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Socially-mediated differences in brain monoamines in rainbow trout: effects of trace metal contaminants

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Abstract

Monoaminergic systems play a crucial role in linking behaviour and physiology. Here the physiological and behavioural effects of metal exposure in relation to monoaminergic systems were considered by exposing rainbow trout dyads, demonstrating stable dominance relationships, to cadmium or lead. Fish exposed to 4 $\mu g\,l^{-1}$ cadmium accumulated more cadmium at the gill than fish held in control water. Fish exposed to 7 $\mu g\,l^{-1}$ cadmium had higher gill, liver and kidney cadmium concentrations. No significant lead accumulation was seen after exposure to 46 $\mu g\,l^{-1}$ for 48 h but exposure to 325 $\mu g\,l^{-1}$ lead caused an increase in gill, liver and kidney lead concentrations. Brain accumulation of both cadmium and lead was only seen after exposure to the highest concentrations. Exposure to 4 or 7 $\mu g\,l^{-1}$ cadmium, or 46 or 325 $\mu g\,l^{-1}$ lead for 48 h did not disrupt established dominance hierarchies. As expected with this stable behavioural situation, in control pairs, animals of different social status displayed different physiological profiles. Subordinate fish had higher concentrations of circulating plasma cortisol and telencephalic 5-hydroxyindoleacetic acid/5-hydroxytryptamine (serotonin) (5-HIAA/5-HT) ratios. However, these physiological profiles were affected by metal exposure, with a trend towards higher serotonergic activity in dominant fish. Dominants exposed to 325 $\mu g\,l^{-1}$ lead had significantly higher hypothalamic 5-HIAA/5-HT ratios when compared with subordinates. The results demonstrate that if stable social hierarchies are established in control water they may not be affected by exposure to cadmium and lead although physiological changes may be evident.

Keywords: Cadmium; 5-HIAA; Lead; Serotonin; Social behaviour

1. Introduction

Monoaminergic neuronal systems, particularly the serotonergic neurons are well conserved within the vertebrate phylum (Parent, 1984) and links between

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monoaminergic systems and behaviour have been established. Food intake (Øverli et al., 1998; De Pedro et al., 1998), cognitive ability (DeNoble et al., 1991; Meneses, 1999), locomotor activity (Genot et al., 1984) and social behaviour (Yodyingyuad et al., 1985; Winberg et al., 1996) are influenced by brain monoamines and links between serotonergic activity and reproduction have also been identified (Elofsson et al., 2000). In mammals, there is a wealth of information supporting a role of serotonin in regulating function of the hypothalamic–pituitary–adrenal axis, with a general assumption that such stimulation occurs at a hypothalamic level (Dinan, 1996).

The importance of monoaminergic systems in linking behaviour and physiology creates the opportunity for toxicants targeting these neurotransmitters to have effects both at the individual and population level. The effect of organic pollutants on brain monoamines has received some attention (e.g. Rozados et al., 1991; Aldegunde et al., 1999; Khan and Thomas, 2000) but there is less known about the effect of trace metals on brain monoamines. In general, metal exposure (including mercury, lead and copper) has been associated with a dose-dependent fall in brain 5-HT levels in fish (Weber et al., 1991; De Boeck et al., 1995; Tsai et al., 1995; Khan and Thomas, 2000).

Dominant-subordinate relationships formed between pairs of salmonid fish represent a stable behavioural situation in which animals of different social status display different physiological profiles (Winberg and Nilsson, 1993; Sloman et al., 2001). A characteristic change in physiology associated with subordinance both in fish and mammals is elevation of circulating plasma cortisol concentrations and substantial activation of the brain serotonergic system (Yodyingyuad et al., 1985; Winberg et al., 1992a, 1996; Øverli et al., 1998). Increased 5-HIAA/5-HT ratios in subordinates are primarily due to increased levels of 5-HIAA (Winberg et al., 1996) caused by the stress, rather than the decreased food intake, associated with subordination (Winberg et al., 1992b). Changes in brain monoamines in dominant fish include elevations of homovanillic acid (HVA), a major dopamine metabolite (Winberg et al., 1991). Additionally, social dominance can be induced by administration of L-dopa (an immediate precursor of dopamine) (Winberg and Nilsson, 1992). Here, rainbow trout dyads were used as a model to investigate the effects of trace metal exposure on individuals with different physiological profiles and their associated behaviours.

The current study aimed to examine the physiological and behavioural effects of the trace metals cadmium and lead. Exposure of fish to cadmium prior to social interaction can affect competitive ability and therefore hierarchy formation (Sloman et al., 2003a,b), but the potential for metals to affect individuals following acquisition of a particular social status and associated physiological profile is unknown.

Two experiments were carried out. In the first, fish were allowed to establish dominance hierarchies and then exposed to two different concentrations of each metal for a 48 h period. To verify whether metal accumulation was occurring in the brain at these concentrations of cadmium and lead, a second experiment exposed groups of 10 fish to the same concentrations for 48 h and then measured brain metal accumulation. For each metal, the lowest concentration was based upon the US EPA National Recommended Water Quality Criteria, a concentration that could realistically be experienced by fish in the wild. The highest concentration was equal to 30% of the 96 h LC_{50} for that metal, a concentration above that known to induce a behavioural effect of cadmium during hierarchy formation (15% of the 96 h LC_{50}; Sloman et al., 2003a,b).

