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Mercury associated neurochemical response in Arctic barnacle goslings (*Branta leucopsis*)

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HIGHLIGHTS

- Arctic coal mine impacted site showed elevated Hg concentrations.
- Differences in soil Hg were reflected in hepatic concentrations of goslings.
- Brain levels of D2-receptors in Arctic birds were related to hepatic Hg levels.
- The use of siblings increased the statistical resolution of the experiment.

GRAPHICAL ABSTRACT

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ABSTRACT

There remains great concern over mercury pollution in the Arctic, though relatively little is known about impacts on biota that inhabit Arctic terrestrial systems. To help address this, the current study was performed with barnacle goslings (*Branta leucopsis*) from a coal mine-impacted site and a control site near Ny-Ålesund, Spitsbergen (Svalbard). The works focused mainly on mercury, as coal contains trace levels of this element. Total mercury concentrations were quantified in soil and vegetation from the two sites, as well as feces and liver from the goslings. Next, the mercury exposures were related to dopamine 2 (D2)- and NMDA-receptors in the brain, given that mercury is a proven neurotoxicant. Soil and vegetation in the mining area contained mercury levels that were approximately 3- and 2.2-times higher than in the control site. Despite a significant difference between the sites, the soil and vegetation mercury levels were within ranges found at other Arctic locations. Goslings grazing in the mine-impacted area contained significantly higher hepatic mercury levels than those sampled from the control site. Compared to other species, the hepatic concentrations were relatively low possibly due to dilution of the mercury in growing goslings (growth dilution) and deposition of mercury in the growing feathers. Hepatic mercury concentrations were positively related to D2-neuroreceptor levels but not to NMDA-receptor levels thus suggesting a possible subtle neurological effect. To our knowledge, this is among the first studies on mercury exposure in Arctic terrestrial organisms, and one of the first to document potential subtle neurological responses associated with exposure to low, environmentally relevant mercury levels, which also can be found at other locations in the Arctic. However, as a pilot effort, the results here need to be examined in

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additional studies that include, for example, larger study designs, different geographic sites and other terrestrial species.

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1. Introduction

Mercury (Hg) is a trace metal found throughout all parts of the world (Driscoll et al., 2013). Depending on its speciation, mercury has the tendency to accumulate in food webs (Douglas et al., 2012), resulting in elevated levels in organisms at higher trophic levels and potentially impacting exposed individuals (Kobiela et al., 2015). Due to biochemical processes, mercury is subject to long-range atmospheric transport and can thus deposit in remote areas such as the Arctic (Douglas et al., 2012). In addition to long-range transport, human activities in the Arctic like coal mining have resulted in locally elevated concentrations of mercury (Poissant et al., 2008). After deposition, mercury can be transformed into organic methylmercury (Gamberg et al., 2015) which is more bioavailable (Poissant et al., 2008) and generally more toxic (Boening, 2000). Over 90% of mercury in Arctic organisms can be attributed to anthropogenic sources (Dietz et al., 2009), ranging from e.g. non-ferrous metal, iron and steel production, cement production, waste incineration and coal fired power generation to artisanal gold production (Muntean et al., 2014). Temporal trends in mercury concentrations vary among Arctic sites, but it has been shown that on average concentrations in Arctic biota have increased 0.5% annually (Riget et al., 2011).

