Perfluorododecanoic acid induces cognitive deficit in adult rats

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ABSTRACT

The brain level of perfluorododecanoic acid (PFDoA) was compared with those of perfluorooctanoic acid (PFOA) and perfluorodecanoic acid (PFDA) in rats 9 days after a single oral dose (50 mg/kg). The PFDoA level in the brain was 44.0 ± 2.0 [mu]g/g, which was higher than that in the serum (24.4 ± 1.0 [mu]g/ml). By contrast, the concentrations of PFOA and PFDA in the brain were low (<0.8 and 4.7 ± 0.4 [mu]g/g, respectively), and less than one-tenth of those in the serum. Next, to investigate the effects on brain function, the cognitive function alterations of PFOA, PFDA, and PFDoA were estimated by the novel object recognition test 5-6 days after dosing. A significant decrease in the discrimination index was observed in PFDoA-treated rats while no significant alteration was observed in PFDA- and PFOA-treated rats. The effects of PFDoA were further assessed by other behavioral tests. PFDoA-associated alteration was observed in the elevated-plus maze test, but not in the Y-maze test, open-field test, and forced swim test. A decrease in the discrimination index of the novel object recognition test was dependent on the PFDoA dose and the PFDoA concentration in the brain. PFDoA concentration in the brain was 28.6 ± 2.6 [mu]g/g 30 days after dosing, and a decrease in discrimination index was observed. Taken together, these results suggest that PFDoA distributes in the brain easier than PFOA and PFDA and causes cognitive deficit.

Key words: perfluorododecanoic acid, brain, cognitive deficit

INTRODUCTION

Perfluoroalkyl substances, including perfluoroalkyl acids (PFAAs) such as perfluorocarboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids, have been manufactured and used for a variety of industrial applications and consumers products, such as surfactants in fire-fighting foam and cleaning agents, protective coatings for textiles, carpets, and electronic and photographic devices, since the 1950s (Buck *et al.*, 2011; Renner 2001). In addition to direct emission through manufacturing process, PFAAs are emitted to the environment as terminal products of the degradation of precursor materials such as perfluoroalkyl sulfonamides, perfluoroalkyl telomer alcohols, and fluoropolymers (Prevedouros *et al.*, 2006; Wang *et al.*, 2013; Wang *et al.*, 2014). PFAAs are widely spread and found in various environmental media, such as air, water, soil, sediment, and sludge from wastewater treatment plants (Kim and Kannan, 2007; Lau *et al.*, 2007; Yamashita *et al.*, 2005), since PFAAs are highly stable and resistant to biodegradation (Key *et al.*, 1997). In 2001, Giesy and Kannan (2001) reported that PFAAs were present in wild life. To date, numerous studies have reported that PFAAs bioaccumulate in a wide variety of wild life, not only in industrialized countries but also in the Arctic and the Antarctic Oceans (Reiner and Place, 2015). In addition, human biomonitoring studies have shown that the general populations in several countries have been exposed to PFAAs (Lau *et al.*, 2007) and that perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexanesulfonic acid, and perfluorononanoic acid have been routinely detected, with PFOS levels being highest (Kato *et al.*, 2011).

Perfluorododecanoic acid (PFDoA), which has chain lengths of 12 carbons, has been reported to be found in human serum (Goralczyk *et al.*, 2015); thus, humans are exposed to PFDoA (Gebbink *et al.*, 2015). The studies on the toxicity of PFCAs with carbon chain lengths longer than 10 are limited (Kato *et al.*, 2015; Liu *et al.*, 2016); therefore, it is necessary to estimate the toxicity of PFDoA. In general, PFCAs are thought to distribute predominantly to the blood, liver, and kidney, while having limited distribution to the brain in experimental animals (Kudo, 2015). The levels of PFOS and PFOA

in the brain are much less than in the serum in experimental animals, indicating that these chemicals hardly cross the blood-brain barrier (Mariussen, 2012). The tissue distribution of PFOS and PFOA observed in wildlife exhibited similar tendencies. It is noteworthy that the brain concentrations of PFCAs with carbon chain lengths longer than 11 were close to those in the serum and liver of marine animals (Ahrens *et al.*, 2009; Mariussen, 2012; Rubarth *et al.*, 2011), suggesting that these PFCAs can easily pass the blood-brain barrier compared with PFCAs with carbon chain lengths shorter than 10. We hypothesize PFDoA passes the blood-brain barrier and affects brain functions.

Various studies on the toxicities associated with exposure to PFAAs have shown hepatotoxicity (Kudo and Kawashima, 2003 a,b), developmental toxicity (Lau *et al.*, 2004; Lau *et al.*, 2007), endocrine disrupting effects (Liu *et al.*, 2015, 2016), and immunotoxicity (DeWitt *et al.*, 2012) in experimental animals. Compared with these toxic effects, less attention has been paid to the neurotoxic potentials. Chronic exposure to PFAAs was associated with small or no effect in adult animals (Butenhoff *et al.*, 2009a, 2012; Fuentes *et al.*, 2007c). By contrast, neurobehavioral alterations were observed in developmentally exposed animals (Butenhoff *et al.*, 2009b; Fuentes *et al.*, 2007a,b; Johansson *et al.*, 2008; Onishchenko *et al.*, 2011; Ribes *et al.*, 2010; Viberg *et al.*, 2013). The high sensitivity seems to be due to both weak blood-brain barrier and vulnerability to the chemicals during the period of high neuronal growth (Eriksson, 1997). It is possible that high concentrations of PFAAs lead to alterations in brain functions.

