Accumulation of Waterborne Mercury(II) in Specific Areas of Fish Brain

ARTICLE *in* ENVIRONMENTAL SCIENCE AND TECHNOLOGY · AUGUST 1999

Impact Factor: 5.33 · DOI: 10.1021/es990001v

4 AUTHORS, INCLUDING:

Claude Rouleau
Université du Québec à Rimouski UQAR
50 PUBLICATIONS **915** CITATIONS

Environ. Sci. Technol. **1999**, *33,* 3384−3389

Accumulation of Waterborne Mercury(II) in Specific Areas of Fish Brain

CLAUDE ROULEAU,*,†,‡
KATHLEEN BORG-NECZAK,†
JAMES GOTTOFREY,† AND HANS TJÄLVE†

Department of Pharmacology and Toxicology, Swedish University of Agricultural Sciences, Biomedicum Box 573, S-751 23 Uppsala, Sweden, and Fisheries and Oceans, Maurice Lamontagne Institute, P.O. Box 1000, Mont-Joli, Québec, Canada G5H 3Z4

We used whole-body autoradiography to study the distribution of ^{203}Hg(II) in the central nervous system of brown (*Salmo trutta)* and rainbow (*Oncorhynchus mykiss*) trout. Fish were either exposed to waterborne Hg(II) for 7 and 21 d or they received an intravenous injection of the metal and were sacrificed 1 and 21 d later. Mercury did not accumulate in the brain after intravenous injection, indicating that the blood−brain barrier is impervious to Hg in plasma. In contrast, Hg was accumulated in specific areas of the brain (olfactory system, eminentia granulares and medulla of cerebellum, optic nerve and tectum, and rhombencephalon) and spinal cord (ventral horn ganglia) following water exposure. The specificity of the accumulation sites strongly suggests that waterborne Hg was taken up by water-exposed receptor cells of sensory nerves and subsequently transferred toward the brain by axonal transport, a normal physiological process for the transport of organelles and dissolved neuronal constituents along nerve axons. Accumulation of Hg in ventral horn ganglia is probably the result of leaching of metal from blood into muscle followed by uptake in motor plates. Axonal transport allows waterborne inorganic Hg, and possibly other xenobiotics, to circumvent the blood−brain barrier. Considering the importance of complex behavior in the life of fish, and the well-known deleterious effects of mercury on the nervous system, the toxicological significance of this uptake route needs to be assessed.

Introduction

Axonal transport in neurons is a physiological process for the transport of organelles and dissolved neuronal constituents along the axons to the nerve terminals (anterograde axonal transport) and back to the cell bodies (retrograde axonal transport). It also constitutes a route by which foreign materials, among them toxic trace metals (Pb, Cd, Hg, Tl, Ag), can circumvent the blood−brain barrier to reach the central nervous system (*1*).

In previous studies on the axonal transport of metals in fish, we showed that ^{109}Cd(II) and ^{54}Mn(II) applied within the nostrils of pike (*Esox lucius*) were taken up in receptor cells of the olfactory epithelium and transported along olfactory nerve neurons toward the brain (*2*, *3*). We also observed that waterborne ^{109}Cd(II) and ^{54}Mn(II) were taken up in the olfactory epithelium and transported to the brain of brown trout (*Salmo trutta*) by axonal transport (*4*, *5*), even at concentrations at the lower end of the range found in freshwater environments (*5*). Such an accumulation of metals from water in the olfactory system may be injurious to the olfactory sense of fish and may disturb processes relying on olfaction, such as migration (*6*)

The neurotoxicity of divalent inorganic mercury is well-known (*7*). We recently observed that ^{203}Hg(II) was transported along the axon of olfactory nerve fibers of pike (*8*). The aim of the present study was to verify if waterborne Hg(II) could also reach the brain of fish by the same way. We used whole-body autoradiography to examine the fine-scale distribution of ^{203}Hg(II) in the brain of brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) following exposure via water for periods of up to 3 weeks. The distribution of the metal after intravenous injection was also studied to determine if Hg(II) could be taken up in the central nervous system directly from the blood over the same period of time.

Materials and Methods

Brown trout (*S. trutta*) were obtained from Kvistforsen's fish farm at Skellefteå River, Sweden. Rainbow trout (*O. mykiss*) were obtained from Skeberga Fiskodling at Alunda, Sweden. Sex was not determined. All fish were held in artificial freshwater (0.15 g of $KHCO_3$, 1.42 g of $CaCO_3$, 0.16 g of MgO, 0.35 g of NaCl, and 6.5 mL of 1 N H_2SO_4 per 100 L of double-deionized water, pH = 7.0−7.5) at 10.0 ± 0.5 °C. They were allowed to acclimate to laboratory conditions for 1 week prior to experiments and were not fed.