In the Arctic, exposure to mercury and associated responses have been illustrated in a number of marine mammals birds, and fish species (Dietz et al., 2011). However, although recent data on mercury concentrations in terrestrial Arctic ecosystems are available on e.g. Arctic soils (Choy et al., 2010; Krajcarová et al., 2016; Wojtun et al., 2013), vegetation (Choy et al., 2010; Krajcarová et al., 2016; Poissant et al., 2008; Wojtun et al., 2013), caribou (*Rangifer tarandus*) (Braune et al., 1999; Gamberg et al., 2015; Riget et al., 2004), Arctic fox (*Vulpes lagopus*) (Bocharova et al., 2013; Dehn et al., 2006; Treu et al., 2017), wolf (*Canis lupus*) (McGrew et al., 2014) and arctic hare (*Lepus arcticus*) (Pedersen and Lierhagen, 2006), we are not aware of studies that have aimed to link responses of Arctic terrestrial species to exposure to environmental mercury. Exposure to mercury may lead to a range of adverse health outcomes in wildlife even at low chronic exposures (Becker et al., 2017; Hawley et al., 2009; Scheuhammer et al., 2012; Spalding et al., 2000), such as embryo toxicity in birds (Yu et al., 2016), immune modulation in birds (Fallacara et al., 2011; Hawley et al., 2009) and mammals (Becker et al., 2017; Kim et al., 2003) and neurochemical and morphological effects in the brains of different species (Arini et al., 2016; Basu et al., 2007b; Nam et al., 2012; Yu et al., 2017). Effects of mercury on levels of neuroreceptors may be induced via different pathways. For examples mercury may affect the stimulation of the *N*-methyl *D*-aspartate receptor (NMDA receptor) via interaction with the uptake of glutamate in synapses (Basu et al., 2007b). Mercury may lower the activity of monoamine oxidase (Berntssen et al., 2003), which is involved in the metabolism of dopamine, a pathway along which mercury may induce dopamine receptor-mediated effects. Several studies have focused on the effects of modulation of D2 receptors on the behavior of animals. For example, female chickens (*Gallus gallus domesticus*) injected with a D2-receptor antagonist showed decreased pecking behavior (Kjaer et al., 2004) and, reduced aggression (Dennis and Cheng, 2011), while exposure to a D2-receptor antagonist suppressed head movements and foraging behavior (Moe et al., 2014). In turkeys (*Meleagris gallopavo*), injection with a D2-receptor antagonist decreased brooding behavior (Thayanunphat et al., 2011), while D2 receptor expression in starlings (*Sturnus vulgaris*) was negatively related

to vocal activation (DeVries et al., 2015). Although this overview on effects of D2-receptor levels is not exhaustive, it illustrates that changes in D2 receptor levels, potentially induced by exposure to mercury, may affect organismal behavior.

To address the knowledge gap on neurotoxic effects that mercury may have on Arctic terrestrial species, an exposure experiment was conducted with barnacle goslings (*Branta leucopsis*) from the Arctic tundra. Barnacle goslings were selected because at this age, they are not exposed to other than local sources of mercury (apart from maternal transfer), they ingest (contaminated) grid and vegetation, and they can be imprinted on humans allowing to guide them to specific locations, and as such their exposure can be manipulated. In this experiment, human-raised goslings were exposed to locally deposited mercury, related to historic coal mining activities, over the course of their development. Two groups of goslings were led systematically to either a mercury contaminated site or a control area, under environmental relevant conditions. Detailed analyses of exposure, internal mercury concentrations and specific levels of neuroreceptors, D2 and NMDA receptors in the brains, were performed to gain a comprehensive insight in the relationships in species specific exposure and effects of mercury. It was expected that tissue concentrations of mercury in goslings are elevated in individuals feeding in the mining areas, and that brain D2 receptor levels are positively, but NMDA receptor negatively related to mercury levels.

2. Materials and methods

2.1. Sites

The experiment was conducted in the vicinity of Ny-Ålesund, Spitsbergen (Svalbard, 78.55°N, 11.55°E) (de Jong et al., 2017). Near Ny-Ålesund, different coal mines were in operation from 1916 to 1963, approximately 1 km SE of the village, with intermittent periods of inactivity. In 1967, the activities terminated after a fatal incident and the mine was abandoned. In the mining area, however, remains of the mining activities are still clearly visible. Large piles of coal and abandoned installations and equipment are littered in the area. Since the area was deserted, vegetation has re-established to a certain extent, which is available for grazing and geese are known to utilize the area (pers. obs). The area was expected to be contaminated by coal-associated chemicals, among which mercury (Hylander and Goodsite, 2006). The control area is a vegetated tundra, 2 km WNW of the village, which is also used by geese to graze (pers. obs.).

2.2. Goslings and experimental design

Barnacle goslings were collected from Indre Brøygane, an uncontaminated island near Ny-Ålesund on June 30th 2014, as described in de Jong et al. (2017). Pipping eggs were marked at June 29th in order to ensure that goslings hatched on the same day. From 8 nests, two siblings each were collected, immediately marked with web-tags as well as a unique color ring, and randomly assigned to either the “control” or “mine” group. Eight goslings per group was the maximum that could be handled, and mimics the high end of natural goose families. Goslings were raised by humans as foster parents, and from day 5 onwards were herded to their respective areas for grazing (Martin and Forsyth, 1998). At the beginning of the experiment, goslings spent about 160 min per day in their respective grazing areas and

this increased to about 360 min per day later on. This gradual shift mainly depended on age of the goslings and weather conditions. On rare occasions, and particularly at the beginning of the experiment, all goslings were provided with measured amounts of supplemental feed (Anseres I waterfowl starter pellets, Kasper Faunafood, Woerden, The Netherlands) during their walks to ensure they were not nutritionally deprived and kept healthy during periods of bad weather.