In the present study, the neurobehavioral effects of PFCAs were estimated in relation to the concentrations of them in the brain of adult rats. We found that PFDoA distributed to the brain easier than PFOA and PFDA and induced cognitive deficit in adult rats.

MATERIALS AND METHODS

Materials

PFDoA (>93%) was purchased from Tokyo Chemical Industry, Co. Ltd (Tokyo, Japan). PFOA (>98%) and perfluorodecanoic acid (PFDA, >96%) were from Sigma-Aldrich (St. Louis, MO., USA). 3-Bromoacetyl-7-methoxycoumarin was prepared as described (Ohya *et al*., 1998). Corn oil was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals were of analytical grade.

Animals and treatments

All animal procedures were approved by the Institutional Animal Care Committee of Josai University in accordance with the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan). Male Wistar rats were purchased from SLC (Hamamatsu, Japan) and were allowed to acclimate to the housing facility for at least 1 week prior to treatment. Animals were given free access to water and food and were kept in a humidity- and temperature- (23 ± 2[degree]C) controlled environment with a 12-h light/dark cycle.

Rats aged 8 weeks old were exposed to a single-oral dose of PFDoA at doses of 5, 20, and 50 mg/kg. A dose of 50 mg/kg was used for comparison of PFOA, PFDA, and PFDoA, since a significant alteration was observed with PFDoA at a dose of 50 mg/kg in the novel object recognition test without severe weight loss or less activity. All PFCAs were suspended in corn oil (4 ml/kg) and dosed to animals. All PFCAs were used without adjusting for the purity of the raw materials since exact purity was not given. These animals were subjected to behavioral tests as described below.

Behavioral testing

Experimental protocols

Three sets of experiments were performed, and separate groups of rats were given each test. First set of experiments: the animals were divided into six groups (n=5-12), namely vehicle alone, PFDoA (5 mg/kg), PFDoA (20 mg/kg), PFDoA (50 mg/kg), PFOA (50 mg/kg), and PFDA (50 mg/kg) and

received an oral gavage on day 1. These animals were then tested in a novel object recognition test on days 6 and 7. Some of the vehicle- or PFDoA-treated animals underwent the test on days 29 and 30 (n=5).

Second set of experiments: the rats which had been treated with vehicle or PFDoA (50 mg/kg) on day 1 underwent an open-field test on day 6, followed by a Y-maze test on day 8 (n=10).

Third set of experiments: the rats which had been treated with vehicle or PFDoA (50 mg/kg) on day 1 underwent an elevated-plus maze test on day 7, followed by a forced swim test on days 8-9 (n=8).

Novel object recognition test

The novel object recognition test was performed to test for attention and memory in a low-motivational state (Galeano *et al.*, 2014). The test was conducted in an opaque plastic enclosure (30 cm x 25 cm x 18 cm). The rat was habituated to the apparatus for 30 min 24 h before testing. Testing began with a 5 min information phase in the enclosure, where two identical objects (A/A') were placed for the animals to explore. After a resting period of 24 h in the home cage, the animal was then placed back in the enclosure, where one familiar object (A) and one novel object (B) were placed, and allowed to explore for 5 min (test phase). The test was recorded by a video camera, and the time that the snout pointed to each object was estimated as exploration time by two reviewers blinded to the conditions of the rats. Between sessions, the objects were cleaned with ethanol followed by water to avoid odor cues. The discrimination index was calculated using the following equation: Discrimination index = (time spent on novel object – time spent on familiar object)/time spent on both novel and familiar objects.

Y-maze test

The Y-maze test was performed to assess spatial working memory (Galeano *et al.*, 2014). The maze consisted of three equal, closed arms (12 cm x 60 cm x 20 cm) connected to the center at equal angles.

The rat was placed on the center and allowed to move freely through the maze for 8 min. The test was recorded by a video camera, and the number of alternations and the number of arm entries were estimated by two reviewers blinded to the conditions of the rats. An arm entry was scored when rats placed four paws within the arm. An alternation was defined as an entry into three different arms on consecutive choice. The percent alternation was calculated as the ratio of actual to possible alternations using the following equation: Alternation (%) = number of alternation/ (number of arm entries – 2) x 100.

Elevated plus-maze test

The elevated plus-maze test was performed to assess anxiety versus risk-taking-like behavior (Galeano *et al.*, 2014). The maze consisted of two open arms (10 cm x 50 cm) and two closed arms with a wall (10 cm x 50 cm x 40 cm) connected to a central platform (10 cm x 10 cm) to form a cross. The maze was elevated to a height of 50 cm from the floor. A video camera was suspended above the maze. The rat was placed on the central platform facing an open arm and recorded for a 10 min period by a computer-based video tracking system (CompACT VAS/DV, Muromachi Kikai Co., Ltd., Tokyo, Japan). The time spent in the open and closed arms of the maze, respectively. The number of entries into the open and closed arms were recorded, respectively. Overall locomotor activity was determined as the distance traveled in the maze.