Brown Trout. Two groups of seven 1-year old brown trout weighting 3−6 g were exposed for either 7 or 21 d to 0.1 μg of Hg(II)·L^{-1}, with 8.5 kBq of ^{203}Hg(II)·L^{-1} as tracer. At the end of the exposure period, fish were briefly rinsed in clean water. Five fish from each group were dissected for quantitative γ counting (*9*). The two others were used for whole-body autoradiography (WBARG) as previously described (*10*). They were embedded in a carboxymethylcellulose gel, frozen in a slurry of hexane and dry ice, and sectioned sagittally at −20 °C (20-μm-thick sections on tape) with a specially designed cryomicrotome (Jung Cryomacrocut, Leica). Sections were freeze-dried and applied to an X-ray film (Structurix, AGFA) at −20 °C for 5 months.

Rainbow Trout, Water Exposure. After analysis of the results obtained in the experiment described above, we wanted to complete our observations by using larger fish and higher ^{203}Hg concentration and to compare the distribution of the metal taken up from water and intravenous injection. Rainbow trout were used since brown trout of appropriate size were not available. Four 4-month old rainbow trout weighting 20−25 g were exposed for 21 d to 2.0 μg of Hg(II)·L^{-1} (22.2 kBq of ^{203}Hg(II)·L^{-1}). They were all used for WBARG as mentioned above. Two fish were sectioned transversally, one sagittally, and one frontally.

Rainbow Trout, Intravenous Injection. Larger rainbow trout were used for intravenous injection (15-month old, body weight 150−200 g). Four fish were injected in the caudal vein with 100 μg of Hg(II) (1.11 MBq of ^{203}Hg(II)) dissolved in 0.3 mL of saline. Following the injection, they were returned

* Corresponding author phone: (*418*) 775-0725; fax: (*418*) 775-0718; e-mail; rouleauc@dfo-mpo.gc.ca. New address September 1, 1999: National Water Research Institute, Aquatic Ecosystem Protection Branch, 867 Lakeshore Road, P.O. Box 5050, Burlington, Ontario, Canada L7R 4A6; phone: (*905*) 336-4928; e-mail: claude.rouleau@cciw.ca.

† Swedish University of Agricultural Sciences.

‡ Maurice Lamontagne Institute.