2. Materials and methods

2.1. Experimental animals

Rainbow trout (140.0 ± 4.1 g; 23.0 ± 0.2 cm (mean $\pm$ S.E.M.)) were obtained from Humber Springs trout hatchery, Ontario and held in 200 l stock tanks fed with flowing, aerated, city of Hamilton tap water from Lake Ontario (hardness = 120 mg l^{-1} as $CaCO_3$; Na^+ = 0.6 mmol l^{-1}; Cl^- = 0.7 mmol l^{-1}; Ca^{2+} = 1.0 mmol l^{-1}; 12 °C; pH = 8; Cd = 0.08 ± 0.009 μg l^{-1}; Pb = 0.5 ± 0.2 μg l^{-1}). Fish were fed to satiation twice daily on commercial trout pellets (Martin Mills Inc., Elmira, Ontario).

2.2. Experiment 1

At the start of this experiment, fish were anaesthetised in benzocaine (0.05 mg ml^{-1}) and individually marked with Alcian Blue dye injected into their fins

(Kelly, 1967). Initial fork lengths and weights were recorded. Fish were then allocated to size-matched pairs as used in many previous behavioural experiments (Winberg et al., 1996; Øverli et al., 1999; Sloman et al., 2001). Fish were placed in their pairs into 26 l plastic tanks but separated from each other by a plastic, opaque partition. Tanks were set up on a flow-through system supplied with control water. After 24 h acclimation to the tanks, the partitions were removed and the fish allowed to socially interact. At the same time a shelter, a small plastic box wrapped in black plastic, was introduced to the tank to allow the submissive fish to escape any continued aggression by the dominant fish. Behavioural observations were made from this point for the remainder of the experiment.

Four daily observations of each pair of fish were made at 08:00 am, 11:00 am, 02:00 pm and 05:00 pm. At each time point, three behaviours were scored. Firstly, the position of each fish within the tank was noted. A fish swimming around in the water column scored three points, resting on the bottom of the tank scored two points, hiding in the shelter scored one point and swimming at the water surface scored zero points. This method for scoring position in the tank was based upon previous studies of salmonid behaviour (Winberg and Nilsson, 1993; Sloman et al., 2000a,b, 2001) where maintaining position in the water column is more indicative of dominance and seeking shelter or swimming at the water surface is indicative of submission. Secondly, the colouration of each fish was noted, the fish lighter in colouration scored one point, the other zero points. Darkening in colour is associated with subordination (O'Connor et al., 1999; Höglund et al., 2000). Thirdly, a single food item was introduced to the tank and the fish that consumed the food item scored one point and the other fish scored zero points. Thus, at the end of the experiment, the fish within each pair that had the highest score was considered to be the dominant fish.

Following 24 h of social interaction, fish were assigned to one of five groups. The first group of pairs remained in control water for the remainder of the experiment. The remaining four groups of pairs were exposed to nominal concentrations of either 4 or 7 $\mu g\,l^{-1}$ cadmium or 46 or 325 $\mu g\,l^{-1}$ lead (measured concentrations: cadmium: $3.57 \pm 0.29\,\mu g\,l^{-1}$ or $7.00 \pm 0.54\,\mu g\,l^{-1}$; lead: $46.12 \pm 3.93\,\mu g\,l^{-1}$ or $325.85 \pm 82.34\,\mu g\,l^{-1}$ lead) for a further 48 h. During this time, fish were not fed with the exception of the single food item introduced during each behavioural observation period. Metal exposure concentrations were achieved by dripping a concentrated metal solution (low concentration of cadmium: $0.0118\,g\,l^{-1}$ of $Cd(NO_3)_2{\cdot}4H_2O$; high cadmium: $0.0181\,g\,l^{-1}$; low concentration of lead: $0.0927\,g\,l^{-1}$ of $Pb(NO_3)_2$; high lead: $0.4795\,g\,l^{-1}$) into a mixing tank at a rate of $0.5\,ml\,min^{-1}$. All stock solutions were acidified with 1% HNO_3 (Fisher Scientific Trace Metal Grade). The mixing tank was supplied with control tap water at a flow rate of $500\,ml\,min^{-1}$ and the flow from the mixing tank into the experimental tanks was $100\,ml\,min^{-1}$. Three water samples were taken daily, acidified to 1% with HNO_3, and analysed for the relevant metal concentration by graphite furnace atomic absorption spectrophotometry (Varian AA-220, GTA 110) using a multi-element standard (Inorganic Ventures).

Following the exposure period, fish were sampled by placing in a lethal dose of benzocaine ($0.5\,mg\,ml^{-1}$) which ensured death within 30 s. Blood samples were withdrawn by caudal venipuncture using heparinised syringes. Blood was spun at $13{,}000 \times g$ for 2 min and the plasma removed, frozen in liquid nitrogen and stored at $-80\,^{\circ}C$ for later analysis of cortisol by radioimmunoassay (ICN pharmaceuticals; Gamperl et al., 1994). The fish were decapitated and the brain dissected out and separated into four parts: telencephalon, hypothalamus, optic tectum, and brain stem (including medulla, cerebellum and part of the spinal cord). Brain parts were immediately wrapped in labelled pieces of aluminium foil and frozen in liquid nitrogen for later analysis of monoamines. Samples of gill, liver and kidney tissue were then taken and placed in pre-weighed Falcon® tubes and digested in five times their volume of 1 N HNO_3 at $50\,^{\circ}C$ for 48 h. Supernatents of these tissue digests were then analysed for the relevant metal concentration by graphite furnace atomic absorption spectrophotometry as before.