Open-field test

The open-field test was performed to assess locomotor activity and anxiety (Galeano *et al.*, 2014). Spontaneous exploratory activity was analyzed using a computer based video tracking system (CompACT VAS/DV). A video camera mounted centrally, above the apparatus, monitored a separate open field chamber (70 cm x 70 cm x 40 cm, divided into 49 square grids). The computer-defined grid lines divided each open field into a center region (30 x 30 cm in the center of the field) and a peripheral region. Each rat was placed individually in the open field area for 30 min. Parameters

evaluated included total distance traveled (m), number of crossings, and time spent in the periphery or in the center.

Forced swim test

The forced swim test was performed to assess behavioral despair (Porsolt *et al.*, 1978). The rat was placed in a glass cylindrical chamber (20 cm D x 50 cm H) filled with water (30 cm height, 25 ± 1[degree]C). Two swimming sessions were carried out with an initial 15 min 'pre-test' followed by a 5 min 'test' after 24 h. Immobility time, defined as the period during which the animal floats in the water with only those movements necessary to keep its head above water, was evaluated by two reviewers blinded to the conditions of the rat.

Tissue collection

Nine days after the oral gavage, the animals were anesthetized with diethyl ether, and blood was withdrawn from the inferior vena cava. Then, the whole body was perfused with approximately 300 ml of ice-cold saline from the left ventricle to remove the blood. The brain and liver were quickly removed, weighed, frozen in liquid nitrogen, and stored at -80[degree]C until use. Serum was prepared by centrifugation and stored at -30[degree]C until analysis. Some rats were sacrificed 30 days after dosing, and serum and tissue samples were obtained as described above. The liver and the brain were homogenized with 9 volumes of 0.25 M sucrose, 1 mM EDTA, and 10 mM Tris-HCl (pH7.4). A portion of the homogenate was subjected to PFCA analysis.

Analysis of PFCAs

The concentrations of PFCAs in the serum and tissue samples were determined as described previously (Ohya *et al.*, 1998). For determination of PFOA, PFDA, and PFDoA, a known amount of perfluorohexanoic acid, PFOA, and PFDA was added to the samples as internal standards, respectively, prior to extraction. In brief, PFCAs were extracted with ethyl acetate:hexane (1:1, v/v)

from an aliquot of serum or tissue homogenates as an ion pair with tetrabutylammonium after adding an internal standard. The extract was then incubated with 3-bromoacetyl-7-methoxycoumarin to yield acetylmethoxycoumarin derivatives of PFCAs. The reaction mixture was separated with high performance liquid chromatography with a fluorescence detector.

Statistical analysis

All data are expressed as means ± SEM. For comparison of dose (0, 5, 20 and 50 mg/kg) and different PFCAs (control, PFOA, PFDA and PFDoA), one-way analysis of variance followed by Scheffé's multiple-range test was used as a *post-hoc* test. For evaluation of the difference in Y-maze test, open-field test, elevated-plus maze test and forced swim test, the statistical significance between control and PFDoA-treated rats was estimated by the Student's *t-test*. The threshold for assessing significance was $P<0.05$, 2-tailed.

RESULTS

Body weights, organ weights, and tissue concentration of PFCAs

Body weights are shown in Table 1 on day 10 for control, PFOA-, PFDA-, and PFDoA-treated rats (50 mg/kg body weight). Body weight in the PFDA group was significantly less than in the control, and those of the PFDoA group were slightly less than the control (Table 1). Hepatomegaly was observed in all PFCA-treated groups (Table 1). The serum concentration of PFDoA was less than those of PFDA and PFDoA (Table 1). The hepatic concentration of PFDoA was comparative to that of PFDA and 4.2 times higher than PFOA (Table 1). The PFDoA concentration in the brain was greater than that of the serum, whereas the PFOA and PFDA concentrations were less than 5 [mu]g/g brain (Table 1).

Effects of PFCA on memory function

To assess attention and memory in a low motivational state, the novel object recognition test was performed. In the information phase, no significant difference was observed in exploration time between two identical objects in all test groups on day 6 (Fig. 1A). In the test phase, rats in the vehicle, PFOA, and PFDA groups preferred the novel object over the familiar object, whereas PFDoA-treated rats did not (Fig. 1B). The discrimination index was not altered in PFOA-treated rats, slightly, but not significantly, declined in PFDA-treated rats, and greatly declined in PFDoA-treated rats (Fig. 1D). The total exploration time was not different between the four experimental groups (Fig. 1C).

The Y-maze test was also employed to assess spatial and working memory. No significant difference was observed in the percentage of alternation behavior and total arm entries between PFDoA-treated rats and vehicle-treated rats (Table 2).

Effects of PFDoA on anxiety behavior

The effects of PFDoA were estimated by the open-field test. No significant differences were observed

in the mean distance traveled, the number of crossings, or the time spent in the central area between vehicle- and PFDoA-treated rats (Table 2).

The effects of PFDoA on anxiety was estimated by the elevated-plus maze test. The treatment with PFDoA significantly increased the time spent in the open arms (Fig. 2A), while the distance traveled, the total number of arm entries, and the percentage of open arm entry were not altered by the treatment with PFDoA (Fig. 2B-D).

Effects of PFDoA on depressive-like behavior

In the forced-swim test, immobility time, which indicates a despair-like state, was not affected by the treatment with PFDoA (Table 2).

