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Absorption, Distribution, and Toxicity of Per- and Polyfluoroalkyl Substances (PFAS) in the Brain: A Review

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Abstract

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic chemicals colloquially known as "forever chemicals" because of their high persistence. PFAS have been detected in the blood, liver, kidney, heart, muscle and brain of various species. Although brain is not a dominant tissue for PFAS accumulation compared to blood and liver, adverse effects of PFAS on brain functions have been identified. Here, we review studies related to the absorption, accumulation, distribution and toxicity of PFAS in the brain. We summarize evidence on two potential mechanisms of PFAS entering the brain: initiating blood-brain barrier (BBB) disassembly through disrupting tight junctions and relying on transporters located at the BBB. PFAS with diverse structures and properties enter and accumulate in the brain with varying efficiencies. For example, compared to long-chain PFAS, short-chain PFAS may not cross cerebral barriers effectively. In addition, according to biomonitoring studies and PFAS exposure experiments, PFAS can accumulate in the brain of humans and wildlife species. With respect to the distribution of PFAS in specific brain regions, the brain stem, hippocampus, hypothalamus, pons/medulla and thalamus are dominant for PFAS accumulation. The accumulation and distribution of PFAS in the brain may lead to toxic effects in the central nervous system (CNS), including PFAS-induced behavioral and cognitive disorders. The specific mechanisms underlying such PFAS-induced neurotoxicity remain to be explored, but two major potential mechanisms based on current understanding are PFAS effects on calcium homeostasis and neurotransmitter alterations in neurons. Based on the information available about PFAS uptake, accumulation, distribution and impacts on the brain, PFAS have the potential to enter and accumulate in the brain at varying levels. The balance of existing studies shows there is some indication of risk in animals, while the human evidence is mixed and warrants further scrutiny.

Environmental Significance

Per- and polyfluoroalkyl substances (PFAS) are a group of persistent man-made chemicals used in products to impart hydrophobic and lipophobic properties. The wide application of these compounds in numerous products has led to ubiquitous exposure. Therefore, they have been detected in multiple tissues, including the brain, of various species. The accumulation and distribution of PFAS in the brain highlight their potential to cause toxic effects. Our review integrates current evidence from multiple perspectives (epidemiological, *in vivo*, and *in vitro*) of PFAS accumulation and their potential toxic effects on the brain. More data are needed to specify the mechanisms by which different PFAS enter the brain, and to more concretely link PFAS accumulation in the brain to neurotoxic mechanisms.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals with useful properties such as water and oil repellency and resistance to extreme temperatures.[1] These properties led to wide industrial and commercial applications, including in semiconductors, firefighting foams, non-stick cookware and food packaging, resulting in exposure of humans and wildlife.[2–5] PFAS are very persistent in the environment, and once in the body, some PFAS accumulate in tissues.[5,6] Studies have detected PFAS in the blood, liver, kidney, heart, muscle and brain of various species.[7–10] Based on previous studies, PFAS accumulate in the blood due to binding between PFAS and serum albumin.[6,11–13] The brain ensures its normal functions through uptake of oxygen, nutrients and other required substances from the blood.[14] Substance exchange in the cerebral circulation creates the opportunity for PFAS to enter the brain. However, xenobiotics usually can't move freely in the brain because of cerebral barriers, such as the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSFB), that protect the central nervous system (CNS, composed of the brain and spinal cord) by allowing needed chemicals in but not toxins and pathogens.[15,16] This barrier function has been shown to also apply to some PFAS.[17]

The BBB is the biochemical boundary of endothelial cells that mediates the exchange of substances between the bloodstream and brain.[18] The link between endothelial cells, known as tight junctions, are responsible for limiting paracellular leakage during substance transport.[19] Various transporters located at the surface of endothelial cells take charge of exchanging chemicals across the cell membrane, such as P-glycoprotein (P-gp), breast cancer resistance

protein (BCRP/Bcrp), multidrug resistance proteins (MRPs/Mrps), organic anion transporting polypeptides (OATPs/Oatps), organic anion transporters (OATs/Oats) and organic cation transporters (OCTs/Octs) (Figure 1). Chemicals bind to transporters and achieve transmembrane transport through the transporters' conformational change.[20] Based on previous studies, PFAS could enter the brain by disrupting tight junctions to permeate into the brain[21–23] or binding to transporters to cross the plasma membrane[24–26]. However, studies related to the interaction of PFAS and transporters mainly focus on renal transporters,[27] while the transport of PFAS through similar transporters at the BBB has yet to be verified. In addition, the specific mechanisms by which different PFAS enter the brain is still unclear, but a number or studies have reported the presence of PFAS in the brain.[7,8,10,17,28–37]

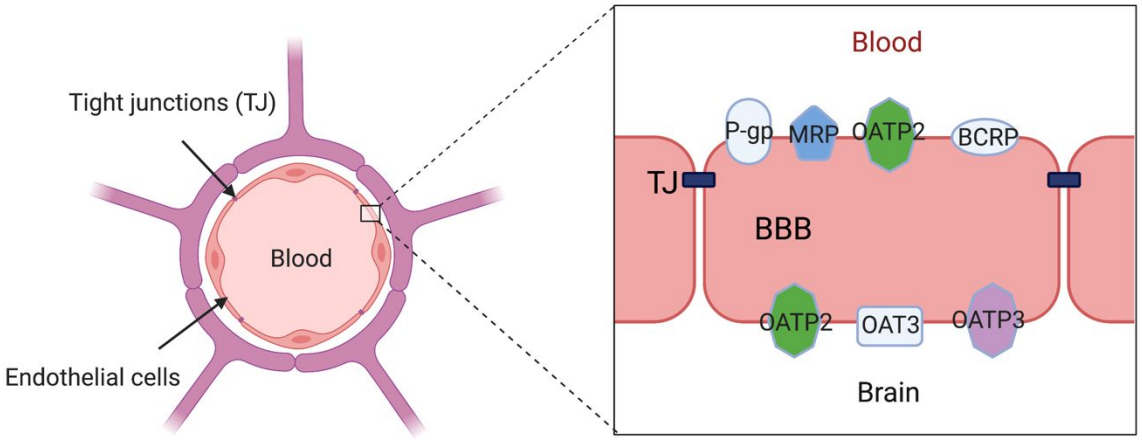

Figure 1 The structure of the blood-brain barrier (BBB), the biochemical boundary of endothelial cells between the bloodstream and brain. The link between endothelial cells (dark blue rectangles in inset) are tight junctions (TJ), responsible for limiting paracellular leakage during substance transport. Various transporters located at the surface of the BBB exchange chemicals across the cell membrane (abbreviations refer to different transporters as discussed in the text).

Biomonitoring studies have detected a broad array of PFAS, including perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFSAs) and PFAS precursors (compounds that have the potential to be degraded to terminal PFAS, including sulfonamides and fluorotelomer substances [38]) in the brain and cerebrospinal fluid (CSF) of humans, and in the brain of wildlife species.[28,8,37,17] The CSF is the fluid surrounding the brain and spinal cord, and PFAS content in this fluid has been used in a small number of studies as a surrogate for PFAS content in the brain

interstitial fluid.[17,37] In addition, various wildlife biomonitoring and animal exposure studies have also detected the accumulation of PFAS, most frequently PFOA and PFOS, in the brain of various species.[7,10,29–36,39] In terms of the PFAS distribution data related to specific brain areas, the brain stem, hippocampus, hypothalamus, pons/medulla and thalamus are dominant for PFAS accumulation.[29,30] These brain region-specific studies are critical for connecting the dominant areas in the brain for PFAS accumulation to the toxic effects of PFAS on the brain, but the available studies are limited. The absorption and accumulation of PFAS in the brain highlight the potential for these substances to cause toxic effects. Studies have reported associations between PFAS exposure and behavioral[40–46] and cognitive[47–52] disorders both in animals and humans, but conflicting results of the direction of the association are present in these studies, and the mechanisms underlying PFAS-induced neurotoxicity remain poorly understood. Various *in vitro* studies proposed two main potential mechanisms, including PFAS-induced intracellular calcium alteration in neurons[53–63] and the impacts of PFAS on neurotransmitters.[64–73] However, most of these *in vitro* studies focus on PFOA and PFOS. The neurotoxicity of perfluoroalkyl acids (PFAAs) and emerging PFAS still needs to be evaluated.

