

Congenital Neuroglial Heterotopia in a Neonatal Harbor Seal (*Phoca vitulina richardsi*) with Evidence of Recent Exposure to Polycyclic Aromatic Hydrocarbons

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ABSTRACT: A male neonatal Pacific harbor seal (*Phoca vitulina richardsi*) stranded off the coast of California, USA, was presented for rehabilitation with numerous partially haired, soft tissue masses around the mouth and in the oropharynx. Because of the extent of the lesions, the seal was humanely euthanized. Histologically, the masses consisted of subepithelial connective tissue and subcutis expanded by a proliferation of streams and bundles of spindle to stellate cells. Morphology of these cells suggested a neural origin, which was confirmed by positive immunohistochemistry for two neural markers, S-100 protein and glial fibrillary acidic protein, so the masses were diagnosed as neuroglial heterotopia. Heterotopic neuroglial tissue is a rare lesion comprised of benign mature neural tissue in an ectopic location with no connection to the central nervous system. Results of polycyclic aromatic hydrocarbon (PAH) metabolite analysis of bile indicated recent exposure to a petroleum source. Although fetal exposure to PAHs in utero can cause neurotoxicity and affect normal embryonic development, it is unknown whether gestational exposure occurred in this case.

Key words: Congenital defect, neuroglial heterotopia, PAH, oil, harbor seal, *Phoca vitulina*.

Congenital defects, defined as developmental abnormalities of structure or function present at birth, are typically diagnosed in free-ranging marine mammals as gross anatomic lesions in young stranded animals (Gulland et al., 2001). Congenital anomalies reported in harbor seals (*Phoca vitulina*) include abnormal tooth number

(Colyer, 1936; Suzuki et al., 1990), alopecia and dental aplasia (King, 1964), ectrodactyly (Tarasoff and Pierard, 1970), cleft palate (Suzuki et al., 1992), penile deviation (Spraker et al., 1994), absence of the right cerebral hemisphere (McKnight et al., 2005), development of a rare conjoined twin known as a fetus in fetu (Buckles et al., 2006), and malformation of the occipital bone (Dennison et al., 2009). Neuroglial heterotopia is an aberrant migration of neural tissue outside the central nervous system (CNS) that occurs rarely in human infants and has been reported in one domestic kitten (Cox et al., 1997). We report the first case of neuroglial heterotopia in a wildlife species and explore the possible role of gestational petroleum exposure in the development of this lesion.

A male neonatal harbor seal pup stranded in San Francisco Bay, California (37°52'12"N, 122°29'48"W) on 14 April 2008 was transported to The Marine Mammal Center, Sausalito, California for medical assessment. The seal was malnourished (7.6 kg), active, and vocal, with a fleshy umbilical stump, suggesting the animal was ≤ 3 days old. Numerous partially haired, soft tissue masses (1–4-cm diameter) were observed around the mouth, apparently originating from the mandibular gingiva (Fig. 1). Several of the



FIGURE 1. Oral soft tissue masses in a neonatal harbor seal stranded off the coast of California, USA.

more ventral masses were covered in spotted tan and gray hair characteristic of harbor seal pelage; others closer to the oral opening were covered in glabrous skin of variable pigmentation with superficial abrasions, presumably from physical trauma. One mass was pedunculated, connected to the gingiva by a thin stalk of tissue. Two additional partially haired masses (1-cm diameter) were seen in the oropharynx, between the base of the tongue and the left tonsil. The masses did not obstruct the airway or prevent orogastric tube feeding for hydration and nutritional support. No obvious craniofacial deformities were noted on physical examination. Blood results were consistent with malnutrition and dehydration, so supportive care and antibiotics were initiated to stabilize the pup prior to further evaluation.

On Day 8, the seal was anesthetized with propofol (5 mg/kg) and isoflurane (1–5%) for closer examination (Gulland et al., 1999). Radiographs of the mandible suggested possible lucencies in the bone underlying the soft tissue masses; however, the bone was poorly calcified so results were inconclusive. Due to the extent and location of the lesions, the seal was humanely euthanized.