10.1021/es990001v CCC: $18.00
Published on Web 08/24/1999

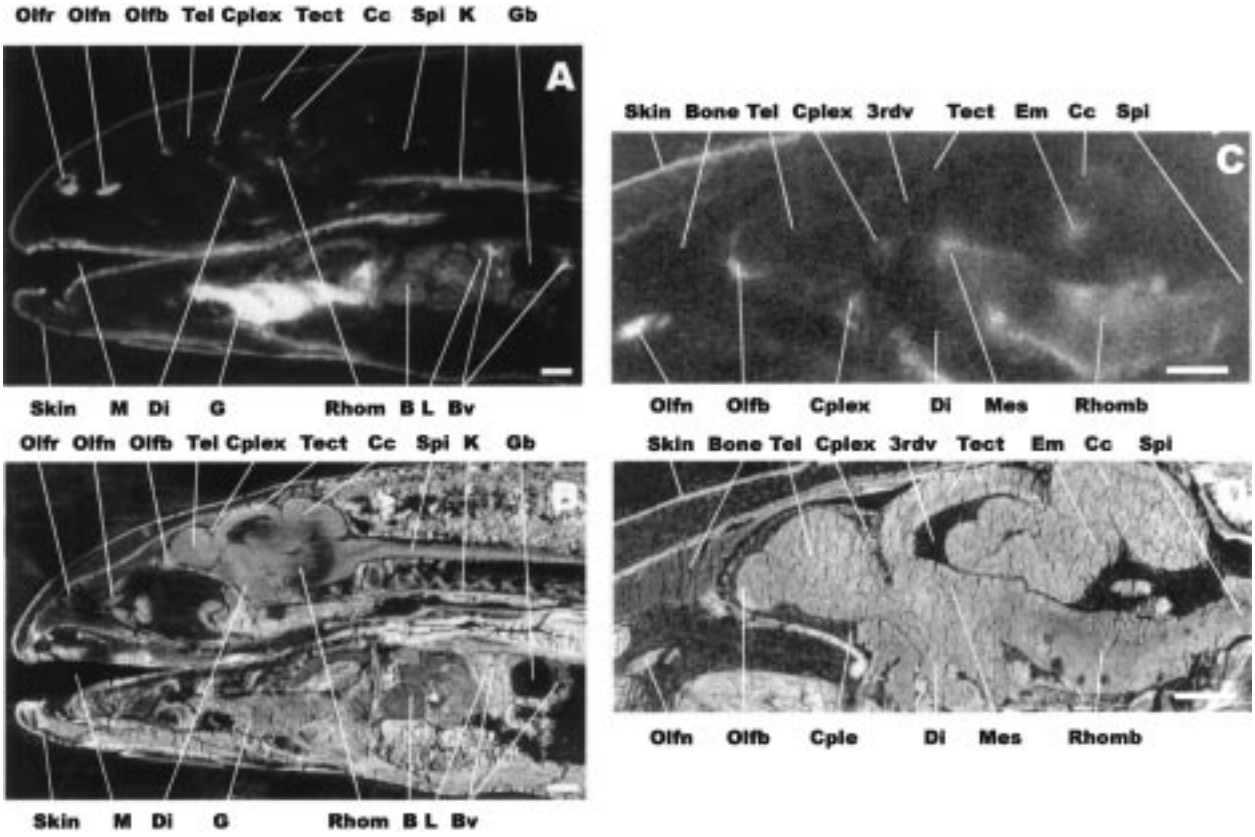

FIGURE 1. (A) Sagittal autoradiogram from a brown trout (*S. trutta*) exposed for 21 d to 0.1 μg of $^{203}Hg(II)\cdot L^{-1}$. (C) Detail of another autoradiogram showing brain area (contrast enhanced). Panels B and D are the corresponding tissue sections. See Table 1 for abbreviations. Bars are 1 mm.

to clean water and two individuals were collected 1 and 21 d after injection. These fish were also used for WBARG. Due to the high amount of ^{203}Hg in the animals, exposure of film was only 14 d.

Results

Brown Trout. Quantitative data of Hg distribution in the tissues of brown trout obtained by γ counting are published elsewhere (*9*). Briefly, the whole-body bioconcentration factor (BCF) was 48 ± 6 after 7 d, and 226 ± 14 after 21 d. Radioactive Hg levels in each tissue sampled followed the same temporal trend. At day 21, gill BCF was the highest (1759 ± 14), followed by kidney (799 ± 60), liver (524 ± 24), mucus (511 ± 35), gastrointestinal tract (325 ± 26), carcass (200 ± 20), brain (125 ± 4), eye (107 ± 6), and muscle (27 ± 2). The relative distribution of Hg was similar at 7 and 21 d, the carcass accounting for 40% of the body burden, gills for 30%, gastrointestinal tract for 15%, kidney and muscle for 5% each, liver for 2.5%, eye for 1%, and brain for 0.6%.

The distribution pattern of radioactive Hg observed in autoradiograms confirmed quantitative data and was similar for both exposure periods (Figure 1). Additional fine details were visible. Oral mucosa and skin, which are in direct contact with metal-containing water, showed a strong labeling. Although the degree of labeling in blood and liver parenchyma was similar, the walls of hepatic blood vessels were more heavily labeled. Nevertheless, the most interesting feature was the specific labeling of certain areas of the brain. The olfactory system of brown trout picked up as much of the label as the gills or kidney. It is noteworthy that the labeling of this part of the brain was limited to the proximal most rostral part, giving the area a crescent-like appearance. Other areas of the brain were specifically labeled in addition to the olfactory system (Figure 1). Labeling of rhombencephalon and mesencephalon was relatively high and heterogeneously distributed. A weaker labeling was also seen in corpus cerebellum, eminentia granulares, and choroid plexus. The radioactivity level in telencephalon, optic tectum, and diencephalon was very low.

Rainbow Trout, Water Exposure. Autoradiograms from rainbow trout exposed to waterborne $^{203}Hg(II)$ are shown in Figure 2. The general distribution pattern observed was similar to that of brown trout, i.e., elevated levels in gills, kidney, skin and oral mucosa (Figure 2D). The distribution of Hg in the liver was also similar (Figure 2A): liver parenchyma and blood had similar levels of the label, while the walls of hepatic blood vessels contained more of the label.

The main features of Hg distribution in the brain of brown trout were also observed in rainbow trout, though more details could be seen. To compare semiquantitatively the labeling of the different brain areas, autoradiograms were digitized as black and white images with 256 values of gray and the average value of gray of various areas was determined (Table 2) (*11*). Values for bony tissue, which were close to background, are also provided for comparison. The features described below were seen in all four experimental fish, regardless of tissue section orientation.

As for brown trout, the entire olfactory system was strongly labeled, with Hg distribution in the olfactory bulb limited to the most anterior part, whereas the telencephalon had a very low labeling (Figure 2B,D). Distribution of ^{203}Hg in the cerebellum was clearly restricted within two specific areas. The autoradiogram in Figure 2B is from a sagittal sectiontaken 1 mm right of the midsagittal plane. It shows the right eminentia granulares, which was strongly labeled. Actually, two distinct labeled structures could be seen in both eminentia granulares in a frontal section taken from another