The brain samples were transported at $-60\,^{\circ}C$ to Uppsala University in Sweden for analysis of monoamine and monoamine catabolite concentrations by HPLC. One sub-set of samples was lost during transit, which accounts for the varying *n* numbers. Brain samples were weighed and homogenised in 4% ice-cold perchloric acid containing 0.2% EDTA, and $40\,ng\,ml^{-1}$ epinine (deoxyepinephrine (internal standard)), using a Potter–Elvenhjem homogeniser (optic

tectum and brain stem) or an MSE 100 W ultrasonic disintegrator (telencephalon and hypothalamus).

2.3. Experiment 2

Five groups of 10 fish were allocated to 200 l tanks containing aerated, flowing, control water. Fish were allowed 24 h to acclimate to the tanks before the experiment was started. One group of fish remained in control water for the remainder of the experiment. The remaining four groups were exposed to nominal concentrations of either 4 or 7 $\mu g\,l^{-1}$ cadmium or 46 or 325 $\mu g\,l^{-1}$ lead (measured concentrations: $3.69 \pm 0.09\ \mu g\,l^{-1}$ or $6.96 \pm 0.25\ \mu g\,l^{-1}$ cadmium, $35.47 \pm 2.63\ \mu g\,l^{-1}$ or $384.73 \pm 18.85\ \mu g\,l^{-1}$ lead) for a further 48 h. During this time, fish were not fed. Metal exposure concentrations were achieved by dripping a concentrated metal solution (low concentration of cadmium: 0.0065 $g\,l^{-1}$ of $Cd(NO_3)_2{\cdot}4H_2O$; high cadmium: 0.0115 $g\,l^{-1}$; low concentration of lead: 0.0441 $g\,l^{-1}$ of $Pb(NO_3)_2$; high lead: 0.312 $g\,l^{-1}$) into a mixing tank at a rate of 1 ml min^{-1}. All stock solutions were acidified with 1% HNO_3 (Fisher Scientific Trace Metal Grade). The mixing tank was supplied with control water at a flow rate of 600 ml min^{-1} and the flow from the mixing tank into the experimental tanks was 500 ml min^{-1}. Three water samples were taken daily, acidified to 1% with HNO_3, and analysed for the relevant metal concentration by graphite furnace atomic absorption spectrophotometry as before.

After 48 h exposure, fish were placed in a lethal dose of benzocaine (0.5 mg ml^{-1}), that ensured death within 30 s. The fish were decapitated and the whole brain dissected out and placed in pre-weighed Eppendorf® tubes and digested in five times their volume of 1 N HNO_3 at 50 °C for 48 h. Supernatents of these brain digests were then analysed for the relevant metal concentration by graphite furnace atomic absorption spectrophotometry.

2.4. Statistical analyses

Data are given as means ± S.E.M. Physiological and behavioural measurements were compared between pairs of fish using paired Student's *t*-tests. Physiological parameters were compared among groups using a one-way ANOVA with Bonferroni post-hoc analysis. Physiological parameters were also compared using linear regression analyses. SPSS® software was used for statistical analyses and the limit of significance in all analyses was $p < 0.05$.

3. Results

Fish were scored according to their behaviour both before and after exposure to cadmium or lead. A total behaviour score was calculated for each fish for both the first 24 h of the experiment (pre-exposure) and for the last 48 h (exposure). There was no significant difference between pre-exposure and exposure behaviour scores in all treatments ($p > 0.1$). Neither was there any significant difference in behaviour scores between treatments ($p > 0.1$; Fig. 1). In each treatment, there was a significant difference in behaviour score between dominants and subordinates in both pre-exposed

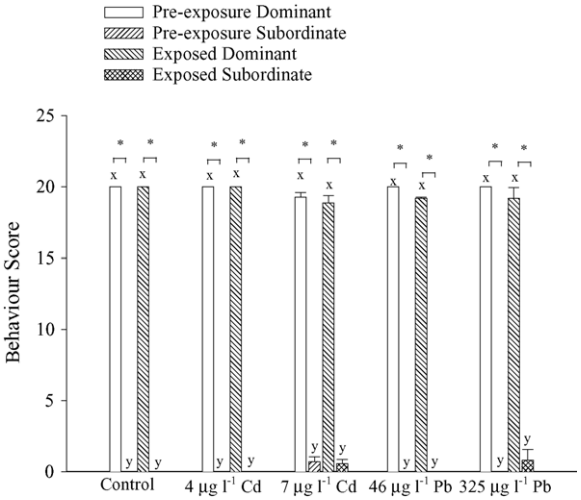

Fig. 1. Behavioural scores calculated by addition of all scores awarded to the fish based upon food acquisition, position in the tank and colouration (see Section 2 for more detail). Scores are divided into the first 24 h of the experiment (pre-exposure) and the last 48 h of the experiment (exposure) where all except the control treatment were exposed to trace metal. Letters denote significant differences where groups sharing the same letter are not statistically different. Subordinate fish had significantly lower behavioural scores than dominant fish in both pre-exposure and exposure groups (ANOVA followed by Bonferroni post-hoc comparisons: $F_{19,332} = 1615$, $p < 0.001$). Asterisks indicate a significant difference within pairs of fish. In all cases, subordinate fish had significantly lower behavioural scores than the dominant with which they were paired (paired *t*-test: $p < 0.001$). Data are given as means ± S.E.M. (control: $n = 18$; 4 $\mu g\,l^{-1}$ Cd: $n = 15$; 7 $\mu g\,l^{-1}$ Cd: $n = 17$; 46 $\mu g\,l^{-1}$ Pb: $n = 17$; 325 $\mu g\,l^{-1}$ Pb: $n = 13$ pairs).