On postmortem examination, 10 individual firm soft tissue masses were identified, clustered along the left ramus of the mandible (Fig. 2). The masses originated



FIGURE 2. Oral masses originating from the mandibular gingiva, clustered along the left ramus of the mandible of a harbor seal stranded off the coast of California, USA.

from the mucocutaneous junction and were tightly adhered to the underlying periosteum. There were two oral masses in the pharyngeal tissue adjacent to the left tonsil (Fig. 3). Closer examination of the mandible revealed smooth cortical bone with appropriate tensile strength and no evidence of lytic lesions or sites of bony invasion. The left mandibular lymph node and salivary gland were markedly enlarged and contained abnormal firm nodules on the cut surface.

Representative tissue samples were



FIGURE 3. Two partially haired oral masses at the base of the tongue, adjacent to the left tonsil of a harbor seal stranded off the coast of California, USA.

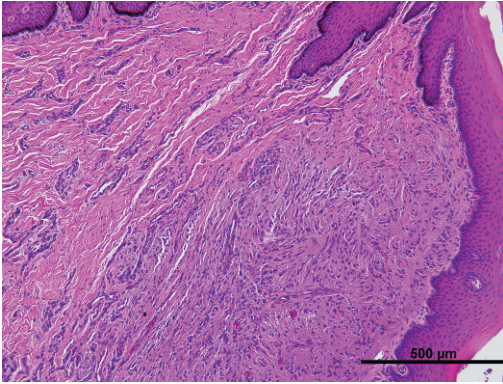


FIGURE 4. Oral mass with a densely cellular proliferation of spindle cells expanding the subepithelial connective tissue in a harbor seal stranded off the coast of California, USA. H&E stain.

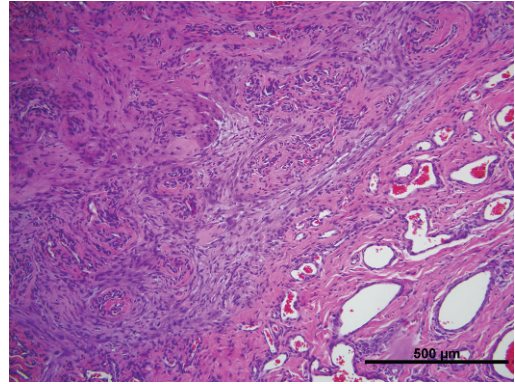


FIGURE 5. Oral mass composed of spindle cells arranged in haphazard streams and bundles in a harbor seal stranded off the coast of California, USA. H&E stain.

fixed in 10% neutral-buffered formalin, routinely processed, embedded in paraffin by conventional methods, sectioned at 4–6 microns, and stained with hematoxylin and eosin (H&E). Immunohistochemical staining was performed with the use of Ventana Autostainers (Benchmark or XT platforms, Ventana Medical System, Inc., Oro Valley, Arizona, USA) S-100 protein (1:4,800, polyclonal rabbit anti-S-100) and glial fibrillary acidic protein (GFAP; 1:8,000, polyclonal rabbit antibody). Histologically, the masses expanded the subepithelial connective tissue (Fig. 4), and less often the subcutaneous adipose tissue. The masses were composed of a moderately cellular proliferation of haphazard streams and bundles of spindle to stellate cells separated by a pale myxomatous matrix (Fig. 5). Cells had indistinct borders and small amounts of pale eosinophilic cytoplasm. Nuclei were elongated and hyperchromatic. Mitotic figures were not observed. Histologic morphology suggested a cell of neural origin, which was confirmed by positive immunohistochemistry for two neural markers, S-100 protein and GFAP. Diffusely, cells had intranuclear and intracytoplasmic positive reactivity to S-100 protein. Multifocally, the cytoplasm was immunoreactive for GFAP. Because of the histologic and immunohis-

tochemical characteristics of these masses and the young age of the seal, specialists in pediatric pathology at the Department of Pathology, Bethesda Naval Hospital were consulted and confirmed a diagnosis of neuroglial heterotopia, which is most commonly diagnosed in human infants. The salivary gland contained a focal infarct, most likely secondary to ischemia.