In this critical review, we surveyed studies related to the absorption, accumulation, distribution and toxicity of a broad array of PFAS in the brain (Table 1 lists those that are the focus of this review; a more exhaustive list for all PFAS analyzed in the reviewed papers can be found in Table S1 in the Supplemental Information, SI). Based on current understanding, we summarized two potential mechanisms for PFAS to enter the brain, including (1) initiating BBB disassembly through the disruption of tight junctions and (2) relying on membrane transporters. To understand the accumulation and distribution of PFAS in the brain of various species, we surveyed studies of PFAS distributions in collected brain samples from biomonitoring studies and controlled exposure experiments. The brain region-specific PFAS distribution may provide links to observed adverse effects. Finally, we reviewed papers discussing the potential neurotoxicity of PFAS, in terms of effects on calcium homeostasis and neurotransmitters, as well as neurobehavioral and cognitive disorders as outcomes of PFAS exposure.

Table 1 The list of PFAS discussed in this review

Class	Name	Acronym	Carbon Chain Length

PFCAs	perfluorobutanoic acid	PFBA	4
	perfluoropentanoic acid	PFPeA	5
	perfluorohexanoic acid	PFHxA	6
	perfluoroheptanoic acid	PFHpA	7
	perfluorooctanoic acid	PFOA	8
	perfluorononanoic acid	PFNA	9
	perfluorodecanoic acid	PFDA	10
	perfluoroundecanoic acid	PFUnDA (PFUDA)	11
	perfluorododecanoic acid	PFDoDA (PFDoA)	12
	perfluorotridecanoic acid	PFTrDA (PFTrA)	13
	perfluorotetradecanoic acid	PFTeDA (PFTeA, PFTA)	14
	perfluoropentadecanoic acid	PFPeDA	15
PFSAs	perfluorobutane sulfonic acid	PFBS	4
	perfluorohexane sulfonic acid	PFHxS	6
	perfluorooctane sulfonic acid	PFOS	8
	perfluorodecane sulfonic acid	PFDS	10
Ethers	6:2 chlorinated polyfluoroalkyl ether sulfonate	6:2 Cl-PFESA (F-53B)	8
	8:2 chlorinated polyfluoroalkyl ether sulfonate	8:2 Cl-PFESA	10
Precursors	perfluorooctane sulfonamide	PFOSA	8
	8:2 polyfluoroalkyl phosphate diester	8:2 diPAP	20

Note: Acronyms in parenthesis are alternative versions used in some reviewed papers.

2. Review scope

In this critical review, we used the Web of Science to search for studies using the following search terms: PFAS, brain, blood-brain barrier (or BBB), transporter, accumulation, distribution,

exposure and neurotoxicity. This resulted in 65 papers published between 2005 and 2020, which we categorized into three major subcategories corresponding to the review sections to follow: (1) absorption of PFAS in the brain, (2) accumulation and distribution of PFAS in the brain, and (3) potential neurotoxicity of PFAS.

In the absorption section, we identified and reviewed 11 papers. In the accumulation and distribution section, we identified and reviewed 25 papers, classified into PFAS in collected brain samples (15 papers, see Table 2) and controlled PFAS exposure experiments (10 papers, see Table 3). We calculated PFAS brain-to-blood (or brain-to-serum) ratios where paired brain and blood (or serum) data were available and/or PFAS brain-to-liver ratios if paired brain and liver (but not blood) concentrations were available. These ratios are useful to understand PFAS uptake and/or retention rates in the brain relative to overall exposure, since accumulation for many PFAS is greatest in blood and liver. Several of the studies reviewed did not report their raw data or substance-specific PFAS concentrations, only the sum of analyzed PFAS, summary statistics, or ranges. For those studies, we contacted the authors and requested the raw data. We received raw data from Dr. Gebbink and Dr. Letcher from their 2012 study[34] and Dr. Verreault from the Verreault et al. 2005 study[10]. Finally, in the section on potential neurotoxicity, we reviewed 34 papers, including 13 on associations of PFAS exposure with behavioral and cognitive disorders, 11 papers on PFAS effects on calcium homeostasis, and 10 papers covering effects of PFAS on neurotransmitters. Since this is an emerging area, we did not perform a systematic review that restricted or characterized studies by quality, but rather tried to show all available evidence.

3. The absorption of PFAS in the brain

Several studies have mentioned barrier functions preventing PFAS from entering the brain.[17,28,37] Specifically, Harada et al. (2007) found that, compared to the transport of PFAS from the serum to the bile, the transport of PFAS from the serum to the CSF is relatively limited in patient samples. The substantial difference in PFOA and PFOS levels between the CSF and serum (the median concentrations of PFOA and PFOS in CSF samples: 0.06 and 0.10 ng/mL, in serum samples: 2.6 and 18.4 ng/mL) suggests that PFOA and PFOS may not cross the BBB freely and/or they are efficiently pumped out from the brain by transporters.[37] Similar findings, that brain-to-blood ratios of PFOA and PFOS are low in humans, based on post-mortem

examinations, were reported in the study of Maestri et al. (2006).[28] In these two early studies, the sample sizes were limited, and the PFAS analysis mainly focused on PFOA and PFOS. Therefore, the results may not necessarily represent the general population, and may not be generalizable to different PFAS given known differences in their toxicokinetics based on animal studies.[74,75]

In 2018, Wang et al. pointed out the barrier effect is one potential factor influencing the penetration of PFAS from the serum into CSF, since PFAS concentrations in CSF are 2 to 3 orders of magnitude lower than in the serum. In addition, they mentioned inflammation could increase the permeability of the brain barriers. Albumin CSF-to-serum ratios are strongly correlated with PFAS CSF-to-serum ratios, which may provide an alternative explanation to the barrier theory, since PFAS are known to bind to albumin in the blood. While their study had a relatively large sample size (223 serum-CSF pairs), and analyzed a broad array of PFAS, including PFCAs, PFSAs and emerging alternatives such as 8:2 Cl-PFESA and 6:2 Cl-PFESA (trade name: F-53B), Wang et al. (2018) noted that the results might not represent the general population, since the paired serum and CSF samples were collected from hospital patients in the Neurological Department. They also mentioned the bias that may come from using the PFAS content in the CSF to represent the PFAS level in the brain interstitial fluid.[17] Obviously, measuring chemicals within the brain interstitial fluid is challenging. Using the drug content of CSF as a surrogate for the drug content of brain interstitial fluid has been demonstrated as feasible, by showing the generated error is less than 3-fold.[76]

With regard to specific barrier functions, studies indicate different PFAS cross the BBB with varying efficiencies.[7,17] For instance, a pilot whale study by Dassuncao et al. (2019) suggested that certain long-chain PFAS, specifically, PFDoA, PFTrA, PFTeA and PFDS, may cross the BBB through a process related to the significantly higher phospholipid levels measured in the brain (though the specific mechanism was not evaluated), while short-chain PFAS may not penetrate the BBB effectively.[7] The current understanding of the potential pathways for chemicals to enter the brain includes (1) initiating BBB disassembly mainly through disrupting tight junctions, and (2) binding to transporters to complete transmembrane transport. However, although PFAS have been detected in the brain and CSF, the mechanisms by which PFAS enter and remain in the brain remain unclear. Understanding the possible mechanisms is critical, both for investigating strategies to block PFAS entering the brain so as to limit their adverse effects,

and for understanding how to select and design safer replacements for these chemicals. In this section, we review what is known about the uptake of PFAS in the brain and CSF.

Several studies have reported PFOS-induced endothelial discontinuity in the brain.[22,23,77] More specifically, PFOS may disrupt tight junctions in brain endothelial cells by triggering the PI3K/Akt signaling pathway. PI3K is a critical regulator of the permeability of endothelial cells. This signaling pathway has been demonstrated via *in vitro* experiments with the PI3K inhibitor, which blocks PFOS-induced endothelial disassembly.[77] In another *in vitro* human microvascular endothelial cell model, PFOS provokes the production of reactive oxygen species (ROS). The existence of ROS induces actin filament remodeling, which is directly associated with increased endothelial permeability.[22] Most recently, Yu et al. (2020) reported that PFOS can penetrate the BBB by disrupting the structure of tight junctions and/or decreasing the expression of tight junction proteins (e.g., Claudin-5 and Occludin). The disrupted tight junctions could then initiate BBB disassembly. Astrocyte hypertrophy and damage have also been found to exacerbate the disassembly of the BBB, as the interaction of endothelial cells and astrocytes is critical for regulating the BBB. PFOS disrupts these interactions and promotes the disruption of the BBB.[23] However, these studies only focused on PFOS. It is still unknown whether other PFAS enter the brain through disrupting the integrity of brain barriers.