The goslings were 23 days old when they were sacrificed through decapitation and immediately dissected (de Jong et al., 2017). Liver tissue was collected for mercury analyses and stored in polyethylene blue cap tubes at -20°C . Because concentrations were likely to be relatively low in most tissues, liver was selected since concentrations were expected to be highest in this tissue (Tsipoura et al., 2011). Brains were snap frozen in liquid nitrogen and stored in blue cap tubes at -80°C for analysis of neuroreceptor levels.

2.3. Soil, vegetation and dropping sampling

Soil was collected during the experiment from both sites randomly ($n = 7$ for mining area, $n = 6$ for control) from the surface (upper 5 cm) with a large PVC spoon. Plant material and stones were removed from the soil samples. Samples were stored in polyethylene bags at -20°C , and shipped frozen to the Netherlands. Vegetation from the same locations of the soil was clipped with scissors to collect above-ground plant parts from the two sites ($n = 4$ each site). Plant material was also stored in polyethylene bags at -20°C . As much as possible, single floral type samples were collected from moss, as well as *Carex* spp., and *Saxifraga* spp.

As an indication of actual exposure, droppings from goslings were collected after they had foraged for a minimum of three hours in their respective areas. Gut passage time in adult barnacle geese is approximately 2–4 h (Prop and Vulink, 1992), and droppings produced after being at a site for such period may be used as a proxy of actual mercury exposure. Within this time frame, droppings of goslings that foraged in the mining area turned dark likely because of the occurrence of coal particles in their droppings. Droppings from birds feeding on the additional feed were also collected at night, after having foraged on the additional feed for a minimum of 4 h, in order to assess potential exposure to mercury related to this feed source. No additional feed was available at the time of mercury analysis, but in this indirect way it is possible to assess the actual exposure of the goslings to the additional feed, relative to the vegetation from the two sites. Droppings were stored at -20°C in polyethylene bags ($n = 6$ for control site; $n = 5$ for mining site; $n = 6$ for additional feed).

2.4. Chemical analyses

Soil, vegetation droppings and liver tissues (concentrations in other tissues like e.g. brains, were expected to be too low for analyses) were analyzed for total mercury with cold vapor/atomic fluorescence spectrometry (Hoogenboom et al., 2015). Approximately one gram of dried sample digested in 10 mL nitric acid (70%) heated in a microwave. After digestion, samples were filled up to 50 mL with Milli-Q ultrapure water. All mercury species were reduced to metallic mercury with Sn(II)Cl_2 released from solution and quantified in their gaseous phase by fluorescence at 253.7 nm. All concentrations given are on total mercury, based on dry-weight (70°C , 48 h). A certified reference material was always included in the analyses. Samples were analyzed under ISO9001 accreditation and ISO 17025:2005 standard. The laboratory participates in inter-laboratory performance studies, including those organized by QUASIMEME (www.quasimeme.org). The results on certified reference materials (fish and fish liver) have always been labelled as “good”, according to the evaluation criteria.

2.5. Biochemical analyses

Membranes were prepared from gosling brains following Arini et al. (2016), with slight modifications. Of each sample, 1 g of cerebrum was homogenized in 10 mL (i.e. 1:10 average weight) of 50 mM Tris buffer (50 mM Tris HCl, 50 mM Tris Base, pH 7.4). Membranes were isolated by centrifugation of the homogenates at 48,000g for 15 min at 4°C . The resulting pellets were resuspended in 10 mL of Tris buffer. This operation was repeated twice for a total of three centrifugations per sample, after which each final pellet was resuspended in 3 mL of Tris and aliquoted. Aliquots were immediately frozen at -80°C until further analysis for neuroendocrine receptor-binding assays.