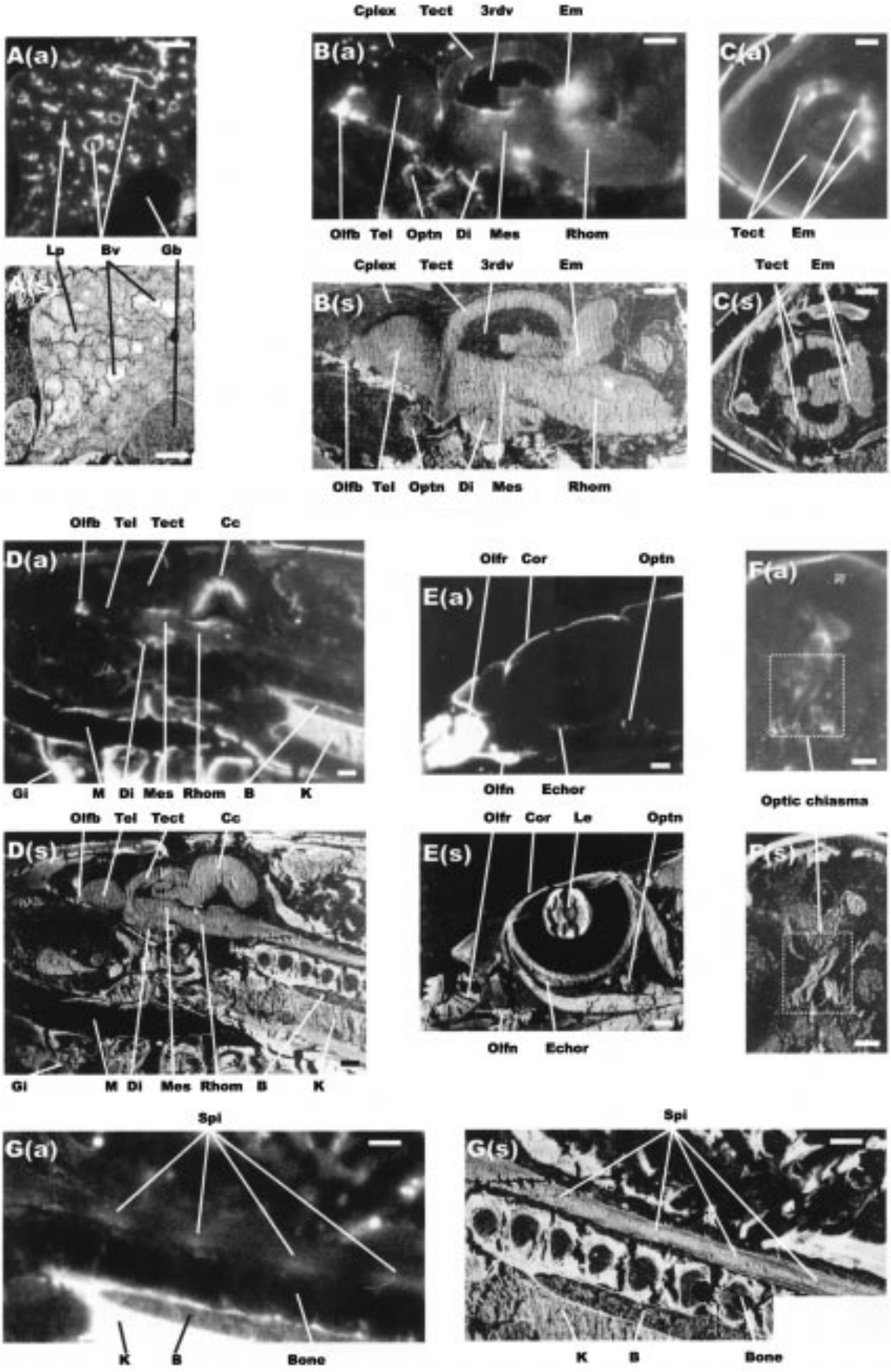

FIGURE 2. Details of autoradiograms from rainbow trout (*O. mykiss*) exposed for 21 d to 2.0 μg of ^{203}Hg(II)·L^{-1}: (A) frontal section showing liver. (B) sagittal section taken 1 mm right of the midsagittal plane; (C) frontal section showing eminentia granulares; (D) midsagittal section; (E) frontal section showing the eye; (F) transversal section showing the optic chiasma; (G) sagittal section showing the spinal cord. (a) = autoradiogram, (s) = tissue section. See Table 1 for abbreviations. Bars are 1 mm.

TABLE 1. List of Abbreviations Used in Figures 1–3 and Table 2

B	blood	Lp	liver parenchyma
Bv	blood vessel	M	mouth
Cc	corpus cerebelli	Mes	mesencephalon
Cor	cornea	Olfb	olfactory bulb
Cplex	choroid plexus	Olfn	olfactory nerve
Di	diencephalon	Olfr	olfactory rosette
Echor	eye choroid	Optn	optic nerve
Em	eminentia granulares	Rhom	rhombencephalon
Gb	gall bladder	Spi	spinal cord
Gi	gills	Tect	optic tectum
K	kidney	Tel	telencephalon
Le	lens	3rdv	3rd ventricle
Li	liver		

fish (Figure 2C). An autoradiogram from a section taken along the midsagittal plane (Figure 2D) also showed that the labeling of corpus cerebelli was limited to the medulla. The cortex had a much lower labeling (Table 2).