The other potential pathway for PFAS entering the brain is by interacting with transport proteins. The traffic of many toxic substances across brain barriers relies on active transport mediated by transporters.[78] Various efflux and influx transporters are expressed at brain barriers, including P-gp, BCRP/Bcrp, MRPs/Mrps, OATPs/Oatps, OATs/Oats and OCTs/Octs, as illustrated in Figure 1.[20] While the transport of PFAS through transporters at the BBB has yet to be verified, previous studies related to the interaction of PFAS and similar transporters expressed in other tissues may provide useful insight. For example, several studies have indicated that PFAS renal clearance is mediated by OATs/Oats,[24,25] and PFAS renal reabsorption is moderated by Oatps.[26] In addition, previous *in vitro* research has investigated the impacts of PFAS on the P-gp transporter, which is one of the most studied efflux transporters at the BBB.[79] Specifically, PFOA and PFOS could significantly inhibit human P-gp, and this inhibition increased with PFAS dose in an *in vitro* experiment, while the interaction of P-gp with other compounds of low molecular weight (less than 300 Da) was not observed.[80] Another *in vitro* study on the marine mussel (*Mytilus californianus)* found that PFOA, PFNA, PFDA and PFHxS have inhibitory effects on P-gp in a

chain-length-dependent manner. That is, longer-chain PFAS caused more severe inhibition of P-gp than shorter-chain PFAS under the same PFAS exposure dose. The mechanism by which PFNA inhibits P-gp is indirect, which means PFNA disrupts the transporter function rather than competing for binding sites with P-gp substrates. But the inhibitory effect of PFNA and PFDA on P-gp is reversible, and exposure of P-gp to PFNA induces the synthesis of new P-gp transporters.[81] Furthermore, an *in vitro* experiment investigating the interaction of PFOA and PFOS with four types of transporters located at the blood-testis barrier[82] showed both PFOA and PFOS inhibited the activity of the BCRP, P-gp, MRP1 and MRP4, among which the BCRP transporter could transport PFOA as its substrate, while P-gp did not transport any of the PFAS analyzed.[83] This finding of P-gp is in line with the *in vitro* P-gp study by Stevenson et al. (2006) mentioned previously.[81] When the PFAS acts as an inhibitor of an efflux transporter, such as with P-gp, it reduces the ability of the transporter to effectively remove xenobiotics (including PFAS) from the tissue where it is expressed. Alternatively, when the PFAS acts as a substrate of a transporter, it could compete for binding sites with the normal substrates of the transporter, and thereby limit the transport of the normal substrates, as with the BCRP and PFOA mentioned here. If the transporter is an efflux transporter, then any PFAS that acts as a substrate will be eliminated as would an endogenous substrate.[84]

Fatty acid transporters are another potential PFAS transporter group. Greaves et al. (2013) first found a correlation between long-chain PFCAs (C10-C15) and nonpolar free fatty acids in the brain of polar bears. However, the method they used could not isolate the specific fatty acid types.[29] A recent study in pilot whales demonstrated that phospholipid (one type of fatty acid) concentrations were predictive of the distribution of long-chain PFAS (C12-C14 PFCAs and PFDS) in the brain.[7] Basically, the brain takes up the majority of its needed fatty acids from the blood. In order to enter the brain, long-chain fatty acids rely on transporters to cross the BBB.[85] Long-chain PFCAs (C10-C15) may have similar mechanisms to long-chain fatty acids to penetrate the BBB due to their similar structures.[29]

Based on the papers discussed in this section, the existence of cerebral barriers prevents xenobiotic chemicals from entering and accumulating in the CNS, but PFAS may enter the brain by initiating BBB disassembly mainly through disrupting tight junctions and/or by relying on transporters to complete transmembrane transport. PFAS are amphiphilic substances composed of a hydrophilic “head’ and a hydrophobic carbon-fluorine “tail”, leading to their ability to cross

the BBB. Based on quantitative structure-activity relationship (QSAR) modelling of the relationship between chemical structures and their ability to cross the BBB, molecular weight less than 400 to 600 Da, lipophilicity and protein binding affinity are major factors in CNS penetration.[86] Different PFAS may match this description: the "smaller molecular weight" that makes it easier to penetrate tight junctions falls within the molecular weight range of PFAAs with chain length between C4 and C11. Moreover, previous studies have emphasized that the hydrophobic interactions between fluorinated carbon "tails" and the binding pocket of proteins allow PFAS to be substrates of membrane transporters.[87] Compared to long-chain PFAS, short-chain PFAS (which are less hydrophobic) may enter the brain less effectively and/or may be efficiently pumped out from the brain by transporters. For long-chain PFCAs, especially C10-C15 PFCAs are likely to enter the brain through interacting with transporters.[7,29] However, the specific mechanisms of PFAS-transporter interactions in the brain are not well understood, and it is also possible that some PFAS may enter the brain through other as yet unidentified mechanism(s). Given the phaseout of many long-chain PFAS and the advent of emerging PFAS alternatives, it is necessary to extend the PFAS types being investigated with respect to uptake in the brain to understand the factors that regulate the absorption of PFAS in the brain. In addition, while *in vivo* and biomonitoring studies are limited due to the invasive nature of sampling the brain, other methods may be complementary to the current focus on *in vitro* experiments. For example, computational simulations are an increasingly powerful tool to provide us a better understanding of the uptake of xenobiotic chemicals[88] at the BBB that could be applied to PFAS.

4. The accumulation and distribution of PFAS in the brain

4.1 The accumulation of PFAS in collected brain samples

To understand the accumulation and distribution of PFAS in the CNS, we reviewed studies reporting PFAS concentration in human brains and CSF, and in the brains of wildlife species. Basic information related to the samples in these studies is listed in Table 2. The mean PFAS concentrations in the brains, blood and livers in these studies are in Table S2 in the SI).

Table 2 Studies of PFAS accumulation in collected samples.

Reference	Organisms	Species	n	Year	Sample collection area
Maestri et al., 2006[28]	human (autopsy)	-	7	-	Northern Italy

Pérez et al., 2013[8]	human (autopsy)	-	20	2008	Spain, Tarragona County
Greaves et al., 2012[39]	polar bear	*Ursus maritimus*	19	2006	East Greenland
Greaves et al., 2013[29]	polar bear	*Ursus maritimus*	19	2006	East Greenland
Pedersen et al., 2015[30]	polar bear	*Ursus maritimus*	9	2011 & 2012	East Greenland
Ahrens et al., 2009[31]	harbor seals	*Phoca vitulina*	4	2007	German Bight
Dassuncao et al., 2019[7]	pilot whale	*Globicephala melas*	7	2016	North Atlantic
Verreault et al, 2005[10]	glaucous gulls	*Larus hyperboreus*	7	2004	Svalbard & Bear Island, Norwegian Arctic
Olivero-Verbel et al., 2006[32]	pelicans	*Pelecanus occidentalis*	5	2004	Cartagena Bay
Rubarth et al., 2011[33]	Red-throated divers	-	4	2005	Usedom, Mecklenburg-West Pomerania, Germany
Gebbink & Letcher., 2012[34]	herring gulls	-	8	2020	Chantry Island, Lake Huron
Shi et al., 2012[35]	common carp	*Cyprinus carpio*	10	2009	Beijing, China (market)
	crucian carp	*Carassius auratus*	13		
	grass carp	*Ctenopharyngodon idellus*	10		
	bighead	*Aristichthys nobilis*	12		
	snakehead	*Ophicephalus argus*	8		
	tilapia	*Tilapia*	7		
Wang et al., 2016[36]	crucian carp	*Carassius carassius*	28	2014	Drainage systems of Beijing International Airport
Harada et al., 2007[37]	patients*	-	7	-	-
Wang et al., 2018[17]	in-patients*	-	223	2017 - 2018	Jiangsu Province, China

Note: - indicates that the information is not provided in the study; * indicates that CSF, not brain tissue, was sampled.

PFAS have been detected in the brain and CSF of humans. Based on these studies, PFAS content in human CSF is relatively lower than in human brain.[8,17,28,37] The mean concentrations of PFAS in these human samples show a consistent trend, namely that PFCA concentrations decrease with

their chain length.[8,17] Another study by Pérez et al. (2013) detected higher mean concentration of PFHxA in the brain of cadavers compared to other PFAS analyzed in their study[8], but this observation is not supported by the patterns we found in other studies for humans and wildlife. Furthermore, a recently published study suggests these observations may need to be taken with some caution due to potential for the analytical method employed and contamination to generate erroneous results for short-chain PFAS like perfluorobutanoic acid (PFBA).[89] In general the number and sample sizes of studies related to the distribution of PFAS in the human brain are limited, and some of these studies only focused on PFOA and PFOS.[28,37] Further, the experimental data in these studies are either from autopsy or hospital patients. It is therefore not clear whether PFAS distribution in these samples can represent the distribution in the general healthy population.