As part of a health survey of Pacific harbor seals, blubber collected from the caudal gluteal region was analyzed for congener-specific polychlorinated biphenyls (PCBs), dichlorodiphenyl trichloroethane (DDT) and DDT metabolites, and polybrominated diphenyl ether (PBDE) flame retardants with the use of a gas chromatography/mass spectrometry method (Sloan et al., 2005). Lipid-adjusted concentrations of these persistent organic pollutants (Σ PCB=20,793 ppb; Σ DDT=19,494 ppb; Σ PBDE=1,863 ppb) were within the range of those measured in other free-ranging harbor seal pups from the central coast of California, but were on the high end of the range (DJG). Concentrations of heavy metals including lead (not detected), mercury (0.087 ppm), and selenium (0.52 ppm) were measured in whole blood with the use of atomic absorption spectrometry for lead (AAAnalyst 800, Perkin Elmer, Waltham, Massa-

chusetts, USA), and hydride vapor generation inductively coupled argon plasma atomic emission spectrometry for mercury and selenium (Fisons-ARL, Accuris, Eschubens, Switzerland). Blood mercury concentration was similar to levels found in other harbor seal pups from central and northern California (0.015–0.555 ppm; Brookens et al., 2007). Bile was analyzed for metabolites of polycyclic aromatic hydrocarbons (PAHs) with the use of high-performance liquid chromatography with fluorescence detection, as described in Krahn et al. (1986). Polycyclic aromatic hydrocarbon metabolites (naphthalene equivalents = 62,000 ppb; phenanthrene equivalents = 10,000 ppb; benzo[a]pyrene equivalents = 230 ppb) were measured in the bile, indicating recent exposure to petrogenic sources such as diesel fuel or crude oil, and a lower level of exposure to pyrogenic sources from the combustion of fossil fuels. Our findings suggest that the neonatal seal was exposed to a source of petroleum.

Neuroglial heterotopia is a rare non-neoplastic lesion comprised of benign mature glial tissue in an ectopic location with no connection to the central nervous system. Other terms used synonymously in the literature include glial choristoma (a mass of normal cells in an ectopic location), neuroglial hamartoma (abnormal growth of tissue that would normally occur at that site), heterotopic brain tissue, accessory brain, and nasal glioma; all but the term *choristoma* should be considered distinct lesions (Al-Nafussi et al., 1990; Harris et al., 1994; Roy and Gungor, 2002; Oya et al., 2005). Neuroglial heterotopia consists of mature neuroectodermal tissue including astrocytes and glial fibers embedded in a fibrovascular stroma (Ide et al., 1997), and may include other cellular components such as neurons, choroid plexus, and ependyma (Madjidi and Couly, 1993). This lesion typically occurs as a firm-to-cystic, solitary, space-occupying mass and may contain cerebrospinal fluid, presumably from autogenous pro-

duction by choroid plexus tissue (Hendrickson et al., 1990). In humans, congenital extracranial heterotopic neuroglial tissue occurs most commonly in the sinonasal region on midline due to anatomic proximity to the central nervous system (Aanaes et al., 2008), but has also been associated with the nasopharynx (Al-Ammar et al., 2006), hard and soft palate (Al-Nafussi et al., 1990; Ide et al., 1997; Anjaneyulu and Deka, 2004), tongue (Fan et al., 2008), lip (Pasyk et al., 1988), buccal region (Aanaes et al., 2008), middle ear (Gyure et al., 2000), face and neck (Hendrickson et al., 1990), chest wall (Shepherd et al., 1987), scalp (McDermott et al., 1996), and lung (Morgan et al., 2003). All existing reports describe a single mass, rather than a cluster of soft tissue masses; however, one facial tumor exhibited fibrous adherence to the periosteum of the facial bones, similar to this harbor seal (Hendrickson et al., 1990). The only report of this lesion in the veterinary literature involves a single pharyngeal mass in a 5-wk-old domestic kitten causing displacement of the tongue without enlargement of the adjacent lymph nodes, with no recurrence after surgical resection (Cox et al., 1997). The only similar congenital lesion in a marine mammal was an intracranial subcortical heterotopia in a juvenile California sea lion (*Zalophus californianus*), with a mass of gray matter in the subcortical white matter of the cerebrum, presumably caused by a neuronal migration defect (Blankenship et al., 2008).

