THE EFFECT OF STRESS AND STARVATION ON BRAIN SEROTONIN UTILIZATION IN ARCTIC CHARR (*SALVELINUS ALPINUS*)

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Summary

The effects of stress and starvation on brain levels of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were studied in Arctic charr (*Salvelinus alpinus*). Three experimental protocols were used to elucidate (1) the effect of stress in fish given food, (2) the effect of starvation, and (3) the effect of stress in fish deprived of food. In the stress experiments, fish were stressed three times a day over a four-week period, and in the starvation experiment the fish were starved for a four-week period. Stressed fish, whether given food or not, showed significantly higher concentrations of 5-HIAA, the main 5-HT metabolite, in both the telencephalon and the brain stem. The 5-HIAA/5-HT ratio (an index of serotonergic activity) was also significantly increased in the brain of stressed fish. In the telencephalon of starved fish, the 5-HT concentration was significantly decreased. However, starvation had no effect on 5-HIAA concentrations or 5-HIAA/5-HT ratios in either the telencephalon or the brain stem. These results suggest that stress increases brain serotonergic activity in Arctic charr, while starvation has no effect on the utilization of this transmitter system. It is suggested that stress could be a mediator of the increased 5-HIAA levels and 5-HIAA/5-HT ratios recently observed in low-ranking Arctic charr in a dominance hierarchy.

Introduction

In a recent study we found that social rank in Arctic charr (*Salvelinus alpinus*) was inversely correlated with the concentrations of 5-hydroxyindoleacetic acid (5-HIAA), a major serotonin (5-hydroxytryptamine, 5-HT) metabolite, present in the telencephalon and the brain stem (Winberg *et al.* 1991). Thus, subordinate individuals showed much higher brain 5-HIAA concentrations than dominant fish, indicating elevated serotonergic activity in subordinate fish. Since the 5-HT levels remained unchanged, the 5-HIAA/5-HT ratios, an index of serotonin utilization (Fuller, 1985), were also increased in low-ranking fish.

In mammals, serotonergic neurones are thought to be involved in stress reactions in the brain (Culman *et al.* 1984). The effect of stress on brain

serotonergic activity in rodents has been the subject of several studies (Bliss *et al.* 1972; Curzon *et al.* 1972; Morgan *et al.* 1975; Morgan and Rudeen, 1976; Adell *et al.* 1988; Mitchell and Thomas, 1988). In general, these studies have reported that stress increases brain levels of 5-HIAA without affecting 5-HT concentrations.

In fish, a low position in a dominance hierarchy has been associated with physiological signs of increased stress (Noakes and Leatherland, 1977; Ejike and Schreck, 1980; Peters *et al.* 1980; Scott and Currie, 1980), implying that the observed increase in 5-HIAA levels in subordinate fish (Winberg *et al.* 1991) could be stress-mediated. However, to our knowledge, there have been no studies on the effect of stress on brain serotonergic activity in fish.

There is also another possible explanation for the increased 5-HIAA levels displayed by subordinate Arctic charr. In a dominance hierarchy, subordinate fish have reduced access to food, since their feeding attempts often induce attacks from the dominant fish. Indeed, fish occupying low positions in a dominance hierarchy show much lower weight gains than dominant individuals (Jobling and Wandsvik, 1983; Abbott *et al.* 1985; Abbott and Dill, 1989), indicating malnutrition in subordinates. In mammals, food deprivation for 24–72 h has been found to elevate brain 5-HIAA levels (Curzon *et al.* 1972; Kantak *et al.* 1978; Fuenmayor and Garcia, 1984). Thus, it is possible that the increase in 5-HIAA levels seen in subordinate Arctic charr (Winberg *et al.* 1991) is an effect of food deprivation.

The aim of the present study was to investigate the effect of stress and starvation on brain levels of 5-HT and 5-HIAA in Arctic charr. In our previous study (Winberg *et al.* 1991) 2–11 weeks of subordinate experience increased brain 5-HIAA levels and 5-HIAA/5-HT ratios. In this study we tried to imitate the chronic stress experienced by a subordinate individual by subjecting the fish to repeated daily stress during a four-week period. The effect of stress was studied both in fish given food and in fish deprived of food during the experiment.