Radioligand binding to the NMDA and D2 receptors were performed using cellular membranes following Arini et al. and Basu et al., respectively (Arini et al., 2016; Basu et al., 2009). For NMDA, 300 $\mu\text{g/mL}$ of membrane preparation was incubated with $[3\text{H}]\text{-MK-801}$ (5 nM, 22.5 Ci/mmol; Perkin Elmer), and slowly vortexed for 120 min at room temperature. Non-specific binding was determined by incubating samples with 100 μM unlabeled MK-801. To minimize non-specific binding, plates for D2 were pre-wetted with a polyethyleneimine buffer (0.1%). Samples (300 $\mu\text{g/mL}$ of membrane preparation) were incubated first with 50 μM ketanserin (to block serotonin receptor binding) and next with $[3\text{H}]\text{-Spiperone}$ (3.2 nM, 15.3 Ci/mmol; Perkin Elmer) and slowly vortexed for 90 min at room temperature. Non-specific binding was determined by incubating samples with 100 μM unlabeled Butaclamol. All samples were assayed in quadruplicate and pooled control samples (chicken brain) were used to monitor variability between plates. Specific binding was defined as the difference between radioligand bound in the presence or absence of the respective displacers.

2.6. Statistical analyses

Analyses of Variance (ANOVA) were performed to assess differences in mercury concentrations in soil and vegetation between site (control/mine as a factor) and to analyze differences in weight, mercury concentrations and receptor levels among siblings (“Sibling” as factor). Least-significant-differences were used as post-hoc test. As mercury levels were not normally distributed (Shapiro-Wilk-test), we log-transformed the residue data prior to an ANOVA. To exclude the effect of sibling on further statistical analyses, receptor levels and hepatic mercury were normalized for the effect of sibling by subtracting the average of each sibling pair from the two corresponding individual sibling observations. The sibling-normalized observations were also analyzed for factorial effect of “Site” by performing ANOVA. To correlate potential relationships between receptor levels (both D2 and NMDA receptor) and log-transformed mercury concentrations, linear regressions were used. All tests are given two-tailed with an α of 0.05. Statistical analyses were performed with GENSTAT version 18.1 (VSN International Ltd., Hemel Hempstead, UK).

3. Results and discussion

3.1. Gosling development

On average, control and mine goslings did not differ in mass at the end of the experiment (de Jong et al., 2017), but the average mass of the siblings marked “Black” was significantly higher than the others (Table S11, ANOVA: $F = 3.63$; $n = 16$; residual d.f. = 8; $p = 0.046$). The lack of differences between sites was likely due to the fact that the goslings received additional feed, which was provided not to initiate growth limitation, potentially affecting the toxicokinetics/dynamics of mercury in the goslings. No additional significant differences in somatic indices e.g. relative organ weights, could be detected between or within the two groups of goslings (Table S11).

3.2. Mercury concentrations

3.2.1. Soil and vegetation

Mercury concentrations were significantly higher in soils from the mining area in comparison to the control area (Fig. 1A; ANOVA: $F = 7.68$; $n = 13$; residual d.f. = 11; $p = 0.018$). The mercury soil concentrations of both areas are approximately an order of magnitude lower than in soils from more industrialized areas in e.g. the Netherlands (Roodbergen et al., 2008) or Slovenia (Gnamuš et al., 2000), and reaching the lower soil concentrations in Switzerland (Ernst et al., 2008). Mercury levels in soil peat in the north of Norway as well as in peat soils from sub-Arctic locations at the Faroe Islands were approximately 5–10 times higher than the ones of the current study (Riget et al., 2000; Shoty et al., 2005). Mercury soil concentrations from East Greenland were below detection limits (<0.01 mg/kg dry), while concentrations ranged from 0.01 to 0.03 mg/kg dry weight in soils from three locations from West Greenland (Riget et al., 2000). The latter concentrations are similar to the levels found in our control site. In a more recent study at the northwestern side of the Hornsund fjord, Svalbard (77.00'N, 15.33'E), without local contamination, soil mercury concentrations ranged from 0.01 to 0.25 mg/kg dry weight, depending on the type of tundra (Wojtun et al., 2013). In a study near the town of Pyramiden, Svalbard (78.39'N, 16.20'E), with known coal mining activities in the past, mercury soil concentrations ranged from 0.004 to 0.736 mg/kg (Krajcarová et al., 2016). The concentrations detected in soils from the control site of the current study are in the lower range of the Pyramiden study, while the soils in the mine area contained mercury in the higher ranges of those found in Hornsund and the lower range of Pyramiden. This indicates that the concentrations detected in soil and vegetation from both our sites may be indicative for larger areas in the Arctic. Although differences between sites were relatively small (though significant), and a more contaminated site may have provided better options to detect toxic effects, it was decided to perform the study at the selected sites, as these are representative for true Arctic conditions.