In addition to humans, PFAS have also been detected in the brain of various wildlife species.[7,10,29–36,39] The dominant detected PFAS are C6-C14 PFCAs, and C6, C8 and C10 PFSAs. The concentration of PFCAs with 6 to 11 carbons increases with chain length in the brain.[7,29–31,34,36] The concentration of PFCAs with 11 to 15 carbons shows a fluctuating trend wherein the concentration of PFCAs with an odd number of carbons are higher than those with an even number of carbons.[7,29,31,33] According to Greaves et al. (2013), the difference between odd and even chain length PFAS may indicate the presence of precursors of PFAS in biota, such as fluorotelomer alcohols (FTOHs), which degrade to both odd and even carbon chain length PFAS.[29] However, this could also be related to the PFAS source: electrochemical fluorination (ECF) and telomerization are the two primary methods of PFAS manufacturing, the former yielding both odd and even chain length PFAS, and the latter producing PFAS with an even number of carbons.[90] Thus, sources containing more ECF-derived PFAS would also have a higher proportion of odd chain-length PFAS. Among the PFSAs, PFOS is always dominant in the brain of wildlife,[10,29–31,33] likely due to the wide application of PFOS historically. Additionally, several studies also detected perfluorooctane sulfonamide (PFOSA), a PFOS precursor, in brain samples.[7,29,31,35] The existence of PFOSA could increase the content of PFOS in brains.

Among all the papers reviewed that were associated with PFAS distribution in the CNS, several of them reported paired blood (or serum) and brain PFAS concentrations and paired liver and brain PFAS concentrations (see SI, Table S2). The calculated PFAS brain-to-blood (or serum)

ratios in wildlife species increased with chain length, suggesting PFAS with longer chain length can enter or remain in the brain more easily.[29,31,33,34] This trend is consistent with the study of Wen et al. (2017) on zebrafish (*Danio rerio*) that long-chain PFAS can outcompete short-chain PFAS for transporters and binding positions, suggesting long-chain PFAS bind to transporters and are transported more effectively than short-chain PFAS.[91] This is in contrast with the more variable data reviewed for humans. This may be because we don't have enough human brain samples to see the trends, or because the mechanisms of accumulation and distribution of PFAS in humans and wildlife species are different, but the former is more likely.

4.2 Brain region-specific PFAS distribution

Among the studies found on PFAS distribution in collected brain samples, Greaves et al. (2013) and Pedersen et al. (2015) focused on the brain region-specific PFAS distribution in polar bears (*Ursus maritimus*).[29,30] Polar bears are the top predators in their food web, and therefore have higher exposure to bioaccumulative chemicals such as long-chain PFAS.[92] Polar bear samples in these two studies were collected in similar geographical locations in East Greenland, although at different times (see Table 2). Compared to the polar bears hunted in 2006, the mean concentration of PFCAs in the brains of polar bears collected from 2011 to 2012 increased, while the mean concentration of PFSAs decreased. This trend was also reflected in the dominant PFAS in each brain region. PFOS was the dominant PFAS in four of eight brain regions in 2006 harvested polar bears, but in only one of the brain regions of polar bears collected from 2011-2012 (Figure 2C & Figure 2D).[29,30] This decline likely results from the phase-out of PFOS production in the early 2000s, as was posited in the study by Rigét et al. (2013), who detected the annual average PFAS concentration in the liver of East Greenland polar bears from 1984 to 2011, and found liver PFOS content decreased since 2006.[93]

Long-chain C11-C15 PFCAs and PFOS are the major PFAS detected in polar bear brains (Figure 2A).[29,30] According to Smithwick et al. (2009) and Greaves et al. (2012), C9-C11 PFCAs and PFOS are the major PFAS in polar bear livers.[39,94] Compared to other tissues, the dominant PFAS in polar bear brains have longer chain lengths. Greaves et al. (2013) mentioned that the high concentration of longer-chain PFCAs may result from unique transport mechanisms into the brain. Their study was the first to explore the relationship between PFAS concentration and nonpolar free fatty acids content. They found a positive correlation between long-chain PFCAs

(mainly C11-C15 PFCAs) and lipid content. The brain is a lipid-rich tissue, providing a more nonpolar environment for the accumulation of long-chain PFCAs, which are more hydrophobic.[29]

In terms of the total PFAS content in each brain region, the brain stem, hippocampus, hypothalamus, pons/medulla and thalamus have higher PFAS content than other brain areas.[29,30] These regions are closer to the incoming bloodstream and receive the freshest blood, providing the PFAS in the blood opportunity to accumulate in these brain regions first.[29] The accumulation of PFAS in these brain regions may have implications for neurotoxicity, as we will discuss in section 5. Further studies are needed to explore the distribution and accumulation of a broad array of PFAS in the brain and connect them to the neurotoxicity of PFAS.

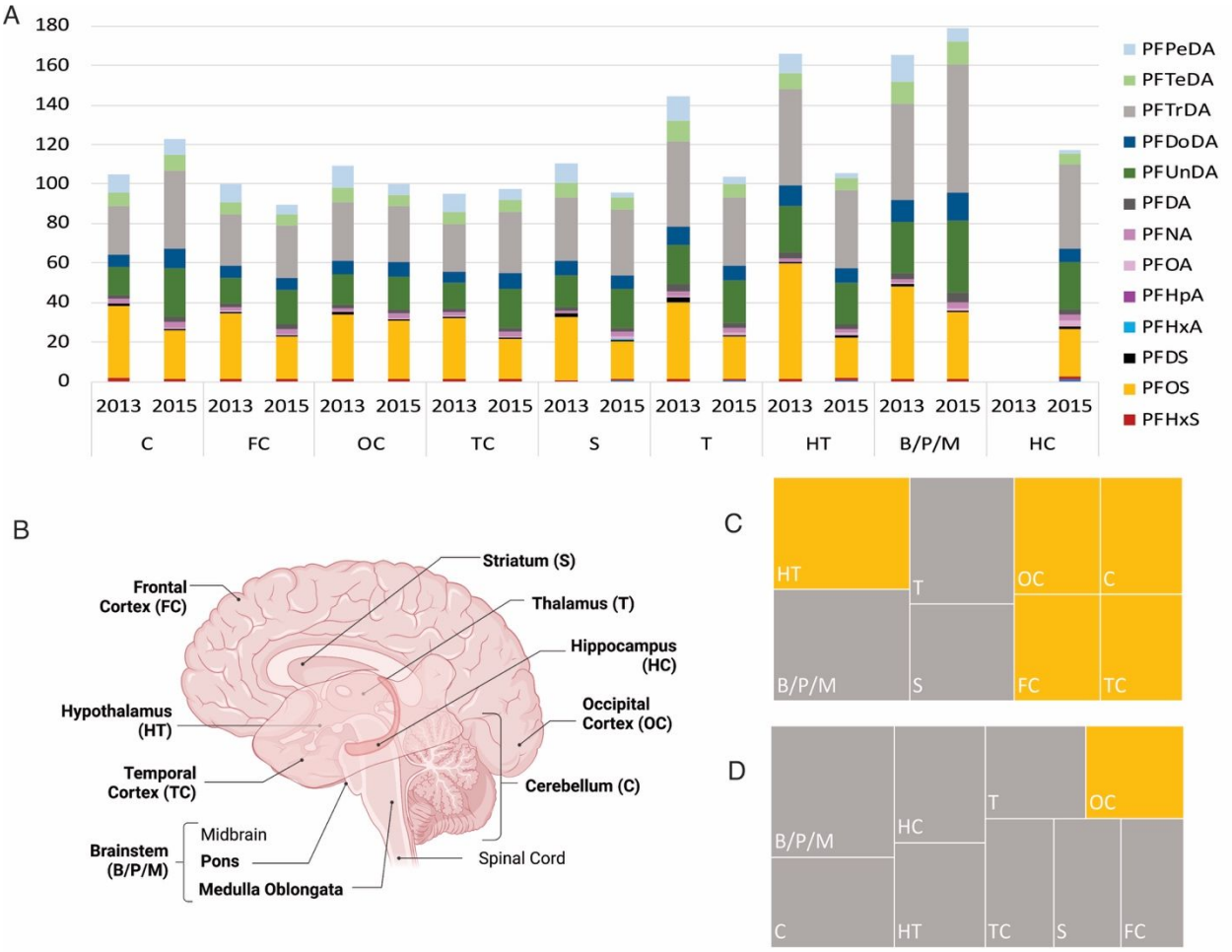

Figure 2 (A) Brain region-specific PFAS distribution in polar bears extracted from Greaves et al. (2013)[29] and Pedersen et al. (2015).[30] These two studies are denoted by 2013 and 2015, respectively, according to the year the study was published. The brain regions are represented by

abbreviations corresponding to the brain regions shown in (B). The size of the boxes in (C) for Greaves et al. (2013) and (D) for Pedersen et al. (2015) represent the total amount of PFAS in each brain region (corresponding to the total height of the columns in (A)), while the color represents the dominant PFAS in each brain region (orange: PFOS, grey: PFTrDA).