Dose-dependency and duration of PFDoA-induced memory deficit

The dose dependency of the PFDoA-induced decrease in the discrimination index was estimated in the novel object recognition test. A decrease in the discrimination index was observed at the doses over 20 mg/kg (Fig. 6B), while the total exploration time was unchanged at any dose (Fig. 3A). The concentration of PFDoA increased in both the serum and brain as the dose of PFDoA increased (Fig. 3C). The relationship between the concentration of PFDoA in the brain and the discrimination index revealed that a decrease in the discrimination index was observed when the PFDoA concentration was more than 20 [mu]g/g tissue (Fig. 3D). Memory deficit in the novel object recognition test was observed on day 30 after an administration of PFDoA (Fig. 4B), while the total exploration time was not altered (Fig. 4A). The PFDoA concentrations in the serum and brain at 30 days after dosing were approximately 70% of those at 9 days after dosing (Fig. 4C). The body weight of PFDoA-treated rats was 355.8 ± 8.3 g, which was slightly less than that of the control rats (379.8 ± 8.3 g, $P<0.05$).

DISCUSSION

In the present study, we demonstrated that PFDoA is highly accumulated in the brain of adult rats that have received a single oral dose. This is different from PFOA and PFDA. PFDoA may distribute equally in the brain, since PFDoA concentrations of various parts in the brain were not different one another when rats were fed a diet admixed with 0.05% (w/w) PFDoA for 7 days (data not shown). To date, PFAAs were thought to accumulate primarily in protein-rich tissues, such as the liver and blood (Kudo, 2015), with little distribution in the brain (Mariussen, 2012). Several studies have revealed that the levels of PFAAs in the brain were lower than those in the liver and serum, indicating that most PFAAs have limited access to the brain. In adult rats, the levels of PFOS, PFOA, and perfluorononanoic acid were shown to be approximately 1-3% of those in the serum at low doses (Austin *et al*., 2003; Benskin *et al.*, 2009; Kudo *et al*., 2007). PFOS levels in the brain were more than 10% of those in the serum when rats were exposed to a sublethal dose of PFOS (Austin *et al*., 2003; Cui *et al*., 2009). In the latter case, the high permeability of the brain to PFOS is thought to be due to membrane perturbation by high concentrations of PFOS in the serum (Wang *et al*., 2011). In studies of the tissue distribution of PFAA in wildlife, the brain levels of PFAA were lower than those in the serum (Mariussen, 2012), except for PFDoA and perfluorotricosanoic acid of which the levels in the brain were equal and higher than those in the serum, respectively (Ahrens *et al.*, 2009; Rubarth *et al*., 2011). These observations on PFDoA are in accordance with the present results. The mechanism underlying the high distribution of PFDoA to the brain remains to be solved. Although the $\log K_{ow}$ of PFDoA is not available experimentally, it was predicted to be 6.30, 8.23, and 10.16 for PFOA, PFDA, and PFDoA, respectively, by EPI suite software (Bhhatarai and Gramatica, 2011). The high lipophilicity may be responsible for the high distribution of PFDoA to the brain.

The neurotoxic effects of PFAAs, especially PFOS and PFOA, were estimated in several studies and summarized by Mariussen (2012). Most studies have shown developmental neurotoxicity, such as delays in neuromotor development, when animals were exposed to the chemicals prenatally or during the neonatal period (Mariussen, 2012). A weak blood brain barrier is thought to be responsible for the

high sensitivity of developing animals. By contrast, studies on neurotoxicity in adult animals are limited. Butenhoff *et al*. (2009a) reported that repeated doses of perfluorohexanesulfonic acid exhibited no effects on the functional observational battery or on motor activity. Butenhoff et al. (2012) have shown that perfluorobutyric acid (PFBA) exhibited no effect on hearing, static righting, grip strength, or motor activity in adult rats that received oral gavages at doses of 30 and 150 mg/kg/day for 28 or 90 days. A delayed direct bilateral pupillary reflex was observed in male rats that chronically received PFOA at a dose of 30 mg/kg/day and PFBA at a dose of 150 mg/kg (Butenhoff *et al*., 2012). In the study by Fuentes *et al*. (2007c), oral exposure to PFOS for 4 weeks exhibited no effect in a functional observation battery, and small effects were observed at doses of 3 or 6 mg/kg/day in the open field test and Morris water maze test, although dose-dependency was not observed. The levels of PFAAs in brain might be low although the levels were not available in these studies. By contrast, the present study demonstrated that treatment with PFDoA was associated with a significant cognitive deficit in adult rats, and that the effect continued for at least 4 weeks after dosing. It seems likely that the magnitude of the cognitive deficit is dependent on the concentration of PFDoA in the brain for the following reasons: i) an inverse relationship was observed between the concentration of PFDoA in the brain and the discrimination index (Fig. 3D); ii) the decrease in the discrimination index was observed 30 days after dosing, when the concentration of PFDoA in the brain remained high (Fig. 4); iii) PFOA, the level of which was less than 0.8 [mu]g/g, did not alter the recognition index, and PFDA slightly, but not significantly, decreased the recognition index and was found in the brain (4.7 [mu]g/g, Table 1).

