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Effects of *Schistocephalus solidus* infection on brain monoaminergic activity in female three-spined sticklebacks *Gasterosteus aculeatus*

Øyvind Øverli[1*], Miklós Páll[3], Bertil Borg[3], Malcolm Jobling[4] and Svante Winberg[2]

[1]*Department of Animal Development and Genetics, and* [2]*Department of Comparative Physiology, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18A, Uppsala SE-752 36, Sweden*
[3]*Department of Zoology, Stockholm University, Stockholm SE-106 91, Sweden*
[4]*Norwegian College of Fisheries Science, University of Tromsø, Tromsø N-9037, Norway*

The three-spined stickleback *Gasterosteus aculeatus* is an intermediate host of the tapeworm *Schistocephalus solidus*. Changes in predator avoidance, foraging and shoaling behaviour have been reported in sticklebacks infested with *S. solidus*, but the mechanisms underlying parasite-induced behavioural changes are not understood. Monoamine neurotransmitters are involved in the control of behaviour and central monoaminergic systems are sensitive to various stressors. Thus, the behavioural effects of *S. solidus* infestation might be a reflection of changes in brain monoaminergic activity in the stickleback host. The concentrations of 5-hydroxytryptamine (5-HT), dopamine (DA), norepinephrine (NE) and their metabolites 5-hydroxyindoleacetic acid (5-HIAA), homovanilic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) were measured in the telencephalons, hypothalami and brainstems of parasitized and non-parasitized female sticklebacks held in the laboratory. The ratios of 5-HIAA:5-HT were significantly elevated in both the hypothalami and brainstems of infected sticklebacks. The concentrations of 5-HT and NE were significantly reduced in the telencephalons of infected fish as compared with controls, but there was no elevation of metabolite concentrations. The results are consistent with chronic stress in infected fish, but may also reflect other alterations of neuroendocrine status resulting from parasite infection.

Keywords: *Gasterosteus aculeatus*; host manipulation; monoamines; parasites; *Schistocephalus solidus*

1. INTRODUCTION

Parasites may influence the behaviour of their hosts (Lambert & Farley 1968; Maeyama *et al.* 1994) and the behavioural changes of parasitized animals often appear to increase the likelihood of the parasite completing its life cycle (Dobson 1988; Moore & Gotelli 1990). Such behavioural alterations may represent the ability of parasites to manipulate host behaviour specifically, e.g. by interfering with host neuroendocrine signalling systems (de Jong-Brink 1995; Adamo & Shoemaker 2000), but may also arise as the side-effects of other parasite activities (Poulin 1995).

A well-documented case of parasite effects on host behaviour is found in teleost fish: three-spined sticklebacks (*Gasterosteus aculeatus*) infected with plerocercoid larvae of the cestode *Schistocephalus solidus* exhibit marked differences in their anti-predator, foraging and shoaling behaviour compared with uninfected conspecifics (Giles 1983, 1987; Godin & Sproul 1988; Barber & Huntingford 1995; Ness & Foster 1999). Another response to *S. solidus* that has been observed in some populations of sticklebacks is a paling of the body and darkening of the eye of the fish during late stages of infection (LoBue & Bell 1993).

Many of the behavioural changes seen in infected sticklebacks could contribute to an increased likelihood of transfer of the parasite to the final hosts (fish-eating birds) and they are therefore consistent with manipulations of host behaviour by the parasite. On the other hand, many of the reported differences in foraging and anti-predator behaviour might result from altered risk taking due to the increased metabolic needs of fish harbouring parasites (Godin & Sproul 1988) rather than specific host manipulation. However, the physiological mechanisms underlying the behavioural changes displayed by sticklebacks infected by *S. solidus* have not been examined.

Similarly to mammals, the brain monoamine neurotransmitters in teleost fishes, namely norepinephrine (NE), dopamine (DA) and 5-hydroxytryptamine (5-HT), influence feeding (De Pedro *et al.* 1995, 1998*a*,*b*), locomotor activity (Winberg *et al.* 1993*a*), aggression (Maler & Ellis 1987; Winberg & Nilsson 1992; Elofsson *et al.* 2000) and social behaviour (reviewed by Winberg & Nilsson 1993). Central monoaminergic systems are also sensitive to a range of stressors (Stanford 1993; Winberg & Nilsson 1993), immunological factors (Lacosta *et al.* 1998, 2000; MohanKumar *et al.* 1999) and nutritional and endocrine status (Levin & Routh 1996; Orosco *et al.* 1996; Leibowitz & Alexander 1998). Thus, it could be hypothesized that infection by *S. solidus* results in altered brain monoaminergic function in sticklebacks and that such changes could be involved in the effects of this parasite on host behaviour. The current study represents a first step in the investigation of the central neuroendocrine mechanisms underlying parasite-induced behavioural modification in three-spined sticklebacks. We examined whether the presence of *S. solidus* plerocercoids in the body cavity was

*Author for correspondence (oyvind.overli@ebc.uu.se).

associated with changes in the concentrations of the monoamines NE, DA and 5-HT and their metabolites (5-hydroxyindoleacetic acid (5-HIAA), homovanilic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG)) in brain tissue samples from infected female sticklebacks as compared with uninfected controls.