4.3 PFAS exposure experiments

Various short-term and long-term exposure experiments at a wide range of PFAS concentrations have been conducted on gilthead bream,[95] rainbow trout,[96] zebrafish,[97–99] carp[100,101] and rats[102–104] to investigate the accumulation and distribution of PFAS in the brain and other tissues. PFAS exposure time, dosage, chain length, functional groups and the age of the test organism have all been shown to affect the accumulation of PFAS in the brain (Table 3).

Table 3 Parameters of exposure experiments exploring the accumulation of PFAS in the brain.

References	Organism	Species	Sample size	Age	Sex	Reagents	Variables	Exposure dosage	Exposure time
Zabaleta et al., 2017[95]	gilthead bream	*Sparus aurata*	70	-	-	8:2 diPAP	exposure time	29 µg/g of diet	2, 4, & 7 days
Vidal et al., 2019[96]	rainbow trout	*Oncorhynchu smykiss*	200	15 months	F, M	PFHxS, PFOS	water temperature, PFAS type	500 µg/kg of water	80 days
Ulhaq et al., 2015[98]	zebrafish	*Danio rerio*	-	-	F, M	^{14}C-PFOA	sex, exposure time	10 µg/L of water; 0.3-30 µg/L of water	40 days
Li et al., 2017[97]	zebrafish	*Danio rerio*	300	fully mature	-	PFOS	exposure time, single-wall carbon nanotubes concentration	200 µg/L of water	24, 48, 72 & 96 hours
Wen et al., 2019[99]	zebrafish	*Danio rerio*	300	4 months	-	PFAAs	exposure time, PFAA chain length	10 µg/L of water	28 days
Giari et al., 2016[101]	common carp	*Cyprinus carpio*	31	2 years	F, M	PFOA	PFOA concentration	200 ng/L of water & 2mg/L of water	56 days
Dong et al., 2019[100]	crucian carp	*Carassius auratus*	150	half-year old	M	PFOA	PFOS concentration	0.2-25000 µg/L of water	7 days
Cui et al., 2009[102]	rat	*Rattus norvegicus*	50	2 months	M	PFOA, PFOS	exposure time	5 & 20 mg/kg of body weight/day	28 days
Liu et al, 2009[104]	rat	-	-	pups	F, M	PFOS	sex, postnatal age	5 mg/ml of subcutaneous injection	24 hours

Gao et al., 2015[103]	rat	*Rattus norvegicus*	40	8 weeks	F, M	PFOA, PFNA, PFOS	sex, PFAS type	0.05-5 mg/L of drinking water	90 days

Note: - indicates that the information is not provided in the study.

In terms of PFAS dosage, some studies reported a positive relationship with PFAS brain accumulation.[100,101] Giari et al. (2016) exposed common carp (*Cyprinus carpio*) to 200 ng/L and 2mg/L PFOA, respectively, for 56 days. They found PFOA concentration in carp brain is lower than the limit of detection (0.4 ng/g wet weight) at 200 ng/L exposure, while they detected PFOA accumulation in brain samples at 2mg/L exposure with the mean concentration of 0.45 ng/g wet weight.[101] Dong et al. (2019) also did not detect PFOA in the crucian carp (*Carassius auratus*) brain at 0.2 μg/L exposure at the seventh exposure day.[100] However, another study on rats (*Rattus norvegicus*) found no obvious difference in PFOA concentration in the rat brain after 28 days of exposure to either 5 mg/kg/day or 20 mg/kg/day PFOA, indicating the saturation of PFOA-protein binding sites at low exposure concentration. PFOA could bind to various proteins in the brain, but increased PFOA elimination through urine or feces will occur when binding sites are saturated. This study also tested the accumulation of PFOS in the brain and found high level of PFOS in the rat brain (146 μg/g) at 20 mg/kg/day PFOS exposure, while the increase in PFOS bioconcentration was not proportional to the increase in PFOS exposure concentration. Cui et al. (2009) suggested that higher PFOS concentration may cause more serious impacts on the integrity of the BBB, leading to more PFOS penetration into the brain. In addition, the concentration of PFOS in the brain is higher than that of PFOA under the same exposure dose and time, indicating the elimination rate of PFOS might be lower than that of PFOA.[102]

The different findings for PFOA and PFOS might result from their different acid functional groups (carboxylate vs. sulfonate) or the presence of an additional fluorinated carbon in PFOS. The study by Wen et al. (2019) pointed out that PFAA accumulation in the zebrafish brain is associated with PFAA chain length and functional group. Specifically, the accumulation of PFAAs in the brain increases with the perfluorinated carbon chain length. This trend may be due to the greater hydrophobic forces that enhance longer chain PFAA binding to proteins.[99] In addition, according to Wen et al. (2017) longer-chain PFAAs might compete for protein binding sites and transporters with shorter-chain PFAAs so as to lead to the observed differences in their bioconcentration potentials.[91] With regard to functional group, compared to PFCAs, PFSAs with

the same perfluorinated carbon chain length are more accumulative in zebrafish brain, since more hydrogen bonds can be formed between amino acid residues and the sulfonate functional group than with the carboxylate functional group.[99]

In addition to PFAS dosage and functional groups, differences among individuals in PFAS exposure experiments could also affect the results of PFAS accumulation in the brain. For example, mice at different postnatal ages were exposed at the same dosage of PFOS (50 mg/kg body weight). Liu et al. (2009) found higher level of PFOS in younger mice after the same PFOS exposure, suggesting the development of the BBB function with age provides added protection from xenobiotic accumulation.[104] However, no obvious sex differences in PFAS brain accumulation has been reported in these PFAS exposure studies.

Exposure experiments with PFAS precursors have also been conducted. For example, the study by Zabaleta et al. (2017) explored the exposure of gilthead bream (*Sparus aurata)* to 29 μg/g 8:2 polyfluoroalkyl phosphate diester (8:2 diPAP), which is a precursor of PFOA, and detected a high level of PFOA (mean concentration 3.7 ng/g) in their brain after 7 days exposure. However, further studies on the accumulation of PFAS precursors in the brain are needed to investigate whether some PFAS precursors may be more toxic than their degradation products,[105] and to improve the understanding of emerging PFAS.

In nature, organisms are exposed to various chemical contaminants through multiple pathways. The presence of other substances may affect the bioaccumulation and distribution of PFAS in organisms. An *in vivo* study on the impacts of single-walled carbon nanotubes on the bioaccumulation of PFOS in zebrafish tissues found the bioaccumulation of PFAS declines with the increase of nanotube dose, because the adsorption of PFOS to the carbon nanotubes reduces the bioavailability of PFOS to zebrafish.[97] This suggests various other environmental contaminants could impact the bioaccumulation of PFAS in organisms, but our understanding of this field is still not well-established.

Experiments associated with PFAS accumulation and distribution in the brain should be done with particular care. Various studies used aquatic organisms to explore the accumulation and distribution of PFAS in the brain. As mentioned by Vidal et al. (2019) water temperature is critical in the design of studies on aquatic organisms since the distribution and accumulation of PFAS may be affected by metabolic rates, which in aquatic organisms is often closely tied to

water temperature. Specifically, they found the brain-to-blood ratios of PFOS increases with water temperature in rainbow trout (*Oncorhynchus mykiss*).[96] Indeed, ectotherms are very sensitive to temperature, which affects their rates of respiration, consumption, and growth and thereby affect most key toxicokinetic parameters.[106] Ulhaq et al. (2015) mentioned that the brain is a tissue with complex blood vessels, leading to the mixing of PFAS in the blood with PFAS in the brain during experiments, which could also affect the interpretation of brain data.[98] In order to reduce invasive experiments, studies have proposed alternative non-invasive biomonitoring methods to measure internal PFAS exposure. For example, Gao et al. (2015) used hair as an indicator of PFAA exposure, indicating the correlation between average concentrations of PFAAs in hair and brain can reach up to 0.86 or more for PFNA and PFOS.[103] However, studies of using hair as a biomarker of PFAS exposure are still quite limited, and results vary by PFAS types,[103,110] subject population[110] and gender[103]. More studies are needed to explore whether hair can be a reliable biomarker for PFAS exposure by testing different PFAS in more species and optimizing the analytical methods for PFAS detection and quantification in the hair. Taken together, differences in analytical techniques in different studies and challenges associated with experiments may impact our ability to compare results across studies.