To assess the neurotoxic effects of PFDoA, 5 behavioral tests were employed in the present study: the novel object recognition test and Y-maze test to assess cognitive function, the open-field test and elevated-plus maze test to assess anxiety, and the forced swim test to assess depression. Treatment of PFDoA was associated with a significant deficit in novel object recognition tests (Fig. 1), while no significant difference was observed between the control and PFDoA-treated rats in Y-maze tests (Table 2). It seems likely that the novel object recognition test is more sensitive to neurotoxic

substances compared with the Y-maze test (Tian *et al*., 2010, Ogundele *et al*., 2014). PFDoA may not be strong enough to cause effects in the Y-maze test. Our results also suggested that PFDoA slightly decreased anxiety in the elevated plus-maze test (Fig. 2), while no significant effect was observed in the open-field test (Fig. 2). Although both the open-field test and the elevated-plus maze test assess anxiety-like behavior, these tests operationalize different aspect of emotional behavior (Ramos, 2008). Alteration with PFDoA treatment may reflect a specific dimension of emotional behavior linked to the elevated-plus maze test. By contrast, PFDoA did not induce depression-like behavior (Table 2).

The mechanisms underlying the cognitive deficit associated with PFDoA treatment remains to be solved. Several *in vitro* studies have proposed mechanisms underlying neurobehavioral alterations associated with PFAAs. It has been shown that PFOS has a modulating effect on ion currents in cerebellar Purkinje cells (Harada *et al.*, 2006) and in primary cultured hippocampal neurons (Liao et al., 2009). PFOS has been shown to alter Ca^{2+} currents and increase the amplitude of field excitatory postsynaptic potentials in the CA1 region of hippocampal slices (Liao *et al*., 2008). Liu *et al*. (2011) demonstrated that PFOS induced a disturbance of Ca^{2+} homeostasis in hippocampal neurons. Ca^{2+} is involved in the synaptic processes underlying both learning and memory, and the perturbation of Ca^{2+} homeostasis interferes with physiological processes in neurons, including synaptic activity (Baker *et al.*, 2013; Berridge *et al*., 1998). PFDoA, like other PFAAs, may disturb Ca^{2+} homeostasis in the brain.

In conclusion, the present study demonstrated that PFDoA passes through the blood-brain barrier and accumulates in the brain. Cognitive deficit is associated with PFDoA levels in the brain.

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FIGURE LEGENDS

Fig. 1

Effects of PFOA, PFDA, and PFDoA on the novel object recognition test. Rats received an oral gavage of PFOA, PFDA, or PFDoA at a dose of 50 mg/kg, and the test was performed on days 6-7. A, Exploration time of object A and object A' in the information phase. B, Exploration time of object A and object B in the test phase. C, Total exploration time in the test phase. D, Discrimination index. Values represent means ± SEM (control, 12; PFOA, 4; PFDA, 7; PFDoA, 7). * Difference compared with object A (P<0.05). $^{a\text{-}c}$, Significant difference without a common superscript (P<0.05).

Fig. 2

Effects of PFDoA on the elevated-plus maze test. Rats received an oral gavage of PFDoA at a dose of 50 mg/kg, and the test was performed on day 7. A, Time spent in open arms. B, Percentage of open arm entries to total arm entries. C, Distance traveled in overall maze. Values represent means ± SEM (control, 6; PFDoA, 8). * Difference compared with control (P<0.05).

Fig. 3

Dose dependency of the effect of PFDoA on the novel object recognition test. Rats received an oral gavage of PFDoA at doses of 5, 20, or 50 mg/kg, and the test was performed on days 6-7. A, Total exploration time in the test phase. B, Relationship between dose and discrimination index. C, PFDoA concentrations in the serum and the brain on day 10. D, Relationship between PFDoA concentration in the brain and discrimination index. Values represent means ± SEM (control, 12; 5 mg/kg, 5; 20 mg/kg, 5; 50 mg/kg, 7). * Difference compared with serum (P<0.05). $^{a\text{-}c}$, Significant difference without a common superscript (P<0.05).

Fig. 4

Effect of PFDoA on the novel object recognition test on day 30. Rats received an oral gavage of PFDoA at a dose of 50 mg/kg, and the test was performed on days 29-30. A, Total exploration time in the test phase. B, Discrimination index. C, PFDoA concentrations in the serum and the brain on day 9 and day 31. Values represent means ± SEM (n=5). * Difference compared with control (P<0.05). # Difference compared with the value on 10 day (P<0.05).

Table 1　Body weight, tissue weight, and PFCA concentrations after oral administration of PFCA.