On average, the labeling of rhombencephalon and mesencephalon was lower than for olfactory bulb and cerebellum (Figure 2B,D). However, their dorsal part and some nucleus-like structures that were visible in some autoradiograms (see in Figure 2B) exhibited a high labeling (Table 2). The optic nerve, optic tectum, and diencephalon were labeled, though to a lesser extent compared to cerebellum and rhombencephalon (Figure 2B,E,F). In the eye, labeling of the water-exposed cornea showed an inward decreasing gradient, and the eye choroid also contained radioactive Hg (Figure 2E). Finally, labeling was visible in the spinal cord as faint and evenly spaced spots, located ventrally (Figure 2G), which probably corresponds to the ventral horn ganglia.

Rainbow Trout, Intravenous Injection. An autoradiogram from a fish maintained in clean water for 1 d after the intravenous injection is shown in Figure 3. Liver and kidney were intensively labeled. The other tissues were labeled to a lesser extent, including the choroid plexus. However, no labeling of brain and spinal cord could be seen (Table 2). The distribution of radioactive Hg in fish maintained in clean water for 21 d after injection was the same.

Discussion

Experimental conditions chosen in this work represent a compromise between environmental realism and technical requirements for successful autoradiography. We needed to use higher dissolved Hg(II) concentrations than those usually found in freshwaters ($<1-10$ ng·L^{-1}) (*12*–*15*) because of the relatively low specific activity of commercially available ^{203}Hg and the short half-life of this isotope (46 d). Nevertheless, water chemistry parameters, e.g., low-hardness neutral freshwater, and Hg speciation (98% $Hg(OH)_2$ and 2% HgOHCl, calculated with the software MINEQL+) (*16*) were environmentally relevant (*17*). It is also noteworthy that fish did not exhibit any sign of toxicity.

To our knowledge, there is no data in the literature about the uptake and distribution of inorganic Hg in fish exposed to the nanogram per liter level that could be compared to our own data. Distribution data from wild fish cannot be used either, since distribution of Hg in the body of wild fish is dependent upon the uptake route (food versus water) and species of mercury taken up (methylmercury versus inorganic Hg). However, the general distribution pattern of inorganic ^{203}Hg (gills > kidney > liver > other tissues) in brown and rainbow trout was the same, despite different metal concentration in exposure water, and it resembled that observed by other workers (*18*). Furthermore, the distribution picture of Hg in the brain of both trout species was the same, indicating that exposure of rainbow trout to higher ^{203}Hg concentration simply resulted in a greater labeling of brain structures compared to brown trout.

The labeling of the olfactory system of trout observed in the present work indicates that dissolved ^{203}Hg(II) was taken up in olfactory receptor cells of olfactory rosettes and transported toward the olfactory bulb via axonal transport, as observed previously for waterborne ^{109}Cd(II) and ^{54}Mn(II) (*4*, *5*). However, the distribution pattern of Hg(II) was clearly different. For instance, Mn(II) has been observed to diffuse caudally in brown trout and pike, due to its apparent ability to cross synaptic junctions between primary and secondary neurons in the olfactory bulbs (*3*, *5*). On the contrary, Cd(II) was not found in any other part of the brain than the olfactory bulbs because it cannot cross the synaptic junctions between interconnecting neurons (*2*, *4*). In the case of Hg(II), we have observed that metal applied in the olfactory chamber of pike (*8*) was unable to cross synaptic junctions, like Cd. The crescent-like labeling of the olfactory bulb observed in this work (Figures 1 and 2) indicates that the same occurred in trout. However, the labeling of rhombencephalon, eminentia granulares, medulla of cerebellum, and optic system was a feature specific to Hg. Since the result from the injection experiment clearly showed that the blood–brain barrier of rainbow trout was impervious to Hg, and if the same is assumed to be true for brown trout also, then the accumulation of ^{203}Hg(II) in areas of the brain other than the olfactory system of trout likely occurred via axonal transport and the possible access routes were probably limited to primary nerve pathways.