2. MATERIAL AND METHODS

Three-spined sticklebacks (*G. aculeatus*) were caught in Öresund, Sweden, on 18 and 19 November 1999. The fish are in a non-reproductive condition at this time of year. The fish were initially held for two weeks under a simulated natural photoperiod in a stock tank containing filtered and aerated natural brackish water at *ca.* 9 °C and were not fed during this period. Infected ($n = 18$) and uninfected control ($n = 18$) fish were then identified by the presence or absence of external signs of *S. solidus* infection and distributed among individual 50 l aquaria containing brackish water (0.8% salinity), sand and a filter. Only adult females were selected for the study. The temperature was adjusted to *ca.* 10 °C and the photoperiod was a simulated natural photoperiod (adjusted weekly from 8.5 L:15.5 D at the start to 7 L:17 D at termination). While in isolation the fish were fed to satiation daily with chironomid larvae. After three weeks, the fish were netted, anaesthetized in 0.1% 2-phenoxyethanol, weighed and measured for length. The fish were decapitated and their brains removed and dissected into the telencephalon, hypothalamus (excluding the pituitary) and brainstem (including the optic tectum and cerebellum) (cf. Winberg *et al.* 1991). The brain samples were rapidly frozen in liquid nitrogen before being transferred to storage at −80 °C. Following brain removal, the fish were dissected in order to confirm the presence or absence of *S. solidus* plerocercoids. The parasites were then removed, counted and weighed. Finally, the sex of the fish was confirmed and their gonads were removed and weighed. The gonadosomatic index (GSI) of each fish was calculated as gonad mass (g) × body mass (g)$^{-1}$ and its condition factor was calculated as body mass (g) × body length (cm)$^{-3}$ × 100. The GSIs and condition factors of infected fish were calculated using both the combined body mass of each fish and its parasite(s) and the mass of the fish after subtraction of the mass of the parasite(s).

The brain samples were homogenized in 0.40 mol l^{-1} ice-cold perchloric acid containing 40 ng ml^{-1} epinine (deoxyepinephrine) (which was used as an internal standard) using a Potter-Elvehjem homogenizer (brainstem) or an MSE 100-W ultrasonic disintegrator (other brain parts). The samples were then centrifuged at 27 000 *g* for 10 min at 4 °C and the supernatants analysed for their concentrations of 5-HT, DA and NE and their metabolites 5-HIAA, HVA and MHPG using high-performance liquid chromatography with an electrochemical detection (HPLC-EC) system (Øverli *et al.* 1999).

Data from infected and uninfected fish were compared using Student's *t*-test, following log transformation when required in order to obtain homogeneity of variance (which was applied to the MHPG concentrations of the hypothalamus and telencephalon). Possible correlations between the monoamine variables and parasite load were assessed by Pearson correlation analysis. Correlation tests were carried out, with the parasite load being expressed as the mass of parasites present in each fish, parasite mass relative to fish body mass, mass of the heaviest individual parasite found in each fish and mass of the heaviest parasite relative to fish body mass.