		Vehicle	PFOA	PFDA	PFDoA
Body weight					
Initial	(g)	261.1 ± 2.6	254.8 ± 1.2	259.9 ± 1.9	258.0 ± 2.9
Final	(g)	294.0 ± 2.9[a]	288.6 ± 0.9[ab]	239.3 ± 4.4[c]	276.4 ± 3.8[b]
Weight gain	(g)	32.9 1.5[a]	33.9 1.3[a]	-20.6 4.1[b]	18.4 3.3[c]
Tissue weight					
Liver	(g)	11.22 ± 0.26[a]	17.13 ± 0.87[b]	15.65 ± 0.69[b]	15.47 ± 0.60[b]
	(% of body weight)	3.82 0.09[a]	5.93 0.30[bc]	6.52 0.21[c]	5.58 0.17[b]
Brain	(g)	1.96 ± 0.02	1.92 ± 0.02	1.92 ± 0.01	1.94 ± 0.02
	(% of body weight)	0.67 0.01[a]	0.66 0.01[ab]	0.80 0.01[c]	0.70 0.01[b]
PFCA concentration					
Serum	([micro]g/ml)	-	33.3 ± 4.4[a]	43.0 ± 1.3[b]	24.4 ± 1.0[c]
Liver	([micro]g/g tissue)	-	58.7 ± 8.1[a]	287.7 ± 11.4[b]	247.7 ± 9.8[c]
Brain	([micro]g/g tissue)	-	ND[a]	4.7 ± 0.4[a]	44.4 ± 2.0[b]
Brain/serum		-	0.000 ± 0.000[a]	0.109 ± 0.008[a]	1.821 ± 0.059[b]
Liver/serum		-	1.774 ± 0.124[a]	6.726 ± 0.301[b]	10.315 ± 0.545[c]

Rats were received an oral gavage of PFOA, PFDA or PFDoA at a dose of 50 mg/kg, or vehicle alone. Blood and tissues were collected on day 10 after dosing. Values represent the mean ± SEM (control, 12; PFOA, 4; PFDA, 7; PFDoA, 7). (-), not determined; ND, less than detection limit (0.8 [micro]g/g). [a, b, c] Means without a common superscript are a significantly different (P<0.05). In the absence of superscript, the means are not statistically significant.

Table 2 Effects of PFDoA on the Y-maze test, open field test, and forced swim test

	Control	PFDoA	*P value*
Y-maze test			
Alternations (%)	65.5 ± 5.6	75.7 ± 5.1	0.194
Total arm entries	16.4 ± 1.6	14.0 ± 2.0	0.362
Open-field test			
Distance traveled (m)	20.3 ± 2.3	16.5 ± 1.5	0.189
Number of crossings	399.1 ± 32.1	322.2 ± 36.4	0.131
Time spent in center region (%)	1.67 ± 0.23	1.44 ± 0.31	0.566
Forced swim test			
Immobility time (s)	165.9 ± 21.9	156.8 ± 11.6	0.718

Rats received an oral dose of PFDoA at a dose of 50 mg/kg. The Y-maze test (n=8), open-field test (n=10) and forced swim test (n=8) were performed on day 8, day 6, and day 8-9, respectively. The difference was not significant.

