreased and increased DA levels and turnover, respectively (Foguth et al., 2019), while the opposite effect was seen in adult rats (*Rattus norvegicus*), where PFOS exposure increased the DA levels (Salgado et al., 2015). These data from experimental studies raise the question of whether the exposure of small mammals to PFAS in the field, such as skiing areas, could lead to neurochemical alterations. In general, there is a paucity of data on PFAS neurotoxicity and especially in relation to environmentally relevant exposure scenarios in nature.

In a previous study, we showed that Bank voles (*Myodes glareolus*) from a Nordic skiing area had significantly higher liver PFAS concentrations, compared to those from a reference area, and that these PFAS were, most likely, derived from skiing products (Grønnestad et al., 2019). However, the reported concentrations were below toxicity threshold levels for laboratory studies on individual PFAS in mice (*Mus musculus*) and rats (Canadian Environmental Protection Act, 1999; Hoff et al., 2004). These results suggest that individual PFAS in ski products may not pose significant risks to biota or the environment. However, we emphasized that the small mammals were exposed to a mixture of PFAS, rather than to individual compounds, thus the issue of mixture toxicity should be considered and addressed in any environmental risk assessment program from skiing areas. Therefore, the aim of the current study was to evaluate the neuro-DA and steroid homeostatic pathways of wild Bank voles, in relation to tissue levels of various PFAS at a Nordic skiing area. Our hypothesis is that PFAS concentrations in Bank voles will affect biological variables in the neuro-DA and steroid hormone metabolic pathways.

2. Materials and methods

2.1. Sampling

Bank voles were sampled in June 2017 in "Granåsen skisenter", located approximately 10 km from the Trondheim city Centre (Norway). Granåsen is the main arena for winter sports in Trondheim and was used as a model contamination site in a skiing area. As a reference site, a natural forest area, not used for ski-sports, was chosen in the vicinity of an ecological farm next to Lake Jonsvatnet. This site is approximately 15 km away from Trondheim city center and 17 km from Granåsen. The sample size (n) was 21 at Granåsen (females (F): $n = 5$, males (M): $n = 16$) and 22 at Jonsvatnet (F: $n = 6$, M: n = 16).

The catching, handling, anesthesia, sampling and euthanizing of the Bank voles were approved by the Norwegian Food Safety Authority (Mattilsynet; references no. 2017/76552) and by the Norwegian Environmental Agency (Miljødirektoratet; reference no. 2017/4061). Permissions for the collection of Bank voles were also given by the landowners. The sampling and handling were performed in accordance with the regulations of the Norwegian Animal Welfare Act and EU legislation; 3Rs (Russell and Burch, 1959). All traps were live traps of type "Ugglan" baited with rye bread dipped in sunflower oil and peanut butter (all food products were sold as "ecological food material"). The Bank voles were sacrificed by cervical dislocation. The animals were weighed, length measured and sexed. The brains (used for DA and transcript analysis), livers (used for PFAS concentrations and steroid metabolism assay) and muscle tissues (used for steroid concentrations) were dissected and snap-frozen in liquid nitrogen and stored at −80 °C. In the current study, muscle tissue was used as a proxy for the free fraction of steroids in blood, since we did not have enough blood sample for this purpose. It has been shown that whole-body homogenate or muscle are suitable tissues for measuring the cellular and circulatory levels of steroid hormones (Arukwe et al., 2008; Preus-Olsen et al., 2014). Despite the limited blood sample size, the interest in measuring steroid hormone levels in muscle or tissue homogenates, rather than in blood plasma is based on the concept that the pattern of steroids release parallels its pattern of secretion into the bloodstream (Sebire et al., 2007; Sebire et al., 2009).

2.2. PFAS analysis

PFAS concentrations were analyzed at the Environmental Toxicology Laboratory, Norwegian University of Life Sciences (NMBU), Oslo, Norway. The analytical procedures were described in (Grønnestad et al., 2016). The samples were analyzed for the following PFAS: 10

perfluoroalkyl carboxylic acids (PFCA): perfluorobutanoic acid (PFBA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUdA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA) and perfluorotetradecanoic acid (PFTeDA), three perfluoroalkyl sulfonic acids (PFSA): perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS) and PFOS, and five perfluoroalkane sulfonamide derivatives (FASA): perfluoro-1-octane sulfonamide (FOSA), *N*-Methyl perfluoro-1-octane sulfonamide (N-MeFOSA), *N*-Ethyl perfluoro-1-octane sulfonamide (N-EtFOSA), 2-(*N*-Methyl perfluoro-1-octane sulfonamido) ethanol (N-MeFOSE) and 2-(*N*-Ethyl perfluoro-1-octane sulfonamido) ethanol (N-EtFOSE). For results on PFAS levels, patterns and biomagnification potential of PFAS in Bank voles from the two areas, see Grønnestad et al. (2019). For more analytical details, see supporting information (SI).

The limits of detection (LODs) were calculated as 3*SD of the procedural blanks and the limits of quantification (LOQs) were calculated as 10 * LOD. Where no PFAS were detected in blank samples, LOQs were determined as 10 * signal-to-noise ratio (S/N).

Contaminants with concentrations above LOD in more than 50% of samples were included in the statistical analyses, and missing values (i.e. < LOD) were assigned a random value between the LOD and zero.

2.3. Quality assurance

The Environmental Toxicology Laboratory is accredited by the Norwegian Accreditation as a testing laboratory according to the requirements of the NS-EN ISO/IEC 17025 (TEST 137).

For each series of maximum 30 samples, 3 blank samples, one blind and 4 recovery samples were run. Mean of procedural blanks, consisting of internal standards and solvents, were subtracted from each series separately, because of variation between series. The relative recovery in Bank voles ranged from 84 to 128% for PFCA, 78–129% for PFSA and 86–115% for FASA.

2.4. Dopamine analysis

For the measurement of brain concentrations of DA and its metabolites (DOPAC and HVA), samples were prepared using methods based on Tareke et al. (2007) and Bertotto et al. (2018) with slight modifications. Samples were kept on ice during handling and extraction. The samples were homogenized prior to extraction. Approximately 200 mg of homogenized brain tissue (right brain half) was placed into a 2 mL centrifuge tube, and internal standards (deuterated dopamine: DA-d4 and deuterated HVA: HVA-d5) were added to yield 1 ng DA-d4 and 2 ng of HVA-d5 per 1 mg of tissue. The samples were extracted twice using ice-cold 0.1% formic acid in water. The extraction was then, performed using a pestle tissue homogenizer. The tubes were centrifuged for 5 min at 4000 rpm and 4 °C. The extracts were then subjected to solid-phase extraction (SPE) with Strata X polymeric reverse-phase cartridges (33 mm, 60 mg, 3 mL; Phenomenex), as described in Tareke et al. (2007). The SPE cartridges were conditioned with 1 mL of 0.1% formic acid in acetonitrile (CH_3CN), followed by 1 mL of 0.1% formic acid in methanol, and 1 mL of 0.1% formic acid in water. The extracts were then added to the column, and the analytes were eluted with 3 mL of 0.1% formic acid in acetonitrile/methanol (1:1, v/v). The resulting extracts were evaporated to dryness with nitrogen gas and reconstituted in 0.4 mL of 0.1% formic acid in water. After vortexing, the extracts were filtered and transferred to autosampler vial inserts for liquid chromatography–tandem mass spectrometry (LC–MS/ MS) analysis. See SI for more details.