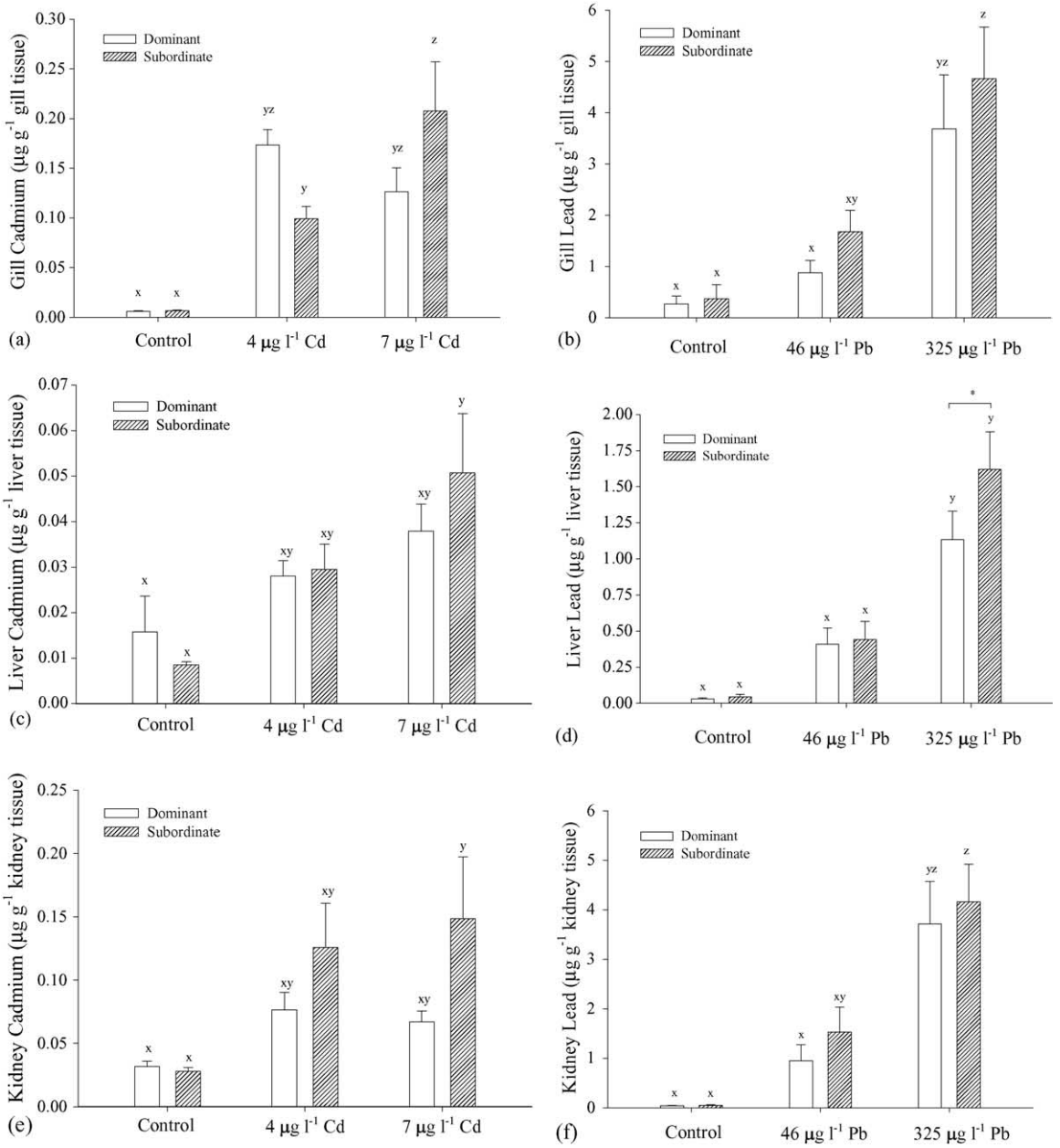

Fig. 2. Gill (a) cadmium, (b) lead, liver (c) cadmium, (d) lead, kidney (e) cadmium and (f) lead concentrations in dominant and subordinate rainbow trout. Letters denote significant differences where groups sharing the same letter are not statistically different. There was a significant effect of metal exposure on all tissue concentrations (ANOVA followed by Bonferroni post-hoc comparisons: gill Cd: $F_{5,94} = 13.058$, $p < 0.001$; gill Pb: $F_{5,70} = 6.909$, $p < 0.001$; liver Cd: $F_{5,94} = 4.489$, $p = 0.001$; liver Pb: $F_{5,70} = 13.093$, $p < 0.001$; kidney Cd: $F_{5,94} = 3.933$, $p = 0.003$; kidney Pb: $F_{5,70} = 8.398$, $p < 0.001$). Asterisks indicate a significant difference using pair-wise comparisons within pairs of fish. Subordinate fish exposed to 325 $\mu g\,l^{-1}$ lead had significantly higher liver lead concentrations than the dominants with which they were paired (paired t-test: $t = -2.229$, $p = 0.046$). Data are given as means ± S.E.M. (control: $n = 18$; 4 $\mu g\,l^{-1}$ Cd: $n = 15$; 7 $\mu g\,l^{-1}$ Cd: $n = 17$; 46 $\mu g\,l^{-1}$ Pb: $n = 17$; 325 $\mu g\,l^{-1}$ Pb: $n = 13$ pairs).