Fig. 1

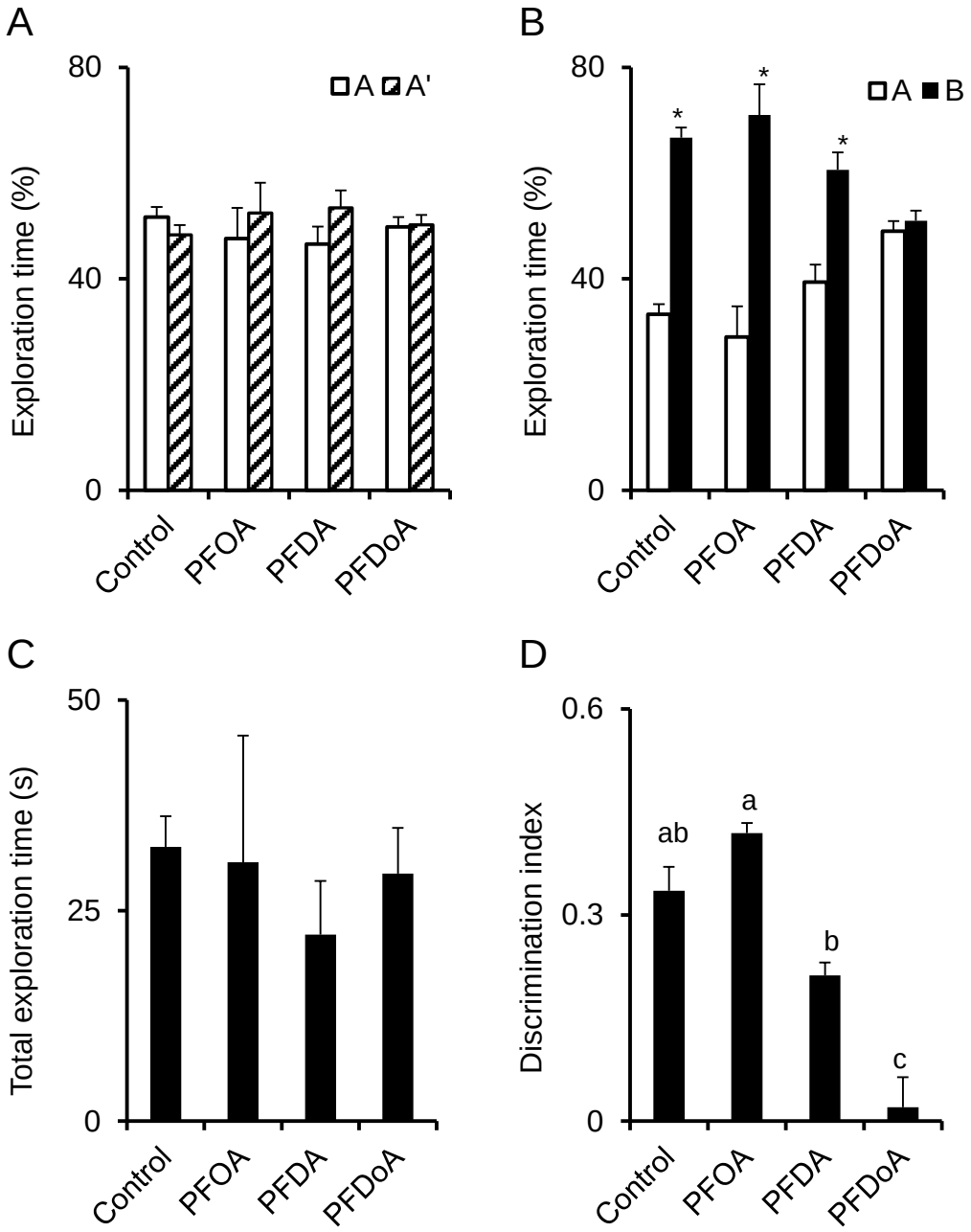

A
80
40
0
Exploration time (%)
A
A'
Control
PFOA
PFDA
PFDoA
B
80
40
0
Exploration time (%)
A
B
*
*
*
Control
PFOA
PFDA
PFDoA
C
50
25
0
Total exploration time (s)
Control
PFOA
PFDA
PFDoA
D
0.6
0.3
0
Discrimination index
ab
a
b
c
Control
PFOA
PFDA
PFDoA

Fig. 2

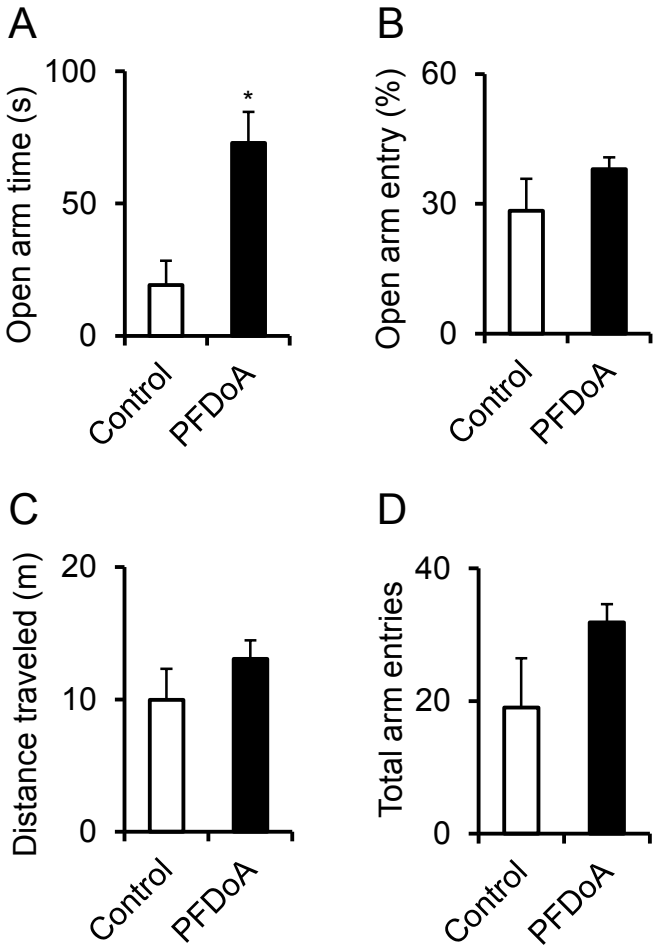

A
Open arm time (s)
100
50
0
*
Control
PFDoA
B
Open arm entry (%)
60
30
0
Control
PFDoA
C
Distance traveled (m)
20
10
0
Control
PFDoA
D
Total arm entries
40
20
0
Control
PFDoA