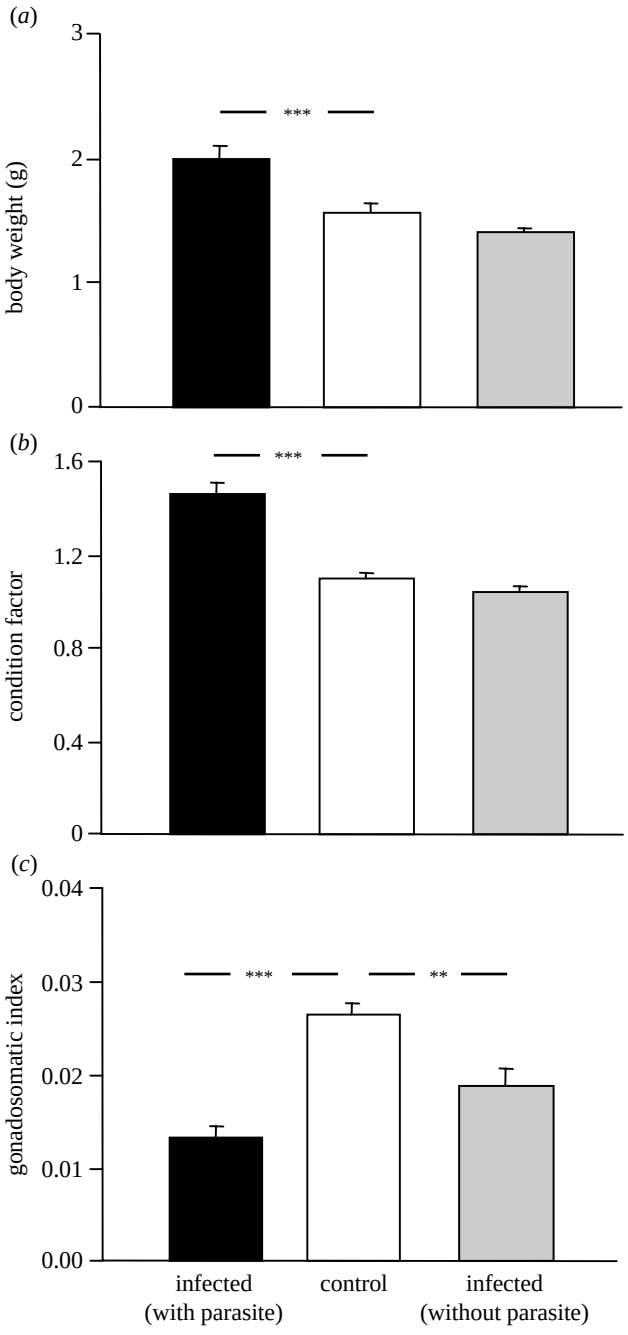

Figure 1. (*a*) Body weight, (*b*) condition factor and (*c*) GSI of sticklebacks infected with *S. solidus*, uninfected controls and infected sticklebacks after removal of the parasite(s). The values shown are means ± s.d.s. Statistically significant differences between groups are indicated by asterisks (*t*-test, $^{**}p < 0.01$, $^{***}p < 0.001$).

3. RESULTS

All fish originally designated as infected hosted between one and five *S. solidus* plerocercoids. One fish was found to be a male and was therefore discounted during the data analysis. The mean body weights, condition factors and GSIs for infected and uninfected fish are shown in figure 1. The data for infected fish are presented as both the mass of the fish plus its parasite(s) and the mass of the fish excluding parasites. The combined masses of infected fish and their parasite(s) were significantly higher than those of controls ($p < 0.001$), but after excluding the parasites the

Table 1. *Monoamine and monoamine metabolite concentrations* ($ng\,g^{-1}$) *and monoamine:metabolite ratios in the brain tissue of* S. solidus*-infected sticklebacks and uninfected controls*

(Values shown are means ± s.d.s. Statistically significant differences between infected fish and controls are indicated in bold type and by asterisks after values for infected fish (*t*-test, $^{*}p < 0.05$, $^{**}p < 0.01$).)

	telencephalon		hypothalamus		brainstem	
	control	infected	control	infected	control	infected
5-HIAA	458 ± 52	397 ± 42	342 ± 76	463 ± 78	294 ± 18	322 ± 24
5-HT	**859 ± 73**	**642 ± 59***	978 ± 198	962 ± 155	642 ± 43	568 ± 40
5-HIAA:5-HT	0.56 ± 0.21	0.64 ± 0.2	**0.34 ± 0.09**	**0.44 ± 0.12***	**0.47 ± 0.1**	**0.57 ± 0.1****
HVA	65.3 ± 17.4	41.5 ± 8.6	109 ± 30	130 ± 21	28.3 ± 2.9	30.8 ± 3.9
DA	496 ± 70	381 ± 39	1464 ± 296	1735 ± 244	432 ± 31	408 ± 47
HVA:DA	0.13 ± 0.03	0.11 ± 0.02	0.07 ± 0.01	0.09 ± 0.02	0.07 ± 0.01	0.09 ± 0.01
MHPG	7.37 ± 1.02	8.71 ± 2.0	20.3 ± 2.6	50.0 ± 17.8	20.2 ± 2.0	16.6 ± 2.6
NE	**1793 ± 222**	**1213 ± 128***	3153 ± 785	3516 ± 640	1543 ± 85	1504 ± 102
MHPG:NE	0.004 ± 0.001	0.007 ± 0.002	0.010 ± 0.002	0.011 ± 0.002	0.013 ± 0.001	0.011 ± 0.002

masses of infected and uninfected fish were similar. The condition factors were also significantly higher in infected fish than in controls when calculated from the masses of the fish plus parasites ($p < 0.001$), but were similar following removal of the parasite. On the other hand, the GSIs of parasitized fish were significantly lower when calculated on the basis of both the mass of fish plus parasites ($p < 0.001$) and when parasite mass was excluded ($p < 0.01$).