The two main theories on the pathogenesis of extracranial heterotopic neuroglial tissue are the sequestration of an encephalocele, in which an irregularity in the neural tube results in the herniation of glial tissue through a bony defect with subsequent loss of connection to the CNS, and the abnormal embryonic migration of pleuripotent neuroectodermal cells from the CNS to an ectopic location during early embryogenesis (McDermott et al., 1996; Oya et al., 2005). Differentials for

the lesion in this neonatal harbor seal included fibrous hamartoma of infancy, dermal fibromatosis, and neoplasia of neural origin.

The etiology of neuroglial heterotopia is unknown, but may be related to in-utero infection, maternal toxin exposure, injury, genetic predisposition, or an ischemic event during the late first or early second trimester of gestation (Barkovich, 1999). The potential relationship between fetal petroleum exposure and the development of morphologic abnormalities is not well understood. In humans, fetal exposure to PAHs during late gestation may disrupt the development of the neonatal immune system, affecting disease susceptibility and altering neurodevelopment (Wormley et al., 2004; Hertz-Picciotto et al., 2008). Short-term exposure of PAHs in pregnant mice during late gestation induced lymphoma in offspring via transplacental and lactational transfer (Castro et al., 2008). Teleost fish embryos exposed to PAH mixtures similar to the composition of crude oil developed cardiac conduction abnormalities, defects in neural tube structure, and deformities of the craniofacial skeleton (Incardona et al., 2004). Pregnant sea otters exposed to crude oil during the Exxon-Valdez oil spill experienced abortion and stillbirth, and transferred petroleum hydrocarbons to their nursing pups via lactation (Tuomi and Williams, 1995). In harbor seals, PAHs affect cell mediated immunity through the suppression of T lymphocyte proliferation and impairment of lymphocyte function *in vitro* (Neale et al., 2002, 2005). It is difficult to establish a causal link between petroleum exposure and direct biologic effects in aquatic wildlife due to confounding variables such as species specific metabolism, individual migration patterns and foraging preferences, and background exposure from natural seeps and pyrogenic sources in the sediment (Lee and Anderson, 2005). The role of PAH exposure in the development of congenital neuroglial heterotopia in this neonatal

harbor seal is unclear, but cannot be ruled out.

Chromatographic patterns of bile from marine animals have been used to propose the possible sources of PAH contamination as pyrogenic or petrogenic, based on the predominance of two–three–ringed vs. four–five–ringed metabolites (Krahn et al., 1993). The chromatographic pattern of the bile of our study animal recorded at phenanthrene wavelengths (260/380 nm) was more similar to the biliary pattern of Atlantic salmon exposed to Monterey crude oil for 48 hr and harbor seals that stranded in Prince William Sound approximately 1–5 mo after the Exxon-Valdez oil spill (Krahn et al., 1992; chromatogram not shown), than to the typical pattern of seals that strand in the urban environment of San Francisco Bay, California (Fig. 6).

Although the timing and route of petroleum exposure for this neonate are unknown, it could have occurred in utero as a result of transplacental transfer from a maternal exposure, or during the first few days of postnatal life via lactational transfer or direct ingestion, inhalation, or dermal absorption. If petroleum exposure occurred after birth, it would negate any connection with this complex congenital lesion, which developed during early to mid gestation. However, the poor nutritional condition of the pup at stranding indicates that it may have had difficulty nursing with its oral masses, making lactational transfer or oral ingestion less likely. Increases in biliary concentrations of petrogenic PAHs reflect recent exposure to petroleum over a period of days to weeks, and may remain elevated for up to a year, according to laboratory studies of fish exposed to crude oil in water (Lee and Anderson, 2005); however, comparable studies are not available for marine mammals. Regardless of the route of exposure, adult vertebrates rapidly metabolize PAHs in the blood stream to more polar compounds through the hepatobiliary system, most likely via induction of the mixed-function oxidases, and then con-