Fig. 3

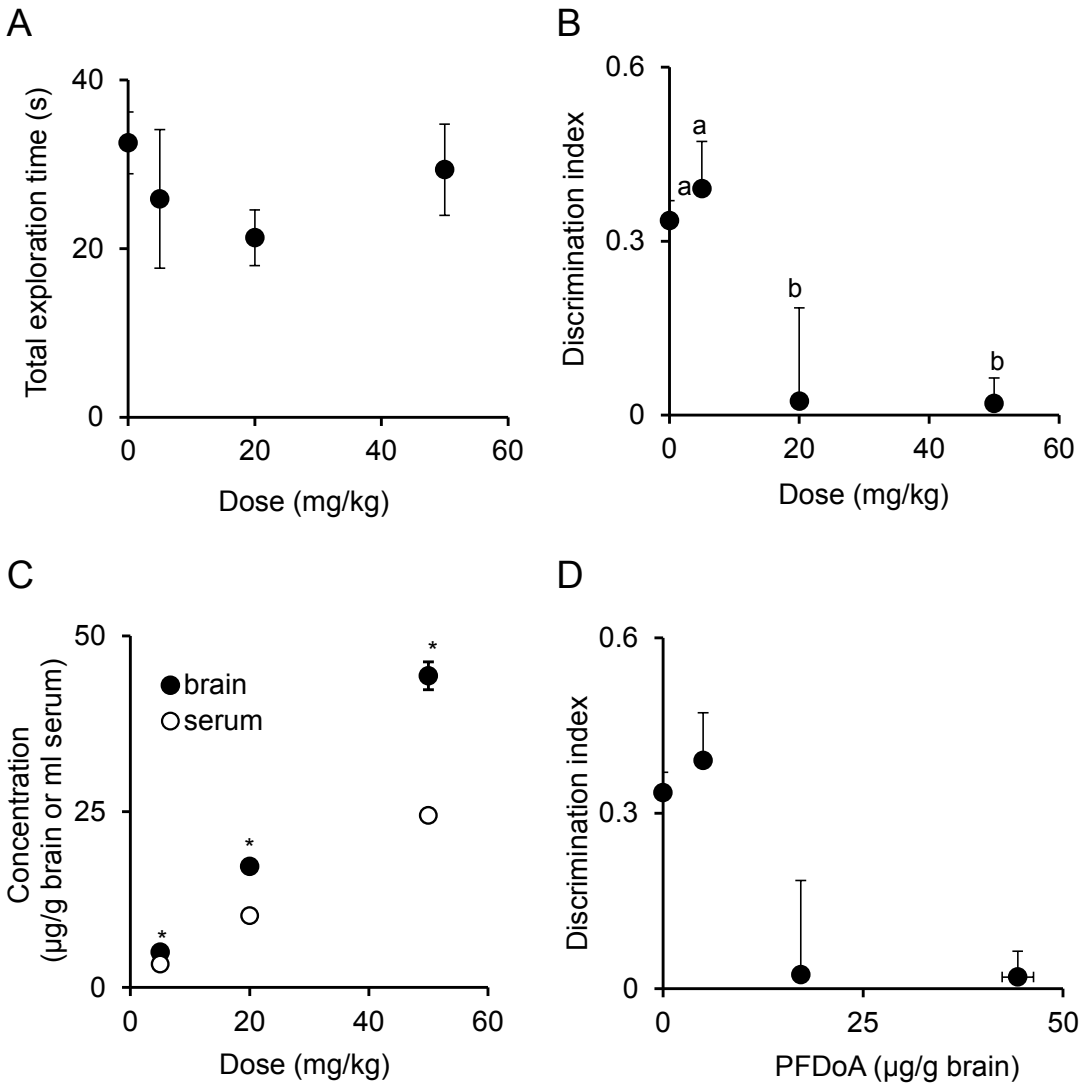
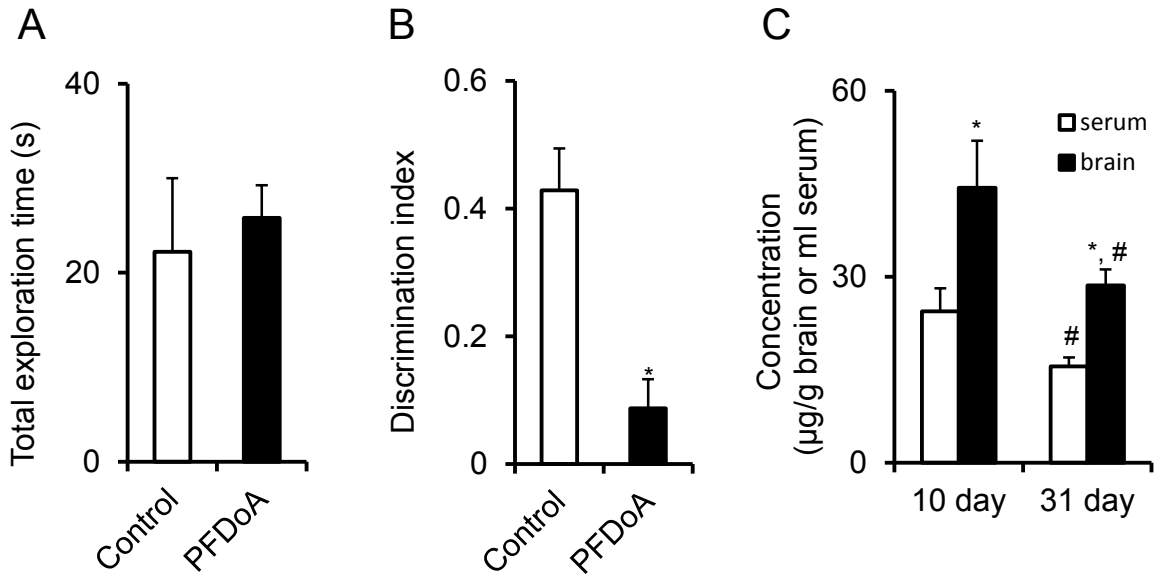

A
Total exploration time (s)
40
20
0
0
20
40
60
Dose (mg/kg)
B
Discrimination index
0.6
0.3
0
a
a
b
b
0
20
40
60
Dose (mg/kg)
C
Concentration (µg/g brain or ml serum)
50
25
0
brain
serum
*
*
*
0
20
40
60
Dose (mg/kg)
D
Discrimination index
0.6
0.3
0
0
25
50
PFDoA (µg/g brain)

Fig. 4


A
Total exploration time (s)
40
20
0
Control
PFDoA
B
Discrimination index
0.6
0.4
0.2
0
*
Control
PFDoA
C
Concentration
(μg/g brain or ml serum)
60
30
0
serum
brain
*
*, #
#
10 day
31 day