The central nervous system has many sensory and motor primary nerve pathways (Figure 4). Prime candidates for the transport of waterborne Hg are sensory nerves innervating water-exposed sensory cells: mechanoreceptors of lateral line system, cutaneous sensory cells (tactile, pressure, nociceptive), and receptor cells of taste buds in oral mucosa. Primary afferent neurons innervating lateral line organs and taste buds terminate in rhombencephalon by branches of VIIth, IXth, and Xth cranial nerves. The eminentia granulares of the cerebellum are the site of termination of many lateral line nerves, whereas spinocerebellar fibers, i.e., afferent nerve fibers from cutaneous sensory cells and proprioceptive receptors, terminate in the medulla of the cerebellum (*19*–*21*). The intense labeling of skin and oral mucosa seen in whole-body autoradiograms (Figures 1 and 2) indicates that Hg uptake may have occurred in water-exposed sensory cells and receptors, possibly followed by axonal transport along neurons toward their termination sites in rhombencephalon and cerebellum. The very specific labeling of cerebellum brings further evidences. It has been shown that eminentia granulares have two divisions, each one receiving fibers from lateral line organs of different origin, e.g., posterior and anterior lateral line nerves (*22*, *23*). The labeling of eminentia granulares seen in Figure 2C corresponds to this anatomical disposition and strongly supports the hypothesis of waterborne Hg uptake in mechanoreceptors of lateral line organs and its subsequent transport in axons. Labeling of the medulla of corpus cerebelli to the exclusion of the cortex is also very specific, since sensory afferents from skin and proprioceptive receptors are the only primary nerves to reach corpus cerebelli. All other afferent and efferent nerves in the medulla and cortex are secondary nerve pathways (*19*) that are unable to carry Hg due to its inability to cross synaptic junctions between primary and secondary neurons (*8*). The fact that telencephalon, which lacks any primary nerve pathways (*19*), was not labeled (Figures 1 and 2) also supports the hypothesis of axonal transport.

Radioactive ^{203}Hg was detected in the optic system of rainbow trout, but not in brown trout. Since labeling of the optic nerve and tectum appears to be lower than that of rhombencephalon and cerebellum (Table 2), this difference

TABLE 2. Average Gray Value ± SD for Various Areas of Digitized Whole-Body Autoradiograms Shown in Figures 2 and 3

Figure 2B		Figure 2D		Figure 2G		Figure 3	
bone	38 ± 11[a]	bone	29 ± 5[a]	bone	20 ± 14[b]	bone	18 ± 7[b]
Olfb	247 ± 4	Olfb	211 ± 13	Spi		Spi	23 ± 8
				ventral ganglia	91−99		
				between ganglia	38−54		
Tel	41 ± 13	Tel	33 ± 5			whole brain	15 ± 7
Em	213 ± 22	Cc					
		medulla	194 ± 24				
		cortex	71 ± 17				
Rhom	104 ± 27[c] to 211 ± 27[d]	Rhom	93 ± 17[c] to 193 ± 41[e]				
Tect	77 ± 25						
Optn	87 ± 48						
3rdv	27 ± 11						

[a] Cranial bone. [b] Vertebrae. [c] Average value. [d] Maximum value for nucleus-like structures. [e] Maximum value for dorsal area. [f] Lowest radioactivity = 1 (black); highest radioactivity = 256 (white).

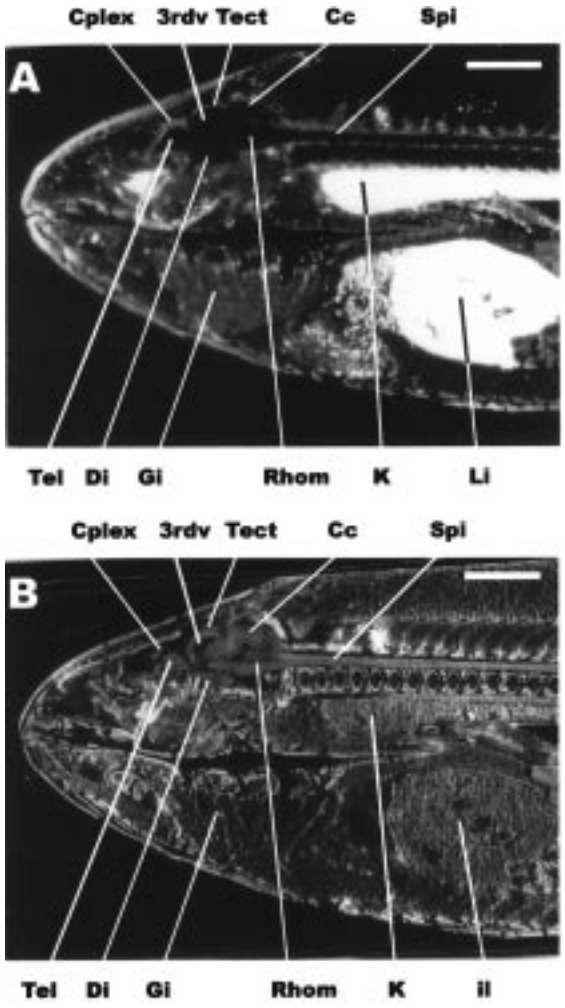

FIGURE 3. (A) Sagittal autoradiogram from a rainbow trout (*O. mykiss*) sampled 1 d after an intravenous injection of ^{203}Hg(II). (B) Corresponding tissue section. See Table 1 for abbreviations. Bars are 1 cm.

between the two fish species is probably related to the lower concentration of radioactive metal to which brown trout was exposed. Axonal transport may also explain the accumulation of ^{203}Hg in the optic system of rainbow trout. The cell bodies of optic neurons are located in the innermost cell layer of the retina, the ganglion cell layer (*24*), and it is known that amino acids, nucleotides, proteins, Ca^{2+}, and Zn^{2+} injected intraocularly, i.e., into the vitreous body, are taken up in optic neurons cell bodies and transported to the optic tectum by