Materials and methods

Fish

The fish were 2-year-old offspring of Arctic charr (*Salvelinus alpinus* L.) caught in Lake Torrön, Jämtland, Sweden. The fish were kept indoors at our department for more than one year before the experiment. The holding tank was continuously supplied with aerated Uppsala tap water (8–11°C) and the photoperiod was kept at 10 h:14 h L:D (light between 08:00 h and 18:00 h). The fish were fed daily with commercial trout pellets (EWOS ST40, Astra-EWOS Sweden) at 1–2% of the body weight.

Aquaria

All experiments were performed in four glass aquaria (1000 mm × 500 mm × 500 mm), each divided into four compartments by black plastic walls, and continuously supplied with aerated Uppsala tap water ($0.80 \, l \, min^{-1}$, 8–10°C).

The water level was maintained at 400 mm by a standpipe. To minimize disturbance of the fish, a fine nylon-mesh screen was attached to the front of the aquaria while black plastic covered the remaining sides. Light was provided by fluorescent tubes (2×20 W, warm white), placed 100 mm above the water surface. The photoperiod was 12 h:12 h L:D with light on between 07:00 h and 19:00 h. The bottom of each compartment was covered with a plastic net that could be lifted by lines. Stress was induced, three times a day (each day), by elevating this net bottom so that the dorsal fin of the fish was above the water surface; the fish was left in the elevated position for 15 min. While lifted, the fish was given three pinches (with fingers) to the caudal fin. During the stress experiments, control fish were kept in identical aquaria but the plastic nets were never lifted.

Experimental protocol

Experiment 1. Effect of stress in fish given food

The fish, initially weighing 46 ± 12 g (mean$\pm$s.D., $N=16$), were kept isolated in individual compartments and daily given commercial trout pellets at 2–4 % of the body weight. Stress was induced as described above during a four-week period (22 May to 12 June).

Experiment 2. Effect of starvation

The fish, initially weighing 54 ± 18 g (mean$\pm$s.D., $N=16$), were kept isolated in individual compartments. Experimental fish were starved for a four-week period (14 June to 12 July) while controls were fed daily with trout pellets (2–4 % of body weight).

Experiment 3. Effect of stress in food-deprived fish

This experiment was performed as for experiment 1 but neither stressed fish nor controls were fed during the experiment. At the start of the experiment the mean mass of the fish was 141 ± 26 g (mean$\pm$s.D., $N=16$). The experiment was run during the period 22 September to 15 October.

Tissue sampling

At the end of each experimental period, fish were decapitated, between 17:00 and 18:00 h. The brain (excluding the olfactory bulbs and the pituitary gland) was rapidly removed and divided into two parts: the telencephalon (weighing 11 ± 3 mg) and the remaining parts of the brain (weighing 156 ± 38 mg), here called the brain stem. The brain samples were wrapped in aluminium foil, frozen in liquid nitrogen (within 2 min of decapitation), and kept at $-80\,°C$. Each fish was weighed after tissue sampling.

Assay of monoamines and their metabolites

The frozen brain samples were homogenized in 4 % (w/v) ice-cold perchloric acid (PCA) containing 0.2 % EDTA, 0.05 % sodium bisulphite and $40\,\text{ng ml}^{-1}$

epinine (deoxyepinephrine, the internal standard), using a Potter–Elvehjem homogenizer (brain stem) or an MSE 100 W ultrasonic disintegrator (telencephalon).

5-HT and 5-HIAA were quantified using high performance liquid chromatography with electrochemical detection as described by Nilsson (1989). As a measure of serotonergic activity, the 5-HIAA/5-HT ratio was calculated for each individual (Fuller, 1985; Winberg et al. 1991).