Deuterated dopamine (DA-d4) was used as an internal standard for dopamine while deuterated HVA (HVA-d5) was used as internal standard for HVA and DOPAC. Relative recoveries varied from 18 to 94% for Da-d4 and 21–66% for HVA-d5 (SI, Table S1). The samples were therefore adjusted for recoveries for each individual sample.

2.5. Steroid hormone analysis

Approximately 200 mg muscle tissue was added to 3× volume of lysis buffer (50 mM Tris base, 0.5% sodium deoxychalate, 1 mM EDTA, 1% triton, 150 mM NaCl, 0.1% SDS) with 10% phenylmethylsulfonyl fluoride (PMSF, to inhibit AChE). This was incubated on ice for 30 min followed by homogenization and centrifuged for 20 min at 15,000*g* and 4 °C. The supernatant was used for steroid extraction.

Steroid hormones were extracted twice using dichloromethane (DCM). DCM was added to a volume of 4× sample volume. The extracts were evaporated to dryness under nitrogen in a water bath at 30 °C and reconstituted with 210 μL of enzyme immunoassay (EIA) buffer. The extracts of muscle tissue were used for the measurement of E2 and T, using EIA kit from Cayman Chemicals (Ann Arbor, MI, USA). All assay solutions were prepared according to kit instructions with deionized water. Absorbance readings were performed on a spectrophotometer (Spectra Max Plus 384, Molecular Devices) at 412 nm. Steroid hormone concentrations were calculated by extrapolating sample absorbance on a linear standard curve, using the analysis tool provided by the kit's manufacturer.

2.6. Microsome extraction

Approximately 100 mg liver tissue was homogenized in 500 μL homogenization buffer (ice-cold 10 mM Tris-HCl buffer with 10% glycerol at pH 7.4). The homogenate was centrifuged for 20 min at 12,000*g* and 4 °C. The supernatant was transferred to new tubes and 1 mL homogenization buffer was added, then centrifuged for 60 min at 38,000 rpm in a vacuum centrifuge at 4 °C. The pellet was then re-suspended in 50 μL microsomal buffer (50 mM Tris-HCl with 0.1 mM EDTA and 20% glycerol, pH 7.4). Total microsomal protein content was determined using the Bradford method (Bradford, 1976), with bovine serum albumin (BSA) as standard.

2.7. Steroid hydroxylation assay

For the steroid hydroxylation assay, 0.4 mg of microsomal protein was incubated with 4 μM testosterone and assay buffer (50 mM Tris–HCl, 10 mM MgCl2, pH 7.4) to a final volume of 240 μL. Duplicates were made for each sample in parallel analysis with and without the addition of NADPH (300 μM). These were vortexed and incubated for 60 min at 30 °C. The reactions were stopped by adding 250 μL acetonitrile (ACN) and centrifuged for 10 min at 10,000*g*. The ACN extracts were filtered and transferred to glass vials and run on an LC-2030 (Shimadzu, Kyoto, Japan) with a UV detector. See more detailed information in SI.

Steroid hydroxylase (OHase) activity was measured as the change in testosterone concentration, after addition of NADPH, compared to when no NADPH was added.

2.8. RNA extraction and quantitative (real-time) polymerase chain reaction (qPCR)

Total brain RNA was isolated from frozen tissues using Direct-zol™ RNA extraction kit. Thereafter, RNA quantity and quality were measured using NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The NanoDrop showed that all samples had high quantity of RNA and good purity (260/280 ratio ranged between 1.97 and 2.0 and 260/230 ratio ranged between 2.0 and 2.1).

Transcripts expression analysis related to the dopaminergic and HPG pathways were performed using qPCR. Briefly, cDNA was synthesized from 1 μg total RNA according to instructions provided with the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA). PCR reaction mix (20 μL) containing 5 μL of 1:5 diluted cDNA, 0.5 μM each of the forward and reverse primer pair sequences (SI Table S2) were amplified using Mx3000P real-time PCR machine (Stratagene, La Jolla, CA). See Khan et al. (2019) for detailed protocol for qPCR analysis of gene

expression patterns. Transcripts tested include - dopamine receptor 1 and 2 (*dr1* and *dr2*), monoamine oxidase (*mao*), vesicular monoamine transporter (*vmat*), dopamine active transporter (*dat*), catechol-*O*-methyltransferase (*comt*), tyrosine hydroxylase (*th*), aromatase (*cyp19a*) and gonadotropin releasing hormone (*gnrh*). However, no positive amplifications were observed for *vmat*, *cyp19a* and *gnrh*.

2.9. Statistical analysis

The program R (version 3.6.3, the R project for statistical computing) was used for the statistical analysis. Normal distribution was tested with Shapiro Wilk's test, and homogeneity of variance was tested with Levene's test. Data were log-transformed prior to data analyses to reduce deviation from normality and homogeneity of variance. Two sample Student's *t*-tests were used to test for significant differences between the skiing and reference areas. The significance level was set at 0.05, and all tests were two tailed.

Multivariate analyses (principal component analyses; PCA) were carried out to investigate for possible relationships between the response variables (DA-related variables or steroid-related variables) and the explanatory variables (individual PFAS) in the skiing area (Granåsen). Explanatory variables were entered as passive variables in the PCA plots. Passive variables do not affect the ordination but are projected onto the unconstrained axes, allowing for visualization of correlations among response and explanatory variables. Variables were standardized to unit variance due to different units. Based on the visualization of possible relationships from the PCAs, general linear models (GLM) were used to quantify the amount of variance explained (R^2) by the respective single explanatory variables. Since there were no effects of gender on the PFAS concentrations, or response variables in the skiing area (*t*-test, $p > 0.05$), the dataset was not divided by sex in the multivariate analysis, to increase the sample size (n).