The concentrations of the monoamines and monoamine metabolites and their ratios are shown in table 1. Of the neurotransmitters analysed, the clearest effect of infection was seen on brain serotonergic activity, as expressed by 5-HIAA:5-HT ratios. The 5-HIAA:5-HT ratios of infected fish were significantly elevated in both their hypothalami ($p < 0.05$) and brainstems ($p < 0.01$). The 5-HT concentrations in the telencephalons of infected fish were significantly lower as compared with controls ($p < 0.05$), but there were no significant differences in their 5-HIAA:5-HT ratios. Telencephalic NE concentrations were also significantly lower in infected than in uninfected fish ($p < 0.05$) and there was a near significant elevation in the MHPG:NE ratios of infected fish ($p = 0.08$). The concentrations of DA and its metabolite HVA and the HVA:DA ratios were not significantly different between groups in any brain region. There were no significant correlations between any measure of monoaminergic activity and parasite load, irrespective of the method used for assessing the parasite load (results not shown).

4. DISCUSSION

Individual growth was not studied, but from weight and condition data (figure 1) it appeared that infected fish were able to maintain their soma at the expense of gonadal development. Presumably, parasitized fish were able to support the 'extra' mass of the parasites by increasing their food intake. Arnott *et al.* (2000) demonstrated that infected sticklebacks (excluding parasite weight) grew faster and maintained a similar or better body condition than uninfected fishes when reared in isolation and given access to adequate food resources.

The results of the present study showed that the presence of *S. solidus* plerocercoids could affect brain monoaminergic systems in the stickleback. Elevated 5-HIAA:5-HT ratios, as seen in the brainstem and hypothalamus of infected sticklebacks, are usually interpreted as indicating enhanced release of the neurotransmitter as a result of increased neural activity (Fillenz 1993; Stanford 1993). An increased brain 5-HIAA:5-HT ratio is a common response to a wide range of stressors in mammals (Stanford 1993). In teleost fish, exposure to predators (Winberg *et al.* 1993*b*), handling stress (Winberg *et al.* 1992) and chronic (Winberg *et al.* 1991) and acute (Øverli *et al.* 1999) social stress are associated with this response.

It seems reasonable to assume that sticklebacks infected with *S. solidus* experience chronic stress and that the elevation in the 5-HIAA:5-HT ratio observed in infected fish could reflect a stress response. The reduced monoamine concentrations seen in the telencephalon of infected fish (table 1) could also be an effect of chronic stress. Monoamine synthesis is usually upregulated as a consequence of increased transmitter use, but depletion of transmitter stores may be observed after repeated or prolonged stress (reviewed by Stanford 1993). Very little is known about the influence of stress on brain catecholamine use in fish. Øverli *et al.* (1999) found that brain MHPG:NE ratios were increased following exposure of rainbow trout (*Oncorhynchus mykiss*) to short-term (less than 24 h) social stress, while NE concentrations were unaffected by this treatment. Höglund *et al.* (2000) obtained similar results with Arctic char (*Salvelinus alpinus*) reared in small groups for five days. With respect to the influence of stress on brain 5-HT concentrations, Winberg *et al.* (1991) found that long-term (more than four weeks) social stress in Arctic char did not alter neurotransmitter concentrations, although brain 5-HIAA:5-HT ratios were elevated in socially subordinate fish.

The observed differences between infected sticklebacks and controls were broadly consistent with chronic stress in infected fish, but infection may also affect brain monoaminergic systems in other ways. Sticklebacks infected with *S. solidus* plerocercoids probably show an immune response (Arnott *et al.* 2000) and a link between

brain monoaminergic systems and the immune system has been reported in mammals (Lacosta *et al*. 1998, 2000; MohanKumar *et al*. 1999). Nutritional and endocrine factors also affect these systems (Levin & Routh 1996; Orosco *et al*. 1996; Leibowitz & Alexander 1998).

In conclusion, the results of the current study indicated that *S. solidus* infection affects brain monoaminergic activity in sticklebacks. The observed differences could reflect a state of stress in infected fish, but they could also be the result of an immune response or of changes in energy or endocrine status. In addition, one cannot exclude active manipulation of host neuroendocrine systems by the parasite, for instance by the release of a neuroactive substance. Further studies are needed in order to determine the mechanism(s) by which *S. solidus* influences host monoamine neurotransmitter systems and the possible role of these changes in controlling the behaviour of infected sticklebacks.

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