and exposed groups ($p < 0.001$; Fig. 1). In all pairs, the fish originally identified as the dominant fish during the pre-exposure period remained dominant during metal exposure. There were also no significant changes in pre-exposure and exposure behaviours of individuals ($p > 0.1$) and within each pair, the dominant fish consistently scored higher than the subordinate with which it was paired both pre-exposure, and following exposure ($p < 0.001$).

Exposure to 4 and 7 $\mu g\,l^{-1}$ cadmium for 48 h resulted in significant elevation of gill cadmium concentrations when compared with controls ($p < 0.001$; Fig. 2). Subordinate fish in the 7 $\mu g\,l^{-1}$ treatment had significantly higher gill cadmium concentrations when compared with the subordinates in the 4 $\mu g\,l^{-1}$ treatment but there was no significant difference between dominant fish exposed to 4 and 7 $\mu g\,l^{-1}$ cadmium. Liver and kidney cadmium concentrations were also significantly affected by treatment (liver: $p = 0.001$; kidney: $p = 0.003$). Subordinate fish in the 7 $\mu g\,l^{-1}$ treatment had significantly higher liver and kidney concentrations than control fish.

There was also a significant effect of treatment on lead tissue concentrations (Fig. 2). Exposure to 325 $\mu g\,l^{-1}$ lead resulted in significant elevation of gill, liver and kidney lead concentrations when compared with control fish ($p < 0.001$). When tissue concentrations were compared within each pair of fish, subordinate animals exposed to 325 $\mu g\,l^{-1}$ lead had significantly higher liver lead concentrations than the dominants with which they were paired ($p = 0.046$). Exposure to 46 $\mu g\,l^{-1}$ lead did not significantly alter tissue lead concentrations. Exposing groups of 10 fish to the same concentrations of cadmium and lead in the second experiment demonstrated that only at the higher concentrations of both metals was there significant metal accumulation in the brain ($p < 0.001$; Fig. 3).

Plasma cortisol did not significantly vary between treatments (Fig. 4). When differences in plasma cortisol were examined between individual pairs of fish, subordinate fish had significantly higher circulating cortisol concentrations than the dominants with which they were paired in the control, low cadmium (4 $\mu g\,l^{-1}$) and high lead (325 $\mu g\,l^{-1}$) treatments (control: $p = 0.003$; 4 $\mu g\,l^{-1}$ cadmium: $p = 0.028$; 325 $\mu g\,l^{-1}$ lead: $p = 0.049$; Fig. 4). Differences between subordinates and dominants were not significant in the other treatments.

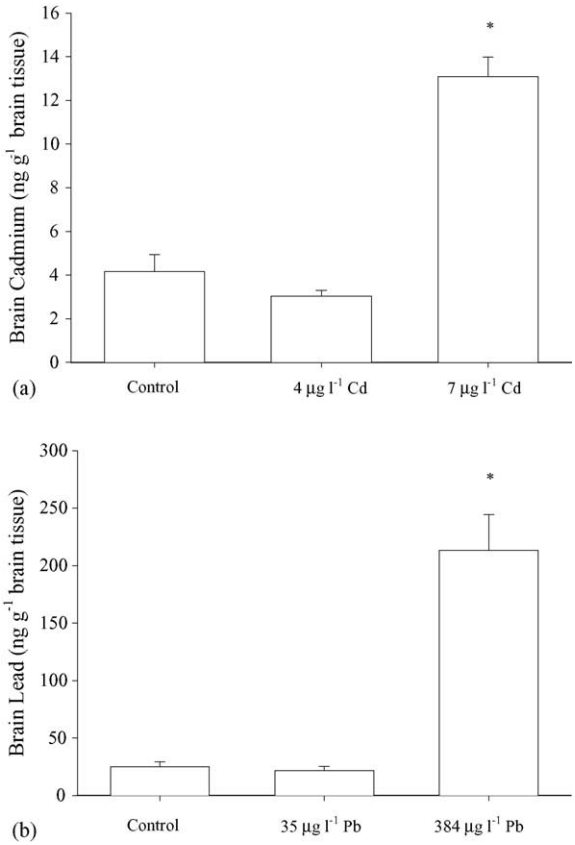

Fig. 3. Brain (a) cadmium and (b) lead concentrations of groups of 10 rainbow trout. Asterisks denote a significant difference from control levels (ANOVA followed by Bonferroni post-hoc comparisons: brain Cd: $F_{2,27} = 35.519$, $p < 0.001$; brain Pb: $F_{2,27} = 61.922$, $p < 0.001$). Data are given as means ± S.E.M. ($n = 10$).