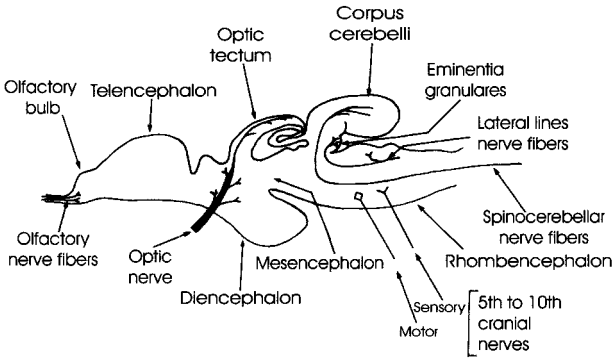

FIGURE 4. Schematic sagittal view of trout brain with main primary nerve pathways. Redrawn from ref 19.

axonal transport (*25*−*29*). The inward decreasing labeling of the water-exposed cornea indicates that waterborne Hg(II) may have diffused toward the intraocular environment, where it was subsequently taken up in optic neurons and axonally transported in the optic system. Labeling of the diencephalon may be also due to the transport of Hg in the optic system, because many optic nerve fibers terminate in this area of the brain (*19*). Though the richly vascularized eye choroid that nourishes and oxygenates the retina was labeled (Figure 2E), it is unlikely that blood Hg reached the retina as no labeling of the optic system was observed in intravenously injected fish (Figure 3).

Axonal transport of Hg may also take place in motor neurons. In rodents, Hg(II) injected intramuscularly is taken up into nerve terminals of motor plates and transported to motor nerve nuclei, both in brain and spinal cord (*30*−*32*). Furthermore, accumulation of Hg(II) in motor neurons of rhombencephalon and spinal cord can occur due to a leakage of the metal from blood vessels into muscle and subsequent uptake in motor nerve terminals (*33*, *34*). In trout, the primary motor neurons that may be involved are those of Vth to Xth cranial motor nerves, which originate from nuclei located in the rhombencephalon, and the spinal motor neurons cell bodies located in the ventral horns of spinal cord (*19*). Fibers from proprioceptive receptors in muscle are also a potential transfer route of Hg toward the medulla of corpus cerebelli. The presence of Hg in blood indicates that metal may have leaked from blood vessels into muscle and then been taken up by motor nerve terminals and proprioceptive receptors. The spotty labeling of the spinal cord (Figure 2G) further supports the occurrence of such a process in fish. In the case of rhombencephalon and medulla of corpus cerebelli, it is not possible to assess the contribution of axonal transport in motor nerve fibers to the labeling of these brain structures with the present set of data, since they both receive primary

sensory and motor nerve fibers. What may appear to be an inconsistency is that no labeling of spinal cord or rhombencephalon was observed in fish that received Hg intravenously (Figure 3), while this was clearly evidenced in rodents (*33*, *34*). It is possible that the short exposure (14 d) of X-ray film to sections from intravenously injected fish was not sufficient to induce film blackening at locations corresponding to motor nerve ganglions. Due to the high ^{203}Hg content of these sections, longer exposure resulted in fogging, especially in areas besides highly labeled liver and kidney.

The specific distribution of Hg in the central nervous system of fish following water exposure strongly suggests that the metal can be taken up in nerve terminals of various sensory and motor systems and subsequently transported toward the brain by axonal transport, thus circumventing the apparently tight blood−brain barrier. Hg causes various perturbations of brain processes (*35*−*37*) and Hg reaching the nervous system has been shown to persist for years in nerve cells (*38*). Fish depend on an intact nervous system for mediating relevant behavior such as searching for food, recognizing predators, communication, and orientation (*37*). If uptake of waterborne Hg in nerve terminals and axonal transport result in a significant accumulation of the metal, the integrity of the nervous system might be jeopardized. Further work on accumulation kinetics of Hg in brain via axonal transport, at an environmentally relevant concentration of the metal in water ($ng \cdot L^{-1}$) and over fish lifetime, are needed to assess the toxicological significance of this process. This should be done with regard to water chemistry, since it strongly affects the speciation of dissolved inorganic Hg(II) and thus its bioaccumulation by aquatic organism (*17*). Axonal transport might also lead to the accumulation in the brain of other xenobiotics that would not pass through blood−brain barrier otherwise.

Acknowledgments

The Swedish Foundation for Strategic Environmental Research and the Swedish Institute funded this work. Authors gratefully acknowledge the comments of three anonymous reviewers, the help of R. Roy for linguistic revision of the manuscript, and the skillful technical assistance of A. Boström.