3. Results and discussion

3.1. Dopamine and dopamine metabolite levels

DA concentrations were significantly higher in brain samples in Bank voles from the skiing area with higher PFAS body burden (Granåsen), compared to the reference area (Jonsvatnet) (t-test, M: $p = 0.05$, F: $p = 0.03$, Fig. 1). The DA metabolite concentrations (DOPAC and HVA), were lower in the skiing area, compared to the reference area. The differences were significant in both sexes for DOPAC (t-test,

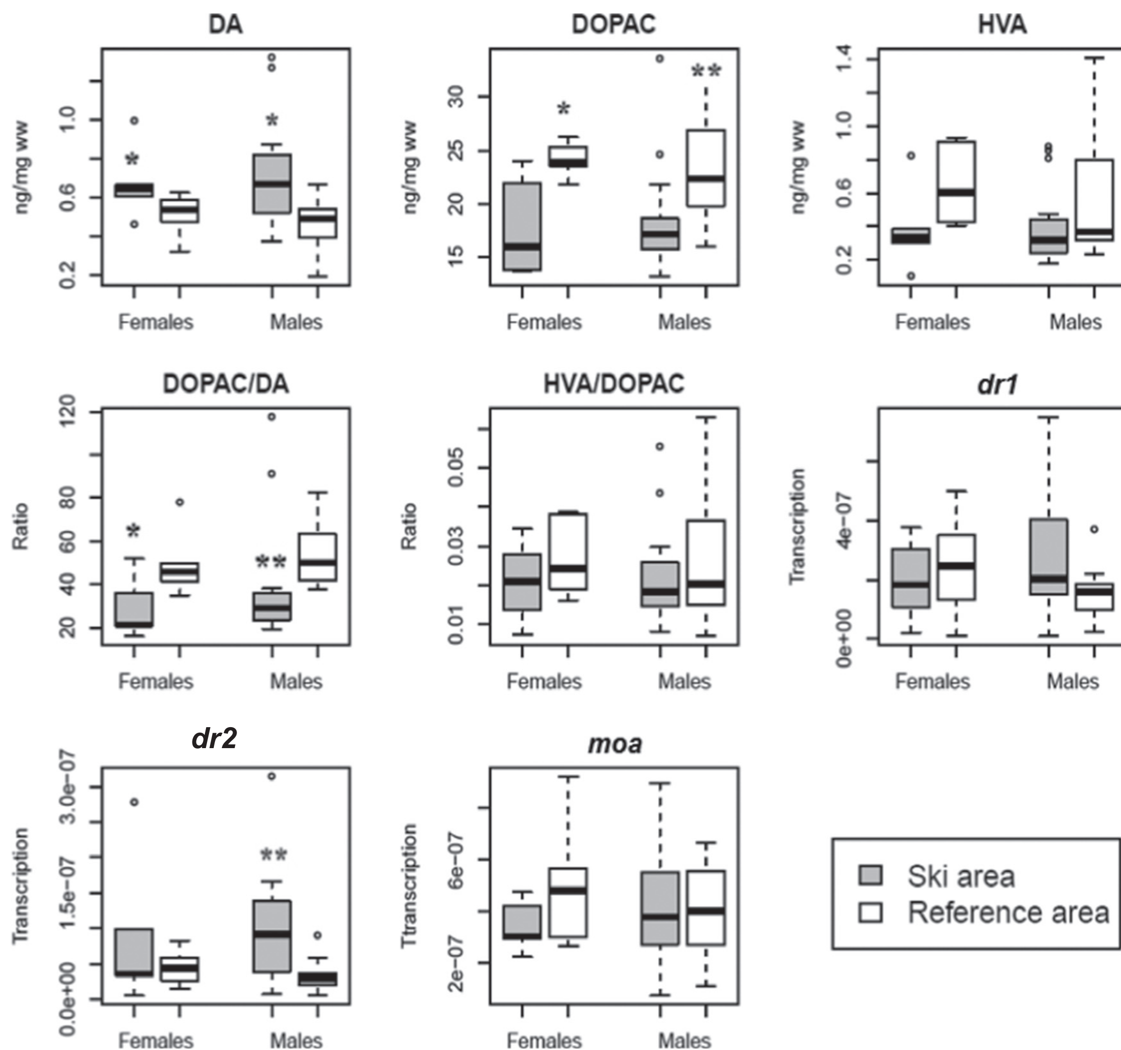


Fig. 1. Boxplots of variables related to the dopamine system in male and female Bank voles from Granåsen skiing area (females: $n = 5$, males: $n = 16$) and Jonsvatnet reference area (females: $n = 6$, males: $n = 16$). Variables are dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), DOPAC/DA ratio, HVA/DOPAC ratio, dopamine receptor 1 (d*r1*), dopamine receptor 2 (*dr2*) and monoamine oxidase (*moa*). Asterisks indicate the significance level: $^*p < 0.05$, $^{**}p < 0.01$.

M: $p = 0.004$, F: $p = 0.04$), but not for HVA (t-test, M: $p = 0.09$, F: $p = 0.1$).

Multivariate analysis (PCA, Fig. 2) was performed to investigate the possible relationship between the different DA variables and PFAS burden in Bank voles from the skiing area. Most of the PFAS showed positive associations with the DA concentrations, and GLM showed that there was a significant positive relationship between all the long-chain PFAS and DA (GLM, $p < 0.05$). This is in accordance with a previous study in adult male rats exposed to PFOS (Salgado et al., 2015). However, in developing Northern leopard frogs, there were lower DA levels in frogs exposed to PFOS and PFOA (Foguth et al., 2019).

It should be noted that we measured PFAS concentrations in Bank vole livers, and not the brain. Previous studies have shown that PFAS concentrations are usually higher in the liver compared to brain tissues of mammals, and that the accumulation of PFAS is tissue specific (Greaves et al., 2012; Greaves et al., 2013). However, in Polar bears PFAS accumulation in the brain was mostly comprised of long-chained PFAS (Greaves et al., 2013) and this finding is in accordance with the present study showing that the long chained PFAS are those associated with changes in dopaminergic endpoints. Long-chained PFAS were the dominant PFAS found in ski waxes, earth worms and soil samples from the skiing area where the Bank voles were sampled (Grønnestad et al., 2019).