The difference in soil concentrations between sites is reflected in the mercury concentrations in vegetation, albeit to a smaller degree (ratio between sites: 2.9 in soil versus 2.3 in vegetation, all samples combined). No significant differences could be detected among species (*Carex* spp. versus *Saxifraga* spp. versus moss; ANOVA: $F = 3.7$; $n = 8$; residual d.f. = 5; $p = 0.103$), hence all species are pooled to assess differences between sites. Concentrations in the vegetation (log-transformed) from the mining area were significantly higher (Fig. 1B, 0.060 versus 0.026 mg/kg dry weight; ANOVA: $F = 8.58$; $n = 8$; residual d.f. = 6; $p = 0.026$). Biota to Soil Accumulation Factors (BSAFs) with spatially matched soil and vegetation samples were significantly

higher in the control versus the mining vegetation (0.89 versus 0.40; ANOVA: $F = 25.16$; $n = 8$; residual d.f. = 6; $p = 0.002$). Mercury levels in moss samples ($n = 2$), which averaged 0.08 mg/kg, were in the same range as found in mosses from the US (Landers et al., 1995) and Canadian Arctic (Choy et al., 2010) and also from locations north of the Arctic circle in Finland (Poikolainen et al., 2004). Concentrations in vascular plants from the Hornsund fjord (Svalbard) ranged from 0.01 to 0.09 mg/kg dry weight (Wojtun et al., 2013), again similar our findings (0.02 to 0.06 mg/kg dry weight).

To summarize, concentrations from both soils and vegetation indicate that the mercury levels from both sites of the current study fell well into the range of previously reported mercury concentrations in soil and vegetation from various Arctic locations. Still, there were significant differences in concentrations in soil and vegetation between the mine contaminated versus control site in our study.

3.2.2. Droppings

Mercury concentrations (log-transformed) in droppings from goslings that fed in the mining area were significantly higher than in droppings collected from control goslings (Fig. 2, 0.086 versus 0.048 mg/kg dry weight; ANOVA: $F = 8.42$; $n = 16$; residual d.f. = 14; $p = 0.004$). The slightly higher mercury concentrations in droppings relative to the concentrations in vegetation indicate that the uptake rate of organic matter from the vegetation exceeds the uptake rate of mercury. Also the droppings of goslings that foraged longer than 4 h on supplemental feed, which both groups received overnight, contained mercury, at levels similar to the droppings from the control site (Fig. 2). This implies that both groups were exposed to mercury as a result of the supplemental feed they received, which might have (partially) decreased potential differences between the groups.

3.2.3. Liver tissue

Overall, hepatic mercury levels in goslings were relatively low, i.e. 0.02–0.04 mg/kg dry weight. Still, mercury levels in goslings which foraged in the mining area were significantly higher than in the ones from the control group (Fig. 1C, 0.030 versus 0.022 mg/kg dry weight; ANOVA: $F = 5.16$; $n = 16$; residual d.f. = 14; $p = 0.039$). After normalization for sibling, the significance of the difference between sites increased considerably (ANOVA: $F = 11.84$; $n = 16$; residual d.f. = 14; $p = 0.004$). Hepatic mercury concentration (log-transformed) did not correlate significantly with gosling mass, however (linear regression: variance ratio = 2.42; $n = 16$; residual d.f. = 14; $p = 0.142$).

Biomagnification Factors (BMFs: Hg ratio between concentrations in liver versus vegetation, based on dry weight) differed between the two sites, i.e. 1.2 for the control and 0.4 for the mining site. The lower BMF for the mining site may be due to goslings receiving supplemental

Fig. 1. A. Average mercury concentrations in soils from control and mining area. B. Average mercury concentrations in vegetation from control and mining area. C. Average mercury concentrations in livers of gosling herded in control and mining area (all: mg/kg dry matter (DM); mean + standard error). All concentrations differ significantly between sites, for (statistical) details see text.