Literature Cited

(1) Arvidson, B. *Toxicology* **1994**, *88*, 1.
(2) Gottofrey, J.; Tjälve, H. *Pharmacol. Toxicol.* **1991**, *69*, 242.
(3) Tjälve, H.; Mejàre, C.; Borg-Neczak, K. *Pharmacol. Toxicol.* **1995**, *77*, 23.
(4) Tjälve, H.; Gottofrey, J.; Björklund, I. *Toxicol. Environ. Chem.* **1986**, *12*, 31.
(5) Rouleau, C.; Tjälve, H.; Gottofrey, J.; Pelletier, É. *Environ. Toxicol. Chem.* **1995**, *14*, 483.
(6) Harden Jones F. R. *Fish migration*; Edwards Arnold Publishers Ltd.: London, 1968.
(7) Clarkson, T. W. In *Mercury Pollution: Integration and Synthesis*; Watras, C. J., Huckabee, J. W., Eds.; Lewis Publishers: 1994; pp 631−642.
(8) Borg-Neczak, K.; Tjälve, H. *Toxicol. Lett.* **1996**, *84*, 107.
(9) Borg-Neczak, K. Ph.D. dissertation, Swedish University of Agricultural Sciences, 1994.
(10) Ullberg, S.; Larsson, B.; Tjälve, H. In *Biological applications of radiotracers*; Gleen, H. J., Ed.; CRC Press: Boca Raton, 1982; pp 56−108.
(11) Oliveira Ribeiro, C. A.; Rouleau, C.; Pelletier, E.; Audet, C.; Tjälve, H. *Environ. Sci. Technol.* **1999**, *33*, 902.
(12) Montgomery, S.; Mucci, A.; Lucotte, M.; Pichet, P. *Can. J. Fish. Aquat. Sci.* **1995**, *52*, 2483.
(13) Mason, R. P.; Sullivan, K. A. *Environ. Sci. Technol.* **1997**, *31*, 942.
(14) Kannan, K.; Smith, R. G., Jr; Lee, R. F.; Windom, H. L.; Heitmuller, P. T.; Macauley, J. M.; Summers, J. K. *Arch. Environ. Contam. Toxicol.* **1998**, *34*, 109.
(15) Miles, C. J.; Fink, L. E. *Arch. Environ. Contam. Toxicol.* **1998**, *35*, 549.
(16) Schecher, W. D.; McAvoy, D. C. *Comput. Environ. Urban Systems* **1992**, 16, 65.
(17) Mason, R. P.; Reinfelder, J. R.; Morel, F. M. M. *Environ. Sci. Technol.* **1996**, *30*, 1835.
(18) Boudou, A.; Ribeyre, F. *Water Res.* **1984**, *18*, 81.
(19) Nieuwenhuys, R. *Am. Zool.* **1982**, *22*, 287.
(20) Paul, D. H. In *Advances in comparative physiology and biochemistry*; Lowenstein, O., Ed.; Academic Press: New York, 1982; pp 111−177.
(21) Hayle, T. H. *J. Comput. Neurol.* **1973**, *149*, 477.
(22) Koester, D. M. *J. Comput. Neurol.* **1983**, *221*, 199.
(23) Szabo, T.; Libouban, S.; Haugede-Carre, F. *Brain Res.* **1979**, *168*, 619.
(24) Hoar, W. S.; Randall, D. J. *Fish physiology*; Academic Press: New York, 1971; Vol. V.
(25) Knull, H. R.; Wells, W. W. *Brain Res.* **1975**, *100*, 121.
(26) Finger T. E.. *J. Comput. Neurol.* **1978**, *181*, 173.
(27) McQuarrie, I. G.; Grafstein, B. *Brain Res.* **1982**, *235*, 213.
(28) Edwards, D. L.; Grafstein, B. *Brain Res.* **1986**, *364*, 258.
(29) Perry, G. W.; Burmeister, D. W.; Grafstein, B. *J. Neurosci.* **1990**, *10*, 3439.
(30) Arvidson, B. *Exp. Neurol.* **1987**, *98*, 198.
(31) Arvidson, B. *Acta Neurol. Scand.* **1990**, *82*, 234.
(32) Arvidson, B. *Neurotoxicology* **1992**, *13*, 277.
(33) Arvidson, B. *Muscle Nerve* **1992**, *15*, 1089.
(34) Schiønning, J. D. *Toxicol. Appl. Pharmacol.* **1993**, *121*, 43.
(35) Miyamoto, M. D. *Brain Res.* **1983**, *267*, 375.
(36) Baatrup, E.; Døving, K. B.; Winberg, S. *Ecotoxicol. Environ. Safety* **1990**, *20*, 269.
(37) Baatrup, E. *Comp. Biochem. Physiol.* **1991**, *100C*, 253.
(38) Hargreaves, R. J.; Evans, J. G.; Janota, I.; Magos, L.; Cavanagh, J. B. *Neuropath. Appl. Neurol.* **1988**, *14*, 443.

Received for review January 4, 1999. Revised manuscript received June 29, 1999. Accepted July 1, 1999.

ES990001V