There was no overall difference in 5-HIAA/5-HT telencephalon ratios among treatments. However, in the control treatment, subordinate fish had a significantly higher 5-HIAA/5-HT ratio than the dominant with which they were paired ($p = 0.019$), whereas this difference was eliminated by all of the metal exposure treatments (Fig. 5). In the control treatments there was a significant positive correlation between plasma cortisol and 5-HIAA/5-HT ratios in the telencephalon ($p = 0.01$) that was also eliminated in the metal treatments. Interestingly, there was a trend towards dominant fish having higher 5-HIAA/5-HT ratios in both the 7 $\mu g\,l^{-1}$ cadmium and 325 $\mu g\,l^{-1}$ lead exposed fish (Fig. 5).

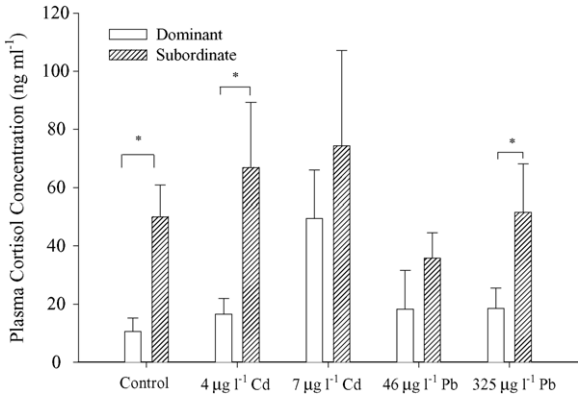

Fig. 4. Plasma cortisol concentrations for dominant and subordinate fish in each treatment group. There were no significant post-hoc differences between groups (ANOVA with Bonferroni post-hoc: $F_{9,146} = 2.527$, $p = 0.10$). Asterisks indicate a significant difference within pairs of fish. In control treatments, subordinate fish had significantly higher circulating concentrations of cortisol compared to the dominant with which they were paired (paired t-test: $t = -3.347$; $p = 0.003$) and the same was true in both the 4 $\mu g\,l^{-1}$ cadmium ($t = -2.450$; $p = 0.028$) and the 325 $\mu g\,l^{-1}$ lead treatments ($t = -2.149$, $p = 0.049$). Data are given as means ± S.E.M. (control: $n = 18$; 4 $\mu g\,l^{-1}$ Cd: $n = 15$; 7 $\mu g\,l^{-1}$ Cd: $n = 17$; 46 $\mu g\,l^{-1}$ Pb: $n = 17$; 325 $\mu g\,l^{-1}$ Pb: $n = 13$ pairs).

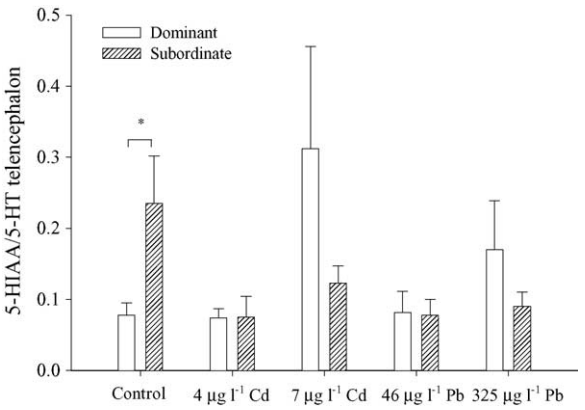

Fig. 5. 5-HIAA/5-HT (5-hydroxyindoleacetic acid/serotonin) ratios in the telencephalon. Asterisks denote a significant difference within pairs of fish. In control treatments, subordinate fish had significantly higher 5-HIAA/5-HT ratios when compared to the dominant fish with which they were paired (paired t-test: $t = -2.791$, $p = 0.019$). Data are given as means ± S.E.M. (control: $n = 18$; 4 $\mu g\,l^{-1}$ Cd: $n = 15$; 7 $\mu g\,l^{-1}$ Cd: $n = 17$; 46 $\mu g\,l^{-1}$ Pb: $n = 17$; 325 $\mu g\,l^{-1}$ Pb: $n = 13$ pairs).

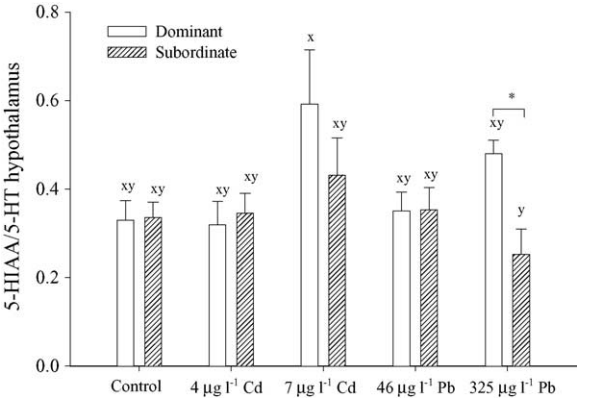

Fig. 6. 5-HIAA/5-HT ratios in the hypothalamus. Letters denote significant differences where groups sharing the same letter are not statistically different (ANOVA followed by Bonferroni post-hoc comparisons: $F_{9,96} = 2.435$, $p = 0.015$). Using paired statistics, asterisks indicate a significant difference within pairs of fish. In fish exposed to 325 $\mu g\,l^{-1}$ lead, dominant fish had significantly higher 5-HIAA/5-HT ratios in the hypothalamus than the subordinates with which they were paired (paired t-test: $t = 3.947$, $p = 0.004$). Data are given as means ± S.E.M. (control: $n = 18$; 4 $\mu g\,l^{-1}$ Cd: $n = 15$; 7 $\mu g\,l^{-1}$ Cd: $n = 17$; 46 $\mu g\,l^{-1}$ Pb: $n = 17$; 325 $\mu g\,l^{-1}$ Pb: $n = 13$ pairs).