Once released in the synaptic cleft, DA can bind to one of its 2 receptor families: dopamine receptor 1-like (Dr1) or dopamine receptor 2-like (Dr2) (Beaulieu and Gainetdinov, 2011). Dr2 plays an important role in regulating DA neuronal activity through synthesis, release and uptake. In addition, activation of Dr2 decreases the excitability of DA neurons and release of DA. Dr1, on the other hand, activates cyclic AMP-dependent protein kinase, stimulating the DA neuron (Jaber et al., 1996). Because of the higher DA concentrations in the Bank vole brains, lower concentrations of DA receptors were expected, in order to counteract the high DA levels and to maintain homeostatic balance. In the current study there were significantly higher *dr2* transcript levels in males from the skiing area, compared to the reference area (*t*-test, $p = 0.005$, Fig. 1). However, there was no significant difference in *dr2* in females, or for *dr1* in either sex (t-test, $p > 0.05$). The multivariate analysis showed a negative relationship between most PFAS and *dr1*

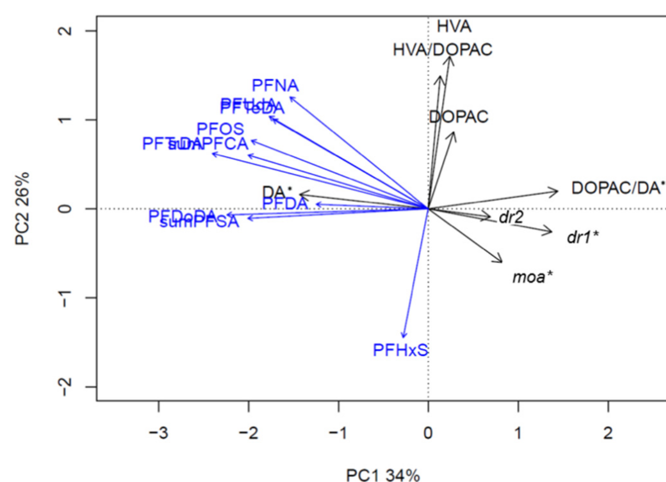


Fig. 2. Biplot of PFAS concentrations and dopamine variables (DA, DOPAC, HVA, DOPAC/DA, HVA/DOPAC, dr1, dr2, moa) in Bank voles from Granåsen skiing area ($n = 21$). Explanatory variables are projected as passive arrows (blue). The % of the total variance explained by each principal component (PCs) is given on each axis. Concentrations of all variables are log transformed and standardized to unit variance. Direction and length of arrows indicate respective strength and increasing variance of loading. Asterisks indicate response variables with significant associations to one or more PFAS.

expression in Bank voles from the skiing area (GLM, ΣPFCA: $p < 0.001$, $R^2 = 0.44$, ΣPFSA: $p = 0.01$, $R^2 = 0.24$, Fig. 2), while no association was observed between *dr2* and PFAS (GLM, $p > 0.5$, Fig. 2). This indicates that the higher *dr2* transcript level observed in males from the skiing area, are most likely not caused by the PFAS. The negative relationship between PFAS and *dr1*, without a corresponding difference between the two study areas, could be explained by other factors not evaluated in our field study, such as other pollutants, human disturbance, predators etc. These un-evaluated factors may be affecting the transcript expression patterns at the reference area. Our results are in accordance with previous findings showing that *dr1* expression was reduced in brain regions of rats exposed to PFOS (Salgado et al., 2016).

A previous study in mice showed that PFAS could modulate the DA system by altering different variables such as synthesis, reuptake, metabolism of DA, transcript and/or protein expression of different receptors (Hallgren and Viberg, 2016). DA is derived from the amino acid tyrosine, which is converted to L-DOPA by the enzyme tyrosine hydroxylase (Th) and further metabolized to DA. Th is considered the rate-limiting step of DA synthesis (Daubner et al., 2011). The plasma membrane DA transporter (Dat) is essential for normal dopamine neurotransmission. Dat terminates the actions of dopamine by rapidly removing DA from the synapse. Inhibition of DA reuptake via Dat thus increases the extracellular and synaptic concentrations and DA lifespan, leading to prolonged stimulation of DA receptors (Shimada et al., 1991). In the current study, *th* and *dat* transcripts did not show consistent positive amplification in the Bank vole brain samples. Overall, only three of 21 samples from Granåsen and two of 22 samples from the reference area showed *th* positive amplification, while only four samples from Granåsen and two samples from the reference area showed *dat* positive amplification (see results in SI Table S3). Other studies have reported that PFOS exposure can disrupt *th* expression in mice, with respective increases and decreases of the neonate and adult mice (Hallgren and Viberg, 2016). However, the authors did not observe any effects on *dat* expression (ibid).

The increased concentrations of DA reported in Bank voles at the Granåsen ski area could lead to alterations in thermoregulation processes (Hasegawa et al., 2000), defense (Sweidan et al., 1991) and aggressive behavior (Ricci et al., 2009), as well as in reproductive pathways (Henderson et al., 2008). DA also plays significant roles in the modulation of fear and anxiety (de la Mora et al., 2010). Consequently, PFAS exposure could alter neurological functions related to these emotional states. In addition, DA is involved in cognitive function, behavioral activation against appetite or aversive events and attention, as well as flexibility responses to stimuli (Seamans and Robbins, 2010). For this reason, the possible PFAS-related changes on the *dr1* and associated signaling pathways might potentially produce neurological disfunctions that may affect individual fitness. Additional studies are needed to confirm these potential linkages.

3.2. Dopamine turnover

The ratios between DA and its metabolites are generally used as a measure of DA turnover (Salgado et al., 2015). The DOPAC/DA ratio is indicative of intra-neuronal metabolism, while HVA/DA provides information on inter-neuronal metabolism of DA in the brain. The DOPAC/DA ratios were significantly lower in Bank voles from the skiing area, compared to the reference area (*t*-test, M: $p = 0.003$, F: $p = 0.05$, Fig. 1). These results are in accordance with previous rodent studies, showing reduced ratios in PFOS exposed rats (Salgado et al., 2015). Thus, PFAS exposure could possibly lead to lower DA turnover. This possibility was supported by the multivariate analysis, showing that there was a negative relationship between DOPAC/DA and several of the long-chained PFAS (Fig. 2), and where GLM confirmed a significant or borderline significant negative relationship (GLM, PFDoDA: $p = 0.05$, $R^2 = 0.15$, PFTrDA: $p = 0.06$, $R^2 = 0.13$). The lower brain metabolism of DA could be caused by a reduction in Mao activity, and thus a build-up of DA in the presynaptic neuron. Mao is responsible for the

metabolism of DA and other amine-containing neurotransmitters (Rutledge and Jonason, 1967). We found a negative association between several PFAS and *mao* mRNA expression (GLM, PFTrDA: $p = 0.04$, $R^2 = 0.17$, PFOS: $p = 0.02$, $R^2 = 0.20$, Fig. 2). There are not many studies that have reported effects of PFAS on Mao activity. However, a study on Polar bears from Greenland reported a positive relationship between Mao activity and PFAS across brain regions (Pedersen et al., 2015). While this finding may contradict our data, it should be noted, that the ΣPFAS concentrations in the Polar bears were almost 100-fold higher than the measured concentration in the Bank voles from the skiing area. Consequently, effects may not only be species-specific, but also dose-dependent. Nevertheless, and regardless of the measured concentration differences, it is possible that Bank voles and Polar bears display possible differences in their sensitivity and mechanisms of action of PFAS on Mao. This uncertainty and possible species-specific differences should be further investigated in rodents and other mammals.