Results

Brain levels of 5-HT and 5-HIAA

Stressed fish, whether given food (expt 1) or starved (expt 3), showed significantly higher 5-HIAA concentrations in both the telencephalon and the brain stem (Fig. 1). The 5-HIAA/5-HT ratio was also higher in stressed fish (Fig. 2) and a significant increase in the 5-HIAA/5-HT ratio was found in the telencephalon as well as in the brain stem of stressed fish deprived of food during the experiment (expt 3, Fig. 2). Furthermore, an increase in the 5-HIAA/5-HT ratio was seen in stressed fish given food during the experiment (expt 1), although, in this experiment, the increase in the 5-HIAA/5-HT ratio was only significant in the telencephalon (Fig. 2).

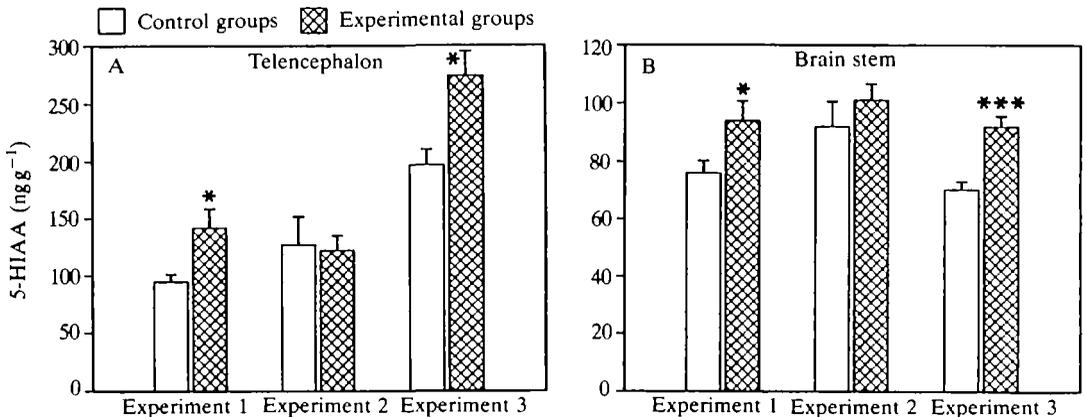There was no effect of starvation on 5-HIAA concentrations in either

Fig. 1. Concentrations of 5-HIAA in the telencephalon (A) and the brain stem (B). Values are mean and s.e.m. from eight individuals. Experiment 1: effect of stress (3×15 min daily) over 4 weeks. Both stressed fish and controls were given food during the experiment. Experiment 2: effect of 4 weeks of starvation. The controls were fed daily. Experiment 3: effect of stress (3×15 min daily) over 4 weeks. Both stressed fish and controls were deprived of food during the experiment. * $P<0.05$, *** $P<0.001$ Mann–Whitney U-test (two-tailed) indicate significant differences from control groups.
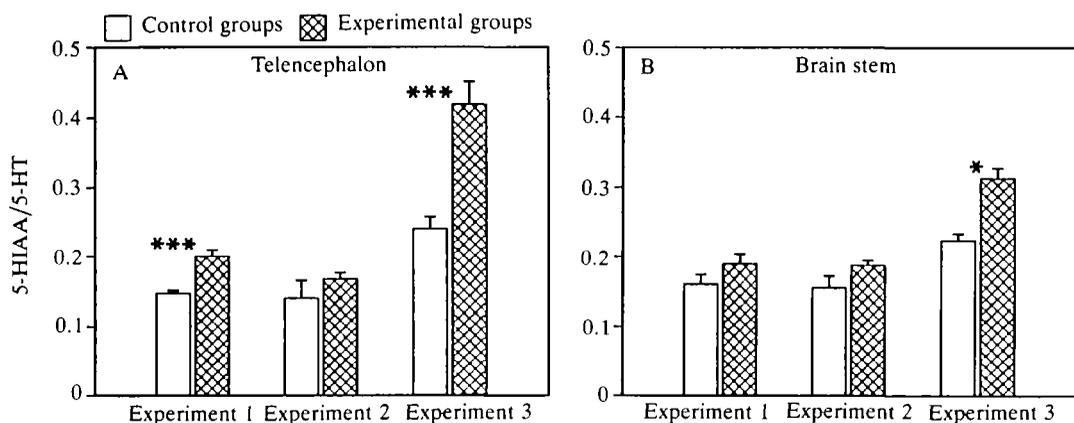