5. The potential neurotoxicity of PFAS

The studies reviewed in the previous sections demonstrate that PFAS accumulate in and distribute through the brain, which highlights the importance to better understand the toxicity of PFAS in the CNS.[29,30] In this review, we surveyed 13 studies related to the associations of PFAS exposure with behavioral and cognitive disorders, mainly including attention deficit hyperactivity disorder (ADHD), fetal congenital cerebral palsy, learning disorders, memory dysfunction, and intellectual disability.[40–52]

In addition, various *in vitro* PFAS exposure experiments have been conducted, mainly on hippocampal neurons, to further explore the mechanisms of PFAS toxicity in the brain. Hippocampal neurons are promising subjects, since the hippocampus is one of the dominant brain areas for PFAS accumulation as discussed above. Also, the hippocampus is related to learning and memory. Here we reviewed 21 papers related to the two most studied potential mechanisms of PFAS neurotoxicity: (1) PFAS-induced intracellular calcium alteration in neurons,[53–63] and (2) the impacts of PFAS on neurotransmitters.[64–73]

5.1 Associations of PFAS exposure with behavioral and cognitive disorders

PFAS have been identified as potential neurobehavioral toxicants, such as inducing behavioral disorders. Multiple studies have explored the prevalence of PFAS exposure and ADHD, but conflicting results exist in these studies, including positive, negative and no associations.[40–45] Specifically, a Norwegian birth cohort study with 1199 mother-child pairs found that higher PFOS concentration in breast milk (collected before infants reached 2 months) increased the odds of ADHD in children (around 13 years old; odd ratio = 1.77, 95% confidence interval: 1.16, 2.72). The positive association between early-life PFOS exposure and ADHD was sex-specific, showing stronger association in girls than boys.[40] Negative associations of PFAS prenatal exposure with ADHD were also reported in multiple studies. For example, A questionnaire-based study with 282 subjects found prenatal PFNA exposure was negatively related to ADHD in 7-year-old children.[41] The study by Stein and Savitz (2011) reported the negative prevalence of PFOA exposure and ADHD in 5-to-18-year-old children living in areas where drinking water was contaminated by PFOA.[42] Stein et al. (2014) indicated that the negative association might be because PFOA could slightly activate peroxisome proliferator-activated receptor (PPAR) gamma, acting like PPAR-gamma agonists, which harbors neuroprotective and anti-inflammatory functions. Similar functions of activating PPAR-gamma between PFOA and PPAR-gamma agonists suggests PFOA might also have neuroprotective function.[43] This explanation of negative association between PFOA exposure and ADHD might be extended to other PFCAs due to their similar structures. In addition, no significant association was found between prenatal PFAS exposure and parent-reported ADHD in 18-month-old children, but the sample size (n= 59) was small in that study.[44] Another study with 4826 mother-child pairs also did not find the prevalence of PFOS and PFOA prenatal exposure and ADHD (odds ratios ranging from 0.96 to 1.02), but in their stratified analyses, increased association of PFAS exposure and ADHD were found in female infants, and in infants from nulliparous or low-educated mothers. The sex-dependent results might result from different endocrine-disrupting effects of PFAS on estrogen, that thereby cause different impacts on males and females.[45] Besides these prenatal PFAS exposure studies, researchers also explored the relationship between PFAS levels in children's blood and their ADHD symptoms. For example, the study by Stein et al. (2014) found sex-specific prevalence of serum PFOA content and ADHD in 6-to-12-year-old children. That is, serum PFOA level was positively associated with ADHD in boys, but negatively in girls.[43] In addition to ADHD, Liew

et al. (2014) conducted a case-cohort study and found higher concentrations of PFOA, PFOS and PFHpS in maternal plasma could increase the risk of cerebral palsy only in male infants, which might result from the limited sample size of female infants and/or the existence of the sex-related mechanisms, which need to be further explored.[46] Evidence is mixed in these human data, indicating further replications is needed to better understand the associations of PFAS exposure with human behavioral disorders.

According to animal exposure experiments, both short-chain and long-chain PFAS could induce cognitive disorders.[47–49] The neonatal exposure of mice to PFHxS affected cognitive function in a long-lasting or even persistent manner.[47] PFDoA decreased the ability of adult rats to recognize novel objects in a dose-dependent manner; the cognitive deficit became more severe as PFDoA concentration increased in the brain.[48] Another PFOS exposure study indicated that both prenatal and postnatal PFOS exposure decreased the spatial learning and memory abilities in rat offspring, and the reduction induced by prenatal PFOS exposure was more severe.[49] In addition, PFAS-induced cognitive deficits have also been reported in humans.[50–52] In the study by Skogheim et al. (2020) with 944 mother-child samples, the PFAS concentration in maternal plasma was used to represent child prenatal PFAS exposure. They observed weak negative associations between non-verbal working memory in preschool children and their prenatal exposure to PFAS, including PFOS, PFOA and PFHpS; and weak positive prevalence of verbal working memory in preschool children and their prenatal exposure to PFAS, including PFNA, PFDA and PFUnDA.[50] Similarly, positive association between higher PFAS serum concentrations and cognition limitations (self-reported difficulty remembering) in 1766 adults between 60-85 years old was reported by Power et al. (2013).[51] However, Vuong et al. (2019) did not observe significant associations between either prenatal or childhood PFOS and PFHxS exposure and the alteration of cognitive functions based on the Full Scale Intelligence Quotient (FSIQ) measurement of 8-year-old children. After stratified analyses, they found positive associations between prenatal PFOA exposure and higher IQ in females, and between childhood PFOS exposure and higher IQ in males.[52] Their findings may reflect some other indicators, rather than a causal relationship.

To sum up, we found conflicting results in these studies focusing on the association of PFAS exposure with behavior and cognitive disorders, especially in humans. This is probably because these studies have diverse sample sizes, and/or diverse experimental subjects with different ages, living areas and health conditions. It is also worth noting that most of the studies related to

ADHD use parentally-reported symptoms, which might compromise the accuracy of the results. Several studies indicated sex-specific associations in stratified analyses, but it is difficult to determine the mechanism underlying these associations since the number of existing studies and sample sizes are limited and their results are inconsistent. Further studies are needed in this field to validate these findings. It is worth further exploring the mechanisms underlying such PFAS-induced neurotoxicity. In the following sections, we reviewed two potential mechanisms of PFAS neurotoxicity.

5.2 Effects on calcium homeostasis and calcium-dependent signaling molecules

Calcium (Ca^{2+}) is responsible for mediating multiple neuronal processes, such as proliferation, synaptogenesis, apoptosis, and neurotransmitter secretion.[112,113] Various PFAS exposure studies have reported effects of PFAS on calcium homeostasis in neurons, which is considered to be one of the potential mechanisms of PFAS neurotoxicity.[53–57] The PFAS-induced calcium increase in neurons is either from extracellular calcium influx or calcium store release (Figure 3).[113] Liao et al. (2008) found PFOS could induce the influx of extracellular calcium through L-type voltage-dependent calcium channels (L-VDCCs) in rat hippocampal neurons.[53] Another study by Liu et al. (2011) found both PFOA and PFOS could significantly increase the calcium concentration in cultured rat hippocampal neurons. The increased calcium was mainly released from intracellular calcium storage organs such as mitochondria and the endoplasmic reticulum (ER),[112] and mediated by inositol 1,4,5-trisphosphate receptors (IP_3Rs) and ryanodine receptors (RyRs) at the surface of calcium stores.[54] Studies have linked calcium overload to neuron dysfunction and even to cell apoptosis.[53,54] Specifically, after acute exposure of hippocampal neurons and brain slices to PFOS, the increased intracellular calcium potentiated synaptic transmission, which represents the communications between neurons. In addition, PFOS-induced intracellular calcium overload also provoked neuronal excitement, which could lead to neuronal injury. In terms of long-term implications, the exposure to PFOS affected the normal structure and functions of neurons.[53] A further study by Liao et al. in 2009 pointed out that the effects of PFAS on rat hippocampal neurons depend on the chain-length, the degree of fluorination and functional groups of PFAS. Specifically, the disturbance of neuronal activities by PFAS increased with the fluorinated carbon chain length and the fluorination level. Compared with perfluorinated carboxylates, perfluorinated sulfonates had stronger effects on neurons.[58] Additionally, Liu et al. (2011) also observed the increase of ROS in calcium-overloaded neurons. ROS could induce oxidative stress

events, which may eventually lead to cell death.[54] Furthermore, Dusze et al. (2018) pointed out the rise of calcium release depends on age, since they found exposure to PFOS increased the calcium release in brain microsomes in adult rats, but not in neonatal rats.[56]