The HVA/DOPAC ratio is a measure of inter-neuronal metabolism in the DA-system. We observed that HVA/DOPAC ratios were lower in the skiing area, compared to the reference area, albeit not significant (*t*-test, M: $p = 0.6$, F: $p = 0.3$, Fig. 1), suggesting that PFAS do not affect the inter-neuronal metabolism of DA from DOPAC to HVA, through the enzymatic actions of Comt. We did not find any correlation between any of the PFAS concentrations in the Bank voles and HVA/DOPAC ratio (Fig. 2). The only exception was PFHxS – although, this relationship was not significant (GLM, $p = 0.08$). These results are in accordance with Salgado et al. (2016), that reported the absence of effects on the inter-neuronal metabolism of DA in PFOS-treated rats.

3.3. Steroid hormone homeostasis

Estrogens and androgens are involved in growth and function of reproductive organs, development of secondary sexual characteristics, and behavioral patterns in vertebrate species. Thus, the balance in various steroid metabolic pathways has been shown to be associated with reproductive health. Consequently, measurement of steroid hormones may help determining the physiological health status of organisms (Gaikwad, 2013). PFAS have been shown to affect several physiological systems, including the endocrine system (López-Doval et al., 2014; Salgado et al., 2015). In the present study, we detected a trend towards lower T concentrations in muscle tissue from male Bank voles in the skiing area, compared to the reference area (t-test $p = 0.06$, Fig. 3), while there were no differences in females (t-test, $p = 0.5$). Specific PFAS associations were observed with a weak negative relationship between T concentrations in muscle tissue and several PFCA (GLM, ΣPFCA = 0.04, $R^2 = 0.17$, Fig. 4). Negative associations between PFAS and T has also been reported in other studies where they found a reduction in T concentrations after PFAS exposure in rats (López-Doval et al., 2014; Zhao et al., 2014). Although some studies have shown that adult male rats exposed to PFOS showed decreased serum E2 levels (Salgado et al., 2015), there was no significant difference in E2 concentrations between the two study areas in either sex in the present study (t-test, M: $p = 0.2$, F: $p = 0.6$), nor significant correlation between the PFAS and E2 concentrations (GLM, $p > 0.05$). Our results indicate that PFAS could be affecting T synthesis, clearance or cellular distribution (such as to the muscle).

The synthesis of T may be regulated by DA through the HPG-axis (Henderson et al., 2008; Bertotto et al., 2018). DA can affect the HPG-axis by decreasing the release of GnRH from the hypothalamus, leading to reduced secretion of FSH and LH from the pituitary, and consequently to decreases in the production and release of T and E2 from the gonads (Henderson et al., 2008; Zohar et al., 2010). Thus, it is possible that PFAS altered the release of T in the Bank voles by increasing DA levels in the brain. However, we did not observe any associations between DA and T or E2 (GLM, T: $p = 0.9$, E2: $p = 0.8$) in the Bank voles, suggesting that the increase in DA levels in voles from Granåsen may not affect the synthesis and release of T and E2. Other neuromodulators of GnRH synthesis, such as noradrenaline, glutamate and γ-aminobutyric acid (Skorupskaite et al., 2014), which are potentially susceptible to PFAS exposure, but not evaluated in the present study, might alternatively be

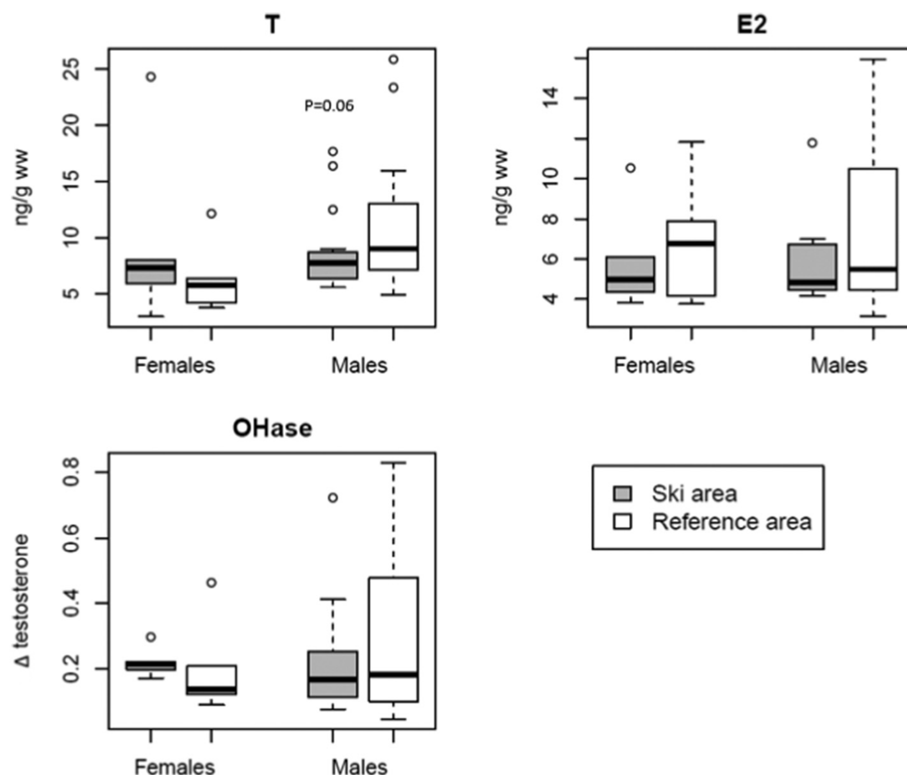


Fig. 3. Boxplots of variables related to the steroid hormone system in male and female Bank voles from Granåsen skiing area (females: n = 5, males: n = 16) and Jonsvatnet reference area (females: n = 6, males: n = 16).