Treatment significantly affected 5-HIAA/5-HT ratios in the hypothalamus ($p = 0.015$; Fig. 6). There were no differences between dominants and subordinates in the control treatment or in the lower concentrations of each metal. However, dominant fish exposed to the highest concentrations of cadmium and lead appeared to have elevated 5-HIAA/5-HT ratios. Between pairs of fish exposed to 325 $\mu g\,l^{-1}$ lead, dominant fish had significantly higher 5-HIAA/5-HT ratios in the hypothalamus than the subordinates with which they were paired ($p = 0.004$). No other statistical differences were found in the other monoamine concentrations measured and the tissue-specific monoamine concentrations, averaged across social status and metal treatment are shown in Table 1.

4. Discussion

Pairs of fish were allowed to establish dominance hierarchies at the start of the experiment and in control pairs, animals of different social status displayed different physiological profiles. Subordinate rainbow trout had higher concentrations of circulating plasma cortisol and elevated 5-HIAA/5-HT ratios in the

Table 1
Concentrations of DOPAC (3,4-dihydroxyphenylacetic acid), HVA (homovanillic acid), DA (dopamine), MHPG (3-methoxy-4-hydroxyphenylglycol), NE (norepinephrine), 5-HIAA (5-hydroxyindoleacetic acid) and 5-HT (5-hydroxytryptamine; serotonin) measured in the various brain regions

	Brain monoamine concentration (ng g^{-1})			
	Telencephalon	Hypothalamus	Optic tectum	Brain stem
DOPAC	9.71 ± 1.86	13.59 ± 2.50	9.90 ± 2.04	6.35 ± 1.25
HVA	6.87 ± 1.13	31.35 ± 10.10	6.50 ± 2.38	5.36 ± 0.75
DA	181.46 ± 19.83	177.76 ± 28.89	222.99 ± 57.07	33.98 ± 6.00
MHPG	6.59 ± 1.13	10.98 ± 2.18	6.75 ± 1.37	5.89 ± 1.72
NE	698.47 ± 42.47	613.76 ± 41.93	285.55 ± 38.08	16.31 ± 1.96
5-HIAA	37.35 ± 6.88	281.79 ± 21.47	38.67 ± 7.92	30.43 ± 3.78
5-HT	314.53 ± 27.73	1045.35 ± 84.88	127.92 ± 18.99	65.47 ± 6.80

Values are averaged across dominance status and metal exposure, due to no significant effects of either treatment group ($p > 0.05$) or social status ($p > 0.05$). Data are given as means ± S.E.M., $n > 70$.

telencephalon in agreement with previous work (Laidley and Leatherland, 1988; Winberg et al., 1996; Fox et al., 1997; Øverli et al., 1998, 1999; Höglund et al., 2000; Sloman et al., 2000a, 2001). The 5-HIAA/5-HT ratio is used as an indicator of serotonergic activity (Winberg et al., 1992b) and is considered a more direct indicator of central serotonergic activity than absolute values of monoamines (Winberg and Nilsson, 1993). Hypothalamic 5-HIAA/5-HT ratios were unaffected by social status in control dyads and although increases in these ratios have been demonstrated previously in subordinate fish they may be less dramatic than in the telencephalon (Øverli et al., 1999).

The differences in physiology that exist between individuals of different social status could potentially affect their vulnerability to the toxic effects of trace metals. For example, subordinate rainbow trout will take up more waterborne copper and silver than their dominant counterparts (Sloman et al., 2003c). Differences in metabolic rate between individuals might provide a logical explanation but it appears that the differences in copper and silver uptake are instead dependent upon elevated sodium uptake rates associated with subordinate trout (Sloman et al., 2004). Both copper and silver cross the gills via sodium transport pathways (Bury and Wood, 1999; Grosell and Wood, 2002) and so increased sodium uptake results in a consequential increase in uptake of these metals. Similar effects are not found with cadmium accumulation because cadmium acts as a calcium analogue, crossing the gill via calcium pathways (Verbost et al., 1989; Wicklund-Glynn et al., 1994) and calcium uptake does not appear to be affected by social status (Sloman and Wood, unpublished data).

As expected, there were no significant differences in accumulation of cadmium between dominant and subordinate fish of the same exposure group in the present study. Cadmium significantly accumulated in the gill tissue following exposure to 4 and 7 $\mu g\,l^{-1}$, and increased in concentration in the liver and kidney, although there was a lot of individual variation. Significant lead accumulation occurred in the gills, liver and kidney of fish exposed to 325 $\mu g\,l^{-1}$ lead, but not at the lower concentration (46 $\mu g\,l^{-1}$). Social status did not affect lead accumulation in the gills or kidney but a significant difference existed between liver lead concentrations of dominant and subordinate fish exposed to 325 $\mu g\,l^{-1}$ lead. The greater accumulation of lead seen in subordinate trout of the present study in part contradicts the hypothesis that only those metals crossing the gill by sodium pathways are affected by social status. Lead is also presumed to cross the gill via calcium pathways (MacDonald et al., 2002; Rogers et al., 2003; Rogers and Wood, 2004) but there is now some indication that partial transport may occur by sodium pathways (Rogers et al., 2003). The effect of social status on lead uptake warrants further investigation, in particular the measurement of actual rates of lead uptake in relation to social status and sodium turnover.