Fig. 2. Mercury concentrations in droppings from goslings that foraged at least three hours on respectively additional feed, in the control or mining area (mg/kg dry matter (DM); mean + standard error). For (statistical) details see text.

feed, which contained low mercury levels, similar to vegetation in the control area (see above). Since all goslings received additional feed, the resulting mercury hepatic concentrations were likely affected, thus lessening any potential difference among the two treatment groups. The BMFs indicate only limited accumulation at both sites. Terrestrial breeding snow buntings (*Plectrophenax nivalis*) from Devon Island Canada showed whole body mercury concentrations of approximately 0.18 mg/kg (Choy et al., 2010), which is considerably higher than concentrations found in the current study. However, in the former study mercury may have been marine derived, as birds bred adjacent to a seabird colony (Choy et al., 2010). Concentrations in livers of Arctic seabirds, which generally feed at a much higher trophic level than geese, ranged from 0.9–9.7 mg/kg dry weight, one or two orders of magnitude higher than concentrations in the current study (Provencher et al., 2014). Threshold levels for seabirds are in the order of >30 mg/kg wet weight in liver (Fisk et al., 2005), but adult seabirds are thought to be less sensitive to mercury exposure than terrestrial species (Thompson, 1996). For non-marine birds, an effect threshold for adult birds has been derived at approx. 2 mg/kg wet weight for reproduction (Shore et al., 2011), which is not applicable for growing goslings, however. As far as we know, there are no threshold levels available for developing chicks. This renders interpretation of our results difficult. Furthermore, mercury concentrations at the moment at which the goslings were sacrificed were presumably considerably lower than concentrations at a younger age. This is due to dilution by increase of somatic mass prior to sacrifice, together with the development of feathers, for which mercury has a high affinity. At the time of sacrifice, feather development was well on the way, but not completed, and therefore it is likely that mercury concentrations would have been higher when goslings were sampled at an earlier age. This idea is corroborated by Ackerman et al. (2011), who found mercury concentrations in chicks of Forster's tern (*Sterna forsteri*), black-necked stilt (*Himantopus mexicanus*) and American avocet (*Recurvirostra americana*) to be highest shortly after hatching, followed by a rapid decline when chicks aged.

3.3. Neurochemical receptors

Levels of D2 and NMDA receptors in the gosling brains were 404 ± 47 and 752 ± 48 fmol/mg protein (mean $\pm$ standard error), respectively. Overall, D2 and NMDA receptor levels did not differ significantly between sites (Table S12). However, when corrected for sibling pair, D2 levels, but not NMDA levels, had a tendency to be higher in goslings from the mining versus the control site (Table S12, D2: ANOVA: $F = 4.37$; $n = 16$; residual d.f. = 14; $p = 0.055$; NMDA: ANOVA: $F = 0.27$; $n = 16$; residual d.f. = 14; $p = 0.609$). There was a significant effect of sibling group on the levels of D2 in the brains of the goslings: D2 levels of both siblings from one pair (marked 'black') were significantly lower, whereas levels of another pair (marked 'red') were significantly

higher in comparison to the other six sibling groups (Table S11, ANOVA: $F = 6.56$; $n = 16$; residual d.f. = 14; $p = 0.008$). We found no such effect on NMDA levels (ANOVA: $F = 1.97$; $n = 16$; residual d.f. = 8; $p = 0.181$). D2 and NMDA levels were negatively correlated with body mass (linear regression: D2: variance ratio = 17.91; $n = 16$; residual d.f. = 14; $p < 0.001$; NMDA: variance ratio = 7.54; $n = 16$; residual d.f. = 14; $p = 0.016$). Moreover, D2 levels in brains correlated positively with hepatic mercury concentrations, (Fig. 3, linear regression: variance ratio = 4.71; $n = 16$; residual d.f. = 14; $p = 0.048$), even more in case of sibling normalized D2 and mercury data (linear regression: variance ratio = 8.15; $n = 16$; residual d.f. = 14; $p = 0.013$). NMDA levels, on the other hand, did not correlate with hepatic mercury levels (linear regression: variance ratio = 0.00; $n = 16$; residual d.f. = 14; $p = 0.985$).