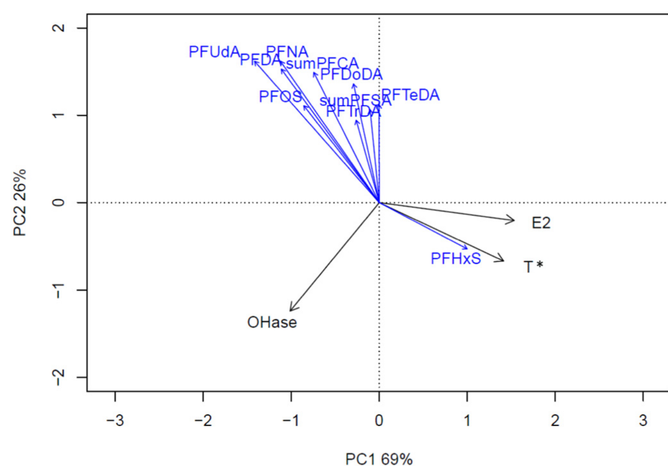


Fig. 4. Biplot of PFAS concentrations and steroid hormone variables (estrogen: E2, testosterone: T and liver steroid hydroxylase activity: OHase) in Bank voles from Granåsen skiing area (n = 21). Explanatory variables are projected as passive arrows (blue). The % of the total variance explained by each principal component (PCs) is given on each axis. Concentrations of all variables are log transformed and standardized to unit variance. Direction and length of arrows indicate respective strength and increasing variance of loading. Asterisks indicate response variables with significant associations to one or more PFAS.

involved in steroid hormone homeostasis through the GnRH signaling pathways (León et al., 2014).

The fact that we found a significant negative association between PFCAs and T (Fig. 4), but not between T and DA, suggests that PFAS could be affecting the synthesis and release of T through other mechanisms. Previously, it has been reported that exposure of male rats to PFOS disrupted the reproductive axis activity through a reduction of T production (Zhao et al., 2014). A study on healthy men also reported a negative association between the concentration of PFOS and T (Joensen et al., 2013). Furthermore, a study by López-Doval et al. (2014) found that oral exposure of male rats to PFOS produced a reduction in *gnrh* mRNA expression and in LH and T secretion. The authors concluded that PFOS exposure disrupted the male reproductive axis at different levels, including the hypothalamus, by decreasing *gnrh* expression and by modifying GnRH release, in the pituitary gland, by inhibiting LH secretion and stimulating FSH release, and, in the testis, by inhibiting T release. A reduction in T concentrations in male Bank voles could eventually affect the reproduction, and thus individual fitness of the Bank voles.

Kang et al. (2016) reported that in vitro exposure to PFOA and PFOS induced and reduced E2 and T levels, respectively, through hepatic CYP-enzyme mediated pathways. They suggested that PFOA and PFOS induced endocrine disruption by affecting the process of steroidogenesis. In the present study, we did not observe significant differences in testosterone OHase activity in Bank vole livers from the skiing area, compared to the reference area (*t*-test, M: $p = 0.9$, F: $p = 0.09$). We did not find any significant association between liver PFAS concentrations and OHase activity (Fig. 4). In addition, no correlation between muscle steroid hormone levels and OHase activity was observed. Steroid hormones serve as endogenous substrates for cytochrome P450 enzymes belonging to the CYP3A subfamily in vertebrate liver microsomes where the major site of hydroxylation is at the 6β-, 16α- and 17α positions and the capacity to hydroxylate steroids is often sex specific (Waxman et al., 1988; Zimniak and Waxman, 1993). In this study, the particular position of testosterone hydroxylation was not determined and as a consequence, our analytical protocol might have omitted the direct effects of PFASs on steroid hydroxylation. Further, it is also possible that PFASs did not directly affect hepatic metabolism of steroids, but rather the synthesis and release of T from the gonads.

4. Conclusion

In the current study, we have addressed the potential effects of PFAS exposure from ski products on the DA and steroid hormone homeostasis in Bank voles inhabiting the environments around a skiing area. We have shown that exposure within the concentration range documented at a Nordic skiing area contaminated by PFAS from ski products showed: a) increased total brain DA level and reduced DA turnover, b) a negative association with *dr1* and *mao* expression, and c) a negative association with cellular T levels in wild male Bank voles. Thus, DA and cellular steroid hormone homeostasis could potentially be altered by environmental PFAS exposure, which could lead to potential consequences on reproduction, general health and fitness of Bank voles from the skiing area.

CRediT authorship contribution statement

Randi Grønnestad: Conceptualization, Methodology, Formal analysis, Visualization, Writing - original draft. **Daniel Schlenk:** Conceptualization, Methodology, Writing - review & editing. **Åse Krøkje:** Conceptualization, Supervision, Writing - review & editing. **Veerle L.B. Jaspers:** Conceptualization, Supervision, Writing - review & editing. **Bjørn Munro Jenssen:** Conceptualization, Supervision, Writing - review & editing. **Scott Coffin:** Methodology, Writing - review & editing. **Luísa Becker Bertotto:** Methodology, Writing - review & editing. **Marissa Giroux:** Methodology, Writing - review & editing. **Jan L. Lyche:** Methodology, Writing - review & editing. **Augustine Arukwe:** Conceptualization, Supervision, Writing - original draft, Writing - review & editing.

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.

Acknowledgements

The work was supported by a PhD fellowship provided by the Faculty of Natural Sciences, NTNU, Norway (project no. 70440378). We thank Randi Røsbak at the Department of Biology at NTNU for assistance at the laboratory. The authors declare no competing financial interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.143745.

References

Arukwe, A., Nordtug, T., Kortner, T.M., Mortensen, A.S., Brakstad, O.G., 2008. Modulation of steroidogenesis and xenobiotic biotransformation responses in zebrafish (Danio rerio) exposed to water-soluble fraction of crude oil. Environ. Res. 107 (3), 362–370.
Ashcroft, G., 1969. Studies in cerebral amine metabolism. Amine metabolism in brain. Proceedings of the Royal Society of Medicine 62, 1099.
Beaulieu, J.M., Gainetdinov, R.R., 2011. The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol. Rev. 63 (1), 182–217.
Bertotto, L.B., Richards, J., Gan, J., Volz, D.C., Schlenk, D., 2018. Effects of bifenthrin exposure on the estrogenic and dopaminergic pathways in zebrafish embryos and juveniles. Environ. Toxicol. Chem. 37 (1), 236–246.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 (1), 248–254.
Canadian Environmental Protection Act, 1999. . Available. https://www.ec.gc.ca/ese-ees/38E6993C-76AA-4486-BAEB-D3828B430A6E/PFOS_En.pdf.
Chang, J.P., Yu, K.L., Wong, A.O.L., Peter, R.E., 1990. Differential actions of dopamine receptor subtypes on gonadotropin and growth hormone release in vitro in goldfish. Neuroendocrinology 51 (6), 664–674.
Dassuncao, C., Pickard, H., Pfohl, M., Tokranov, A.K., Li, M., Mikkelsen, B., Slitt, A., Sunderland, E.M., 2019. Phospholipid levels predict the tissue distribution of poly-