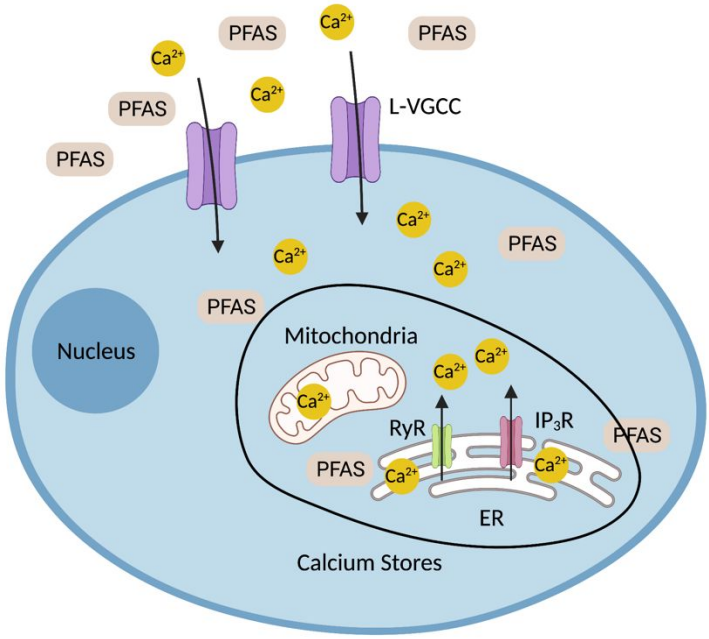

Figure 3 Proposed mechanisms of PFAS-induced intracellular calcium increase, based on extracellular calcium influx and/or calcium store release. The extracellular calcium influx is mediated by L-VGSSs at the surface of neurons. Intracellular PFAS could induce the release of calcium from intracellular calcium storage organs such as mitochondria and the ER. Intracellular calcium release is mediated by IP_3Rs and RyRs at the surface of calcium stores.

Studies have also reported PFAS-induced alteration of calcium-dependent signaling molecules, a potential molecular mechanism of PFAS-induced neurotoxicity since these molecules are critical for determining the structure and functions of neurons.[54,57,59–61] Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), cAMP-response element binding protein (CREB) and calcineurin are critical calcium signaling down-stream molecules.[54,59] CaMKII participates in synaptogenesis and plays important roles in learning and memory.[59,61] CREB is critical in neuronal growth and the formation of long-term memory.[114] Calcineurin is important for neuron survival and cognition.[54] PFOS was shown to increase the expression of CaMKIIα and phosphorylated CREB in adult male rat cortex and hippocampus.[59] The expression of calcineurin (CaM) significantly increased in both PFOA and PFOS treated rat hippocampal neurons.[54] Furthermore, to probe the

developmental neurotoxicity of PFAS, studies investigated various calcium-dependent signaling molecules in different developmental stages of mice after PFAS exposure.[60,61] Liu et al. (2010a) detected that the expression of N-methyl- D-aspartate receptor subtype-2B (NR2B), CaM, CaMKIIα and CERB changed both under prenatal and postnatal PFOS exposure in mice. NR2B expression is related to learning ability and memory; CaM can respond to calcium concentration changes, which has been detected in a PFAS exposure study we mentioned previously;[54] the change of CaM will further impact its downstream molecule CaMKIIα; and CERB is related to neuronal growth. They suggested the relationship between the alterations of the expression of these calcium-related signaling molecules and cognitive deficits. Based on their results, PFOS could reach the brain at the embryo stage, and further induce adverse effects to the CNS postnatally.[60]

In addition to these molecules, PFOS and PFOA were also shown to increase the level of growth-associated protein-43 (GAP-43), synaptophysin and tau in mouse hippocampus and cerebral cortex after neonatal exposure. These proteins play important roles in synaptogenesis, neuronal development and growth. The neonatal stage is a critical brain development period, and PFAS-induced overexpression of these proteins at the neonatal stage affects the healthy development of the mouse brain.[61] Finally, PFNA could also induce increased intracellular calcium concentration and CaMKII expression in rat pheochromocytoma-12 (PC12) cells. These alterations could result in oxidative stress in cells and ultimately lead to cell apoptosis.[57] This observation is in line with Wang et al. (2015), who found prenatal and postnatal PFOS exposure could increase hippocampal neuron apoptosis in rat offspring. The increase of apoptosis is in a similar manner to the calcium increase in neurons, suggesting the rise of intracellular calcium is one of the potential mechanisms of neuron apoptosis. Specifically, Wang et al. (2015) indicated that PFOS-induced calcium disturbance in neurons injured calcium signaling pathways, then induced neuronal apoptosis, and eventually could cause behavioral deficits, such as ADHD and response inhibition.[55] However, studies also found approaches to reduce PFAS-induced neuronal dysfunctions. For example, Oh et al. (2018) pointed out phycoerythrin-derived peptide of *Pyropia yezoensis* (PYP) could alleviate PFOS-induced calcium disorder.[62] A recent study by Zhang et al. (2020) found blueberry anthocyanins (ANT) could reduce PFOA-induced neurotoxicity in *Dugesia japonica* in terms of locomotion reduction, oxidative stress and neurotransmitter dysregulation.[63] These studies provide insights for alleviating PFAS-induced

neuronal toxic effects, but the mechanisms underlying these protective strategies remain to be explored.

5.3 Effects on neurotransmitters

The second most studied potential mechanism of PFAS neurotoxicity is neurotransmitter dysfunction. Neurotransmitters are chemicals generated by neurons that are responsible for signal transmission.[115] Neurotransmitter levels in the brain are related to the activation of neurons and signal transmission among neurons.[64] Studies have reported the implications of PFAS on neurotransmitters in the brain, mainly dopamine,[64–70] glutamate,[64,66,68,70–72] acetylcholine and the cholinergic system.[66,68,69,73]

According to various exposure experiments, PFOS and PFOA could alter dopamine concentration in the brains of rat, mouse and frog, but the direction of the alteration was not consistent across studies.[64–66] Yu et al. (2016) applied a high-throughput targeted metabolomics approach to analyze the PFOA-induced neurotoxicity in male mice, and found the increase of dopamine concentrations in 0.5 mg PFOA/kg body weight/day exposure group.[64] In terms of different brain regions, PFOS increased the dopamine concentration in the prefrontal cortex and hippocampus in adult mice after 28 days PFOS exposure, but the alteration of dopamine content in amygdala was not significant.[65] However, another PFAS exposure study on Northern leopard frog (*Lithobates pipiens*) found PFOS and PFOA decreased dopamine in the brain. In addition, this study suggested long-term developmental PFAS exposure could reduce the amount of dopaminergic neurons. Leopard frogs can be more relevant for the study of these neurons compared to rodents, since leopard frogs have neuromelanin-containing dopaminergic neurons, similar to those affected by Parkinson's disease in humans. Therefore, Foguth et al. (2019) considered that further studies on frogs are needed to explore the relationship between PFAS-induced dopamine alteration and Parkinson's disease.[66] In addition to monitoring dopamine content in the brain, detecting alterations of the gene expression of dopamine receptors further helps to explore the potential molecular mechanism of PFAS neurotoxicity.[65,67] To understand the effects of PFOS on the development of CNS, neonatal mice were exposed to PFOS during development. After 24 hours of PFOS exposure, the transcription of dopamine receptor-D5 decreased in mouse cerebral cortex. At 2 months post exposure, the transcription of dopamine receptor-D2 was reduced in mouse hippocampus.[67] Similar findings have been reported in the

study by Salgado et al. (2016), namely the gene and protein expression of D1 and D2 receptors in rat prefrontal cortex and hippocampus changed after exposure to PFOS.[65] Both D1 and D2 receptors play important roles in cognition and memory. Another study by Hallgren and Viberg (2016) considered the decreased transcription of dopamine receptorr-D2 in hippocampus may be related to the cognition disorder in adult mice. However, they did not explain the reduced D5 receptor in cerebral cortex due to the lack of developmental roles of D5 receptor in cerebral cortex among literature studies.[67]