Mercury-associated changes in D2 receptors have been observed in previous studies, although the mode of toxicity is somewhat equivocal. For example, exposure of Sprague–Dawley rat dams (*Rattus norvegicus domesticus*) to methylmercury resulted in a significant increase in D2-receptor densities in male offspring (Coccini et al., 2011). Similarly, the density of hypothalamic D2 receptors was positively correlated with Hg concentrations in yellow perch (*Perca flavescens*) exposed in the laboratory to dietary methylmercury (0.5 to 50 ppm) (Arini et al., 2016). In contrast, D2-receptor densities in field-exposed mink (*Mustela vison*) were negatively correlated with mercury exposure (Basu et al., 2005). None-unidirectional effects of mercury on dopaminergic endpoints have been illustrated in the literature before. For instance, in yellow perch, exposure from 0 to 5 PPM of MeHg in the feed resulted in increased D2-receptor levels, which however at 50 PPM decreased again (Arini et al., 2016). In Atlantic salmon (*Salmo salar*), superoxide dismutase levels in brain increased at low exposure levels mercury, however at higher exposure this was decreased, which was accompanied by decreased activity of monoamine oxidase (a neural enzyme) (Berntssen et al., 2003). Such decrease in monoamine oxidase activity was also evident in river otters (*Lontra canadensis*) exposed to mercury (Basu et al., 2007a). These literature data indicate that, although there is a relationship between mercury exposure and D2 densities, the direction of the association may vary according to species or exposure scenarios, which makes it difficult to speculate on specific further impacts on e.g. behavior of the goslings.

In contrast to the D2 levels, NMDA receptor levels were not correlated with hepatic mercury concentrations in goslings. *In ovo* exposure of thick-billed murre (*Uria lomvia*) and Arctic terns (*Sterna paradisaea*) to mercury did show any changes in NMDA levels in the chicks' brain (Braune et al., 2012). Furthermore, in herring gulls (*Larus argentatus*), there was no relationship between mercury levels and NMDA receptors (Rutkiewicz et al., 2010). In contrast, however, mercury concentrations

Fig. 3. Relationship between hepatic mercury concentrations (mg/kg dry matter (DM)) and levels of D2 in the brain of barnacle goslings (fmol/mg protein; all observations from control and mining sites combined). For (statistical) details see text.

correlated negatively with NMDA levels in discrete regions of the brain of mink (Basu et al., 2007b). At much higher hepatic mercury concentrations (average 8 mg/kg dry weight), NMDA receptor levels were negatively correlated with mercury concentrations in bald eagles (*Haliaeetus leucocephalus*) (Rutkiewicz et al., 2011). Polar bears (*Ursus maritimus*) containing relatively low levels of brain mercury, i.e. 0.3 mg/kg dry weight, also showed a decrease in NMDA levels at elevated mercury concentrations (Basu et al., 2009). Hence, the absence of a relationship between NMDA receptor levels and total hepatic mercury concentrations in the current study may be due to a number of reasons. Foremost is that the mercury levels are just too low to elicit any response. In addition, here we did not measure mercury in the brain (hepatic mercury was used) while NMDA was measured in the whole brain versus discrete regions.

Our data show that contaminants related to historic mining activities may be linked to decreased levels of D2 receptor levels in gosling brains. In this first study we focused on mercury, as this is one of the major contaminants in coal that has neurotoxic properties. Other contaminants, such as polycyclic aromatic hydrocarbons (PAHs), may also affect organisms which feed in coal contaminated sites. PAHs, however, express toxic modes of action other than neurotoxicity. The hepatic mercury levels at which D2 receptor levels showed declines were lower than found in other studies. In our study, birds were systematically exposed to elevated, although environmentally relevant, levels of mercury. The assignment of siblings from the same nest to each treatment group allowed to include this source of variation in the statistical analyses. The results indicate that such background may need to be considered when interpreting (neurotoxic) effects of chemicals under field conditions. Nevertheless, some issues warrant further investigations, e.g. the limited design of the current (pilot) study, the potential influence of e.g. selenium or other compounds on the toxicokinetics/dynamics of mercury in developing goslings and further potential impacts of mercury associated decreases in D2 levels on e.g. behavior of goslings. Notwithstanding these limitations, we conclude that in this Arctic breeding goose species, relatively low mercury exposures may be associated with subtle changes in D2 receptor levels. As the effect concentrations were low and effects subtle, there is a need for larger, more detailed field experiments for further assessment of actual risks. Classical monitoring studies, missing a systematic inclusion of individual background of the organisms involved, may lack the power for such assessment and may therefore overlook potential impacts.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2017.12.191>.

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