The repeatable physiological profiles associated with social status were in part disrupted by exposure to cadmium and lead. Cortisol concentrations were higher in metal-exposed subordinates compared to their

dominant counterparts as seen in control pairs of fish. However, there was more inter-individual variation among the metal-exposed fish and so this relationship was only significant in dyads exposed to 4 $\mu g\,l^{-1}$ cadmium and 325 $\mu g\,l^{-1}$ lead. In contrast, the characteristic elevation of telencephalic 5-HIAA/5-HT ratios in subordinate animals was eliminated following metal exposure. Indeed, in fish exposed to the highest concentrations of cadmium and lead, there was a trend towards higher 5-HIAA/5-HT ratios in dominant fish, the opposite of that seen in control fish. This effect was also seen in the hypothalamus and reached significance in lead-exposed fish.

Social interaction is believed to increase functional 5-HT release and turnover in submissive animals resulting in higher levels of 5-HIAA, the metabolite of 5-HT (Winberg and Nilsson, 1993) and a consequential elevation of 5-HIAA/5-HT ratios. In mammals, both cadmium and lead cause decreases in 5-HT hypothalamic concentrations (Pillai et al., 2003) and a similar effect of copper exposure has been documented in fish (De Boeck et al., 1995). One-week exposure of common carp to waterborne copper resulted in a dose-dependent decrease in 5-HT resulting in increased 5-HIAA/5-HT ratios in the telencephalon, hypothalamus and brain stem. This would suggest a reduced 5-HT synthesis rate rather than increased 5-HT turnover (De Boeck et al., 1995). In addition, cadmium causes a depression in serotonin metabolism in mammals (Lafuente and Esquifino, 1999) and decreases in hypothalamic 5-HT have been associated with lead (Khan and Thomas, 2000) and mercury (Tsai et al., 1995) exposure in fish.

There are several mechanisms by which metals may affect brain monoamine levels. De Boeck et al. (1995) suggested that hypoxia may underlie the fall in 5-HT seen in copper exposed carp. Copper is known to cause gill damage and as the authors could detect no copper accumulation in the brain, direct effects of copper on post-synaptic receptor mechanisms were discounted. Cadmium and lead, like copper, are known to cause gill damage (Sorensen, 1991), which could lead to mild hypoxia known to initiate decreases in 5-HT levels (Freeman et al., 1986). However, in the present study both cadmium and lead were seen to accumulate in the brain at the higher exposure concentrations and so could also exert more direct neurological effects. Damage to brain tissue occurs following dietary exposure of trout to copper, including oedema and vacuolation in the cell body layers of the tectum close to the hypothalamic region (Handy, 2003). Alternatively, cadmium and lead may cause specific neuro-endocrine adjustments, for example by interfering with binding of endogenous ligands to their receptors, or potentially altering intracellular calcium regulation (Pillai et al., 2003). In the present study, it is not clear why the greatest changes in 5-HIAA/5-HT ratios occurred in dominant individuals, but changes in brain monoamine ratios occurred in the higher exposure concentrations where brain accumulation of metals occurred. Socially-mediated differences in brain metal accumulation were not measured in the present study, and this remains a feasible explanation and area for future research.

The close involvement of serotonin in the regulation of hypothalamic–pituitary–adrenal (interrenal) axis has been well documented (Dinan, 1996; Winberg et al., 1997). In particular, the release of corticotrophin-releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus is known to be under serotonergic control, and in addition serotonin may have a direct action on the anterior pituitary and adrenal cortex/interrenal cells (Dinan, 1996). Stressors other than social status that activate the hypothalamic–pituitary–interrenal (HPI) axis in fish also increase the 5-HIAA/5-HT ratio in the same way (e.g. repeated netting, predation: Winberg et al., 1992a, 1993). In the present study, exposure to cadmium and lead only altered the differences in brain monoamines between dominants and subordinates, with no apparent effects on cortisol concentration. Lafuente and Esquifino (1999) noted in their study on the effects of cadmium on rats that changes in pituitary hormone secretion did not correlate with the modifications of central nervous system metabolism of the neurotransmitters involved in their regulation.

In all the treatments, no status switches were observed and dominant fish had significantly higher behaviour scores than the subordinate with which they were paired, both prior to and during metal exposure. This lack of behavioural change may seem surprising given the observed changes in physiology. However, as differences in brain serotonergic activity associated with social rank are a consequence rather than a cause of social behaviour (Winberg et al., 1992a) and changes in aggression are generally associated with long-term increases in brain monoamines (Winberg et al., 2001; Øverli et al., 2004), it is perhaps not surprising

that physiological changes were not manifested in behavioural changes, particularly if the behaviour of the fish was relatively stable. Nevertheless, implications of these physiological changes in subsequent periods of hierarchy stability remain unknown. An increasing number of studies are trying to integrate behavioural and physiological measures of toxicity (reviewed by Scott and Sloman, 2004) and it is becoming obvious that for complex behaviours such as social status, the detectable effects of toxicants may vary from physiological to behavioural, critically depending upon the timing of exposure. Understanding how behavioural and physiological thresholds vary in this way is crucial to the potential use of behaviour as an indicator of toxicity.

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