and perfluoroalkyl substances in a marine mammal. Environmental Science & Technology Letters 6 (3), 119–125.
Daubner, S.C., Le, T., Wang, S., 2011. Tyrosine hydroxylase and regulation of dopamine synthesis. Archives of Biochemistry and Biophysics 508, 1–12.
de la Mora, M.P., Gallegos-Cari, A., Arizmendi-García, Y., Marcellino, D., Fuxe, K., 2010. Role of dopamine receptor mechanisms in the amygdaloid modulation of fear and anxiety: structural and functional analysis. Prog. Neurobiol. 90 (2), 198–216.
Eriksson, P., Viberg, H., 2005. Tiered Testing in Mammals-The Neonatal Animal Model.
Foguth, R.M., Flynn, R.W., de Perre, C., Iacchetta, M., Lee, L.S., Sepúlveda, M.S., Cannon, J.R., 2019. Developmental exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) selectively decreases brain dopamine levels in Northern leopard frogs. Toxicol. Appl. Pharmacol. 377, 114623.
Gaikwad, N.W., 2013. Ultra performance liquid chromatography-tandem mass spectrometry method for profiling of steroid metabolome in human tissue. Anal. Chem. 85 (10), 4951–4960.
Glüge, J., Scheringer, M., Cousins, I., DeWitt, J.C., Goldenman, G., Herzke, D., Lohmann, R., Ng, C., Trier, X., Wang, Z., 2020. An Overview of the Uses of Per- and Polyfluoroalkyl Substances (PFAS).
Goschke, T., Bolte, A., 2014. Emotional modulation of control dilemmas: the role of positive affect, reward, and dopamine in cognitive stability and flexibility. Neuropsychologia 62, 403–423.
Greaves, A.K., Letcher, R.J., Sonne, C., Dietz, R., Born, E.W., 2012. Tissue-specific concentrations and patterns of perfluoroalkyl carboxylates and sulfonates in East Greenland polar bears. Environ. Sci. Technol. 46 (21), 11575–11583.
Greaves, A.K., Letcher, R.J., Sonne, C., Dietz, R., 2013. Brain region distribution and patterns of bioaccumulative perfluoroalkyl carboxylates and sulfonates in East Greenland polar bears (Ursus maritimus). Environ. Toxicol. Chem. 32 (3), 713–722.
Grønnestad, R., Villanger, G.D., Polder, A., Kovacs, K.M., Lydersen, C., Jenssen, B.M., Borgå, K., 2016. Maternal transfer of perfluoroalkyl substances in hooded seals. Environ. Toxicol. Chem. 36 (3), 763–770.
Grønnestad, R., Vázquez, B.P., Arukwe, A., Jaspers, V.L.B., Jenssen, B.M., Karimi, M., Lyche, J.L., Krøkje, Å., 2019. Levels, patterns, and biomagnification potential of perfluoroalkyl substances in a terrestrial food chain in a nordic skiing area. Environ. Sci. Technol. 53 (22), 13390–13397.
Hallgren, S., Viberg, H., 2016. Postnatal exposure to PFOS, but not PBDE 99, disturb dopaminergic gene transcription in the mouse CNS. Environ. Toxicol. Pharmacol. 41, 121–126.
Hasegawa, H., Yazawa, T., Yasumatsu, M., Otokawa, M., Aihara, Y., 2000. Alteration in dopamine metabolism in the thermoregulatory center of exercising rats. Neurosci. Lett. 289 (3), 161–164.
Henderson, H.L., Townsend, J., Tortonese, D.J., 2008. Direct effects of prolactin and dopamine on the gonadotroph response to GnRH. J. Endocrinol. 197 (2), 343–350.
Hoff, P., Tony Scheirs, J., Van de Vijver, K., Van Dongen, W., Esmans Eddy, L., Blust, R., De Coen, W., 2004. Biochemical effect evaluation of perfluorooctane sulfonic acid-contaminated wood mice (Apodemus sylvaticus). Environ. Health Perspect. 112 (6), 681–686.
Houde, M., Martin, J.W., Letcher, R.J., Solomon, K.R., Muir, D.C.G., 2006. Biological monitoring of polyfluoroalkyl substances: a review. Environ. Sci. Technol. 40 (11), 3463–3473.
Jaber, M., Robinson, S.W., Missale, C., Caron, M.G., 1996. Dopamine receptors and brain function. Neuropharmacology 35 (11), 1503–1519.
Joensen, U.N., Veyrand, B., Antignac, J.-P., Blomberg Jensen, M., Petersen, J.H., Marchand, P., Skakkebæk, N.E., Andersson, A.-M., Le Bizec, B., Jørgensen, N., 2013. PFOS (perfluorooctanesulfonate) in serum is negatively associated with testosterone levels, but not with semen quality, in healthy men. Hum. Reprod. 28 (3), 599–608.
Johansson, N., Eriksson, P., Viberg, H., 2009. Neonatal exposure to PFOS and PFOA in mice results in changes in proteins which are important for neuronal growth and synaptogenesis in the developing brain. Toxicol. Sci. 108 (2), 412–418.
Kang, J.S., Choi, J.-S., Park, J.-W., 2016. Transcriptional changes in steroidogenesis by perfluoroalkyl acids (PFOA and PFOS) regulate the synthesis of sex hormones in H295R cells. Chemosphere 155, 436–443.
Khan, E.A., Bertotto, L.B., Dale, K., Lille-Langøy, R., Yadetie, F., Karlsen, O.A., Goksøyr, A., Schlenk, D., Arukwe, A., 2019. Modulation of neuro-dopamine homeostasis in juvenile female Atlantic cod (Gadus morhua) exposed to polycyclic aromatic hydrocarbons and perfluoroalkyl substances. Environ. Sci. Technol. 53 (12), 7036–7044.
Kissa, E., 2001. Fluorinated Surfactants and Repellents. Marcel Dekker, New York.
Kotthoff, M., Müller, J., Jürling, H., Schlummer, M., Fiedler, D., 2015. Perfluoroalkyl and polyfluoroalkyl substances in consumer products. Environ. Sci. Pollut. Res. 22 (19), 14546–14559.
Lee, I., Viberg, H., 2013. A single neonatal exposure to perfluorohexane sulfonate (PFHxS) affects the levels of important neuroproteins in the developing mouse brain. Neurotoxicology 37, 190–196.
León, S., García-Galiano, D., Ruiz-Pino, F., Barroso, A., Manfredi-Lozano, M., Romero-Ruiz, A., Roa, J., Vázquez, M.J., Gaytan, F., Blomenrohr, M., van Duin, M., Pinilla, L., Tena-Sempere, M., 2014. Physiological roles of gonadotropin-inhibitory hormone signaling in the control of mammalian reproductive axis: studies in the NPFF1 receptor null mouse. Endocrinology 155 (8), 2953–2965.