In addition to decreasing dopamine, Long et al. (2013) found the exposure of adult mice to PFOS could increase hippocampal glutamate, which is another critical neurotransmitter related to learning and memory.[70] Additionally, another study found the glutamate concentration in the brain decreased after exposing mice to 2.5 mg PFOA/kg body weight/day for 28 days.[64] Similar results have been reported by Foguth et al. (2020), who included both PFOS alone (10 ppb) and PFAS mixture (4 ppb PFOS, 3 ppb PFHxS, 1.25 ppb PFOA, 1.25 ppb PFHxA and 0.5 ppb PFPeA) exposure groups in their study and found both of these exposures resulted in significantly decreased glutamate concentrations in the brains of Northern leopard frogs in a similar degree.[68] The low glutamate level in the brain could cause adverse effects to synaptic plasticity and memory.[116] Furthermore, an *in vitro* study on rat cerebellar granule neurons mentioned that PFOS and PFOA increased glutamate concentration, and in turn induced glutamate excitotoxicity, which means excessive glutamate leads to excessive stimulation of its receptors, and even to cell injury and eventually death.[71,117] However, the degree of excitotoxicity induced by PFOS and PFOA were different, which the authors suggest may be due to the different mechanisms of neurotoxicity caused by PFOS and PFOA. In addition, the glutamate excitotoxicity also varied from the developmental stages of cultured neurons.[71] Liao et al. (2009) found PFOS ranging from 0.1 to 100 μM altered the glutamate-activated current in rat hippocampal neurons.[72] However, the exposure of leopard frog to PFOA and PFOS did not significantly change the glutamate level in the brain.[66]

The study by Foguth et al. (2020) detected the alteration of diverse neurotransmitters in Northern leopard frogs exposed to PFAS, among which, they found PFAS could alter these tested neurotransmitters, especially acetylcholine. Specifically, PFOS and the PFAS mixture described above significantly increased acetylcholine level in the later developmental stage of frogs, but

the mechanism behind this acetylcholine rise was not clear.[68] In addition, the exposure of neonatal mice to PFOS and PFOA damaged the adult cholinergic system, even at low PFAS exposure dose (1.4 mmol/kg body weight).[73] However, Foguth et al. (2019) did not observe significant change of acetylcholine levels in leopard frog brain after PFOA and PFOS exposure.[66] Based on these observations, the effects of PFAS on neurotransmitters are complex. As Slotkin et al. (2008) mentioned, the mechanism of PFAS impacts on neurotransmitters vary by PFAS types.[69] In addition to PFAS type, the alterations of PFAS to neurotransmitters may also depend on PFAS exposure time and dosage, animal species, and brain/neuro-developmental stages, and these factors are important to consider when comparing across studies.

To sum up, PFAS-induced intracellular calcium alteration in neurons and the impacts of PFAS on neurotransmitters are two major potential mechanisms of PFAS neurotoxicity. There is also a potential link between the effects of PFAS on calcium homeostasis and its effects on neurotransmitters because it is known that the increase of intracellular calcium can trigger neurotransmitter secretion.[54] With respect to all of these PFAS neurotoxicity studies, the majority of them focus on PFOS and PFOA, but researchers found long-chain PFAS can enter the brain more effectively than short-chain PFAS, as was also highlighted in the preceding sections on PFAS absorption, accumulation, and distribution in the brain. Although long-chain PFAS have been phased out and been replaced by diverse emerging PFAS, they are still present in tissues and the environment. Information on the neurotoxicity of long-chain and emerging PFAS is still lacking. In addition, many studies have mentioned the different adverse effects resulting from diverse PFAS types, but it is necessary to further explore the specific mechanisms behind observed differences.[54,69] This review has focused primarily on observations of direct effects of PFAS on the brain and associated outcomes, but there are additional, potentially important, indirect impacts of PFAS, for example through disruption of thyroid hormone function in pregnant women which could affect neurodevelopment of the fetus.[118–120] Such indirect effects also merit further consideration. Furthermore, more studies are needed to explore PFAS neurotoxicity at the molecular level. Currently, it is still difficult to connect the potential neurotoxic mechanisms to specific brain diseases. Taken together, critical data gaps remain not only for neurotoxicity, but also in the whole field of PFAS toxicology. Exploring these toxicological issues faces similar dilemmas: the existence of thousands of untested PFAS, and the lack of the quantification of the potential health effects associated with PFAS exposure. Data

and tools are needed to establish the link between PFAS exposure and toxicity. The framework of adverse outcome pathways (AOPs), for example, which identify the specific molecular events required to cause a toxic effect, could help to analyze the risk of more PFAS more accurately with fewer resources, since *in vitro* experiments and *in silico* approaches can be alternatives to *in vivo* studies to test and screen molecular events linked to specific toxic effects.[121]

5. Conclusion

Research on PFAS absorption, accumulation, distribution and toxicity in the brain is increasing, but many critical gaps remain. PFAS may enter the brain through initiating BBB disassembly and/or relying on transporters located at the BBB, but diverse PFAS with different chain-length and functional groups have different abilities to enter the brain. Future studies are needed to specify the mechanism of each PFAS entering the brain, and how the uptake efficiencies are affected by differences in PFAS structure and properties. After entering the brain, PFAS have the potential to distribute to and accumulate in different areas of the brain. The available studies related to PFAS distribution in various brain regions are quite limited, as are PFAS accumulation data in human brains. Indeed, the experiment on the brain is invasive and should be done with particular care since the brain is a vulnerable tissue with complex blood vessels. As a result, to reduce the invasive experiments and to make the PFAS-related brain studies more accessible, it will be helpful to find surrogates (such as the CSF and hair) that can represent PFAS concentration in the brain. In addition to *in vivo* methods, 3-D tri-culture models have been used in the brain for drug screening and disease modeling in the brain.[122] Although this technology has not yet been used in the study of PFAS, it is a potentially powerful path forward to explore the absorption, accumulation and effects of PFAS in the brain with an in vitro system that more closely mimics in vivo activity. Computational methods may likewise be useful alternatives or complements to experiments to explore PFAS toxicokinetics in the brain. More studies are needed to explore the characteristics of the accumulation of PFAS in the brain and the brain region-specific PFAS distribution. These studies help to understand the specific toxic effects to the CNS induced by PFAS since the brain is composed of various regions which are responsible for mediating different functions, such as learning, memory, emotions and movement. In this review, we summarized PFAS-induced toxic effects including behavioral and cognitive deficits. Although learning and memory disorders have been observed in studies, the link between PFAS exposure to specific diseases such as Alzheimer disease and Parkinson's disease remains to be

explored. Two primary mechanisms of PFAS-induced neurotoxicity have been proposed: disrupting calcium homeostasis and the alteration of neurotransmitters. However, the existence of disconnects across studies on the toxicity of PFAS in the brain and those on potential neurotoxicity mechanisms makes interpretation difficult. For example, studies related to the prevalence of prenatal/postnatal PFAS exposure and behavioral and cognitive disorders and *in vitro* studies exploring mechanisms of PFAS neurotoxicity do not evaluate consistent PFAS type, exposure concentrations or model organisms. Finally, to understand PFAS in the brain more comprehensively, we expect future studies to be better aligned between the accumulation of PFAS and PFAS toxicity in the brain. Currently, PFOA and PFOS are overrepresented in the literature. Given that other PFAS have been shown to accumulate in the brain, and the general lack of studies on emerging PFAS, it is important to identify the neurotoxicity of these other important environmental contaminants.

In this review, we show the existing evidence from multiple perspectives (epidemiological, in vivo, and in vitro) that PFAS do enter and accumulate in the brain, and they may have an effect. The importance of addressing gaps in our understanding is that there are potentially thousands of PFAS (with at least hundreds in active use) that haven't been tested: the lack of toxicity of some of them does not mean that the others will be safe. It is important to determine whether "emerging" or replacement PFAS may have more profound neurological effects than others, and to connect the understanding of the absorption, distribution and toxicity of PFAS in the brain.

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Conflicts of interest

The authors declare no competing financial interest.

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