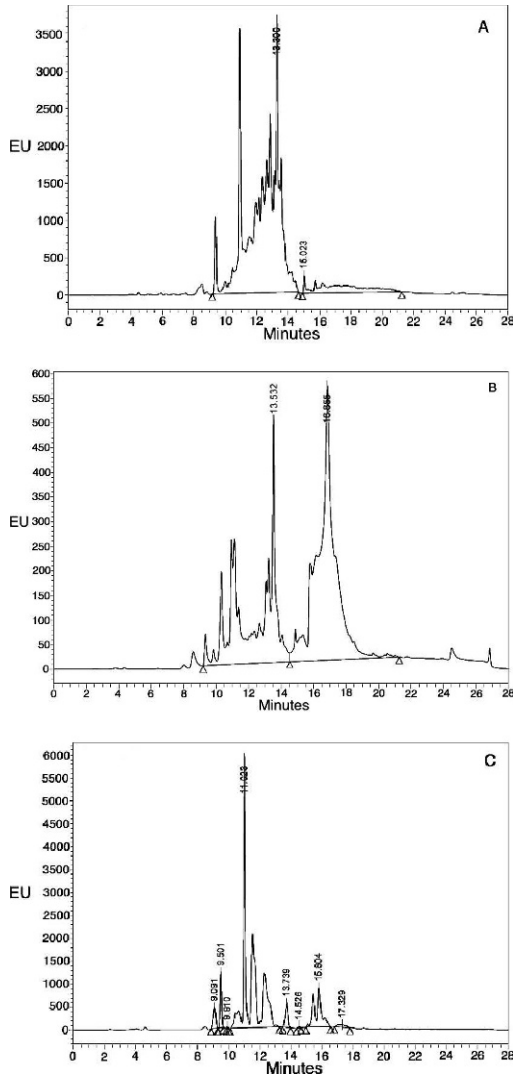


FIGURE 6. Biliary polycyclic aromatic hydrocarbon (PAH) metabolite chromatograms collected at the phenanthrene wavelength pair for (A) an Atlantic salmon exposed to 25 ppb Monterey crude oil for 48 hr in a laboratory, (B) our study animal (a stranded harbor seal), and (C) a free-ranging adult female harbor seal from San Francisco Bay, California, USA. Note the characteristic two-hump (9–15 min and 15–21 min) chromatographic pattern of oil exposure in both the salmon and our study animal, as compared with the pattern of multiple peaks with no humps associated with urban pyrogenic PAH exposure.

concentrate metabolites in the bile for elimination (Varanasi et al., 1989; Hellou, 1996). However, if an animal has been exposed to a large amount of petroleum,

such as an oil spill, the liver enzyme system can become overwhelmed such that it cannot efficiently metabolize the PAHs, so parent and alkylated petroleum-related PAHs might be measured in concentrations well above background levels in bile and tissues such as liver and muscle. Although maternal transfer of petroleum hydrocarbons to the developing fetus has been documented in humans, laboratory animals, and sea otters during the Exxon-Valdez oil spill (Tuomi and Williams, 1995; Perera et al., 1999; Tozuka et al., 2004), little is known about fetal metabolism of these compounds. During human fetal development, the immature hepatobiliary excretory system is not yet functional, so the placenta functions to metabolize and excrete toxins back to the maternal system for elimination and also acts as a barrier to protect the fetus from potentially toxic compounds in maternal blood (Macias et al., 2009). However, if one or both of these mechanisms is impaired or overwhelmed by an acute exposure, toxins could enter fetal circulation, resulting in deleterious effects to the fetus (Macias et al., 2009). A large bunker fuel spill (58,000 gallons) occurred in San Francisco Bay in November 2007, which corresponds roughly with the late first trimester to early second trimester of this pup's gestation (Reidman, 1990); however, exposure may have occurred from petroleum products that entered the bay via nonpoint-source discharges. Although some harbor seals travel extensively during gestation (Miller, 1988; Greig, 2002), others are more residential (Nickel, 2003), so it is unknown whether this pup's dam was present in San Francisco Bay during the November spill. Although there is not a clear link between the unusual lesions and PAH exposure in this animal, the recent documentation of three rare developmental anomalies in harbor seal pups from central California (McKnight et al., 2005; Buckles et al., 2006) raises concerns about the role of potential teratogenic contaminants in this

region and highlights the need for further investigation.

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Submitted for publication 29 December 2009.

Accepted 8 October 2010.