López-Doval, S., Salgado, R., Pereiro, N., Moyano, R., Lafuente, A., 2014. Perfluorooctane sulfonate effects on the reproductive axis in adult male rats. Environ. Res. 134, 158–168.
Maestri, L., Negri, S., Ferrari, M., Ghittori, S., Fabris, F., Danesino, P., Imbriani, M., 2006. Determination of perfluorooctanoic acid and perfluorooctanesulfonate in human tissues by liquid chromatography/single quadrupole mass spectrometry. Rapid Commun. Mass Spectrom. 20 (18), 2728–2734.
Nakajima, S., Gerretsen, P., Takeuchi, H., Caravaggio, F., Chow, T., Le Foll, B., Mulsant, B., Pollock, B., Graff-Guerrero, A., 2013. The potential role of dopamine D_3 receptor neurotransmission in cognition. Eur. Neuropsychopharmacol. 23 (8), 799–813.
Onishchenko, N., Fischer, C., Wan Ibrahim, W.N., Negri, S., Spulber, S., Cottica, D., Ceccatelli, S., 2011. Prenatal exposure to PFOS or PFOA alters motor function in mice in a sex-related manner. Neurotox. Res. 19 (3), 452–461.
Pedersen, K.E., Basu, N., Letcher, R., Greaves, A.K., Sonne, C., Dietz, R., Styrishave, B., 2015. Brain region-specific perfluoroalkylated sulfonate (PFSA) and carboxylic acid (PFCA) accumulation and neurochemical biomarker responses in east Greenland polar bears (Ursus maritimus). Environ. Res. 138, 22–31.
Plassmann, M.M., Berger, U., 2013. Perfluoroalkyl carboxylic acids with up to 22 carbon atoms in snow and soil samples from a ski area. Chemosphere 91 (6), 832–837.
Preus-Olsen, G., Olufsen, M.O., Pedersen, S.A., Letcher, R.J., Arukwe, A., 2014. Effects of elevated dissolved carbon dioxide and perfluorooctane sulfonic acid, given singly and in combination, on steroidogenic and biotransformation pathways of Atlantic cod. Aquat. Toxicol. 155, 222–235.
Ricci, L.A., Schwartzer, J.J., Melloni, R.H., 2009. Alterations in the anterior hypothalamic dopamine system in aggressive adolescent AAS-treated hamsters. Horm. Behav. 55 (2), 348–355.
Russell, W.M.S., Burch, R.L., 1959. The Principles of Humane Experimental Technique. Methuen.
Rutledge, C.O., Jonason, J., 1967. Metabolic Pathways of Dopamine and Norepinephrine in Rabbit Brain In Vitro. 157(3) pp. 493–502.
Salgado, R., Pereiro, N., López-Doval, S., Lafuente, A., 2015. Initial study on the possible mechanisms involved in the effects of high doses of perfluorooctane sulfonate (PFOS) on prolactin secretion. Food Chem. Toxicol. 83, 10–16.
Salgado, R., López-Doval, S., Pereiro, N., Lafuente, A., 2016. Perfluorooctane sulfonate (PFOS) exposure could modify the dopaminergic system in several limbic brain regions. Toxicol. Lett. 240 (1), 226–235.
Seamans, J.K., Robbins, T.W., 2010. Dopamine Modulation of the Prefrontal Cortex and Cognitive Function. The Dopamine Receptors. K. A. Neve. Humana Press, Totowa, NJ, pp. 373–398.
Sebire, M., Katsiadaki, I., Scott, A.P., 2007. Non-invasive measurement of 11-ketotestosterone, cortisol and androstenedione in male three-spined stickleback (Gasterosteus aculeatus). Gen. Comp. Endocrinol. 152 (1), 30–38.
Sebire, M., Katsiadaki, I., Scott, A., 2009. Further refinement of the non-invasive procedure for measuring steroid production in the male three-spined stickleback Gasterosteus aculeatus. J. Fish Biol. 75 (8), 2082–2094.
Shimada, S., Kitayama, S., Lin, C.L., Patel, A., Nanthakumar, E., Gregor, P., Kuhar, M., Uhl, G., 1991. Cloning and expression of a cocaine-sensitive dopamine transporter complementary DNA. Science 254 (5031), 576–578.
Skorupskaite, K., George, J.T., Anderson, R.A., 2014. The kisspeptin-GnRH pathway in human reproductive health and disease. Hum. Reprod. Update 20 (4), 485–500.
Sweidan, S., Edinger, H., Siegel, A., 1991. D2 dopamine receptor-mediated mechanisms in the medial preoptic-anterior hypothalamus regulate affective defense behavior in the cat. Brain Res. 549 (1), 127–137.
Tareke, E., Bowyer, J.F., Doerge, D.R., 2007. Quantification of rat brain neurotransmitters and metabolites using liquid chromatography/electrospray tandem mass spectrometry and comparison with liquid chromatography/electrochemical detection. Rapid Commun. Mass Spectrom. 21 (23), 3898–3904.
Waxman, D.J., LeBlanc, G., Morrissey, J., Staunton, J., Lapenson, D., 1988. Adult male-specific and neonatally programmed rat hepatic P-450 forms RLM2 and 2a are not dependent on pulsatile plasma growth hormone for expression. J. Biol. Chem. 263 (23), 11396–11406.
Xing, L., Esau, C., Trudeau, V., 2016. Direct Regulation of Aromatase B Expression by 17β-Estradiol and Dopamine D1 Receptor Agonist in Adult Radial Glial Cells. vol. 9 p. 504.
Yu, K.L., Rosenblum, P.M., Peter, R.E., 1991. In vitro release of gonadotropin-releasing hormone from the brain preoptic-anterior hypothalamic region and pituitary of female goldfish. Gen. Comp. Endocrinol. 81 (2), 256–267.
Zhao, B., Li, L., Liu, J., Li, H., Zhang, C., Han, P., Zhang, Y., Yuan, X., Ge, R.S., Chu, Y., 2014. Exposure to perfluorooctane sulfonate in utero reduces testosterone production in rat fetal leydig cells. PLoS One 9 (1), 78888.
Zimniak, P., Waxman, D., 1993. Liver cytochrome P450 metabolism of endogenous steroid hormones, bile acids, and fatty acids. Cytochrome P450. Springer, pp. 123–144.
Zohar, Y., Muñoz-Cueto, J.A., Elizur, A., Kah, O., 2010. Neuroendocrinology of reproduction in teleost fish. Gen. Comp. Endocrinol. 165 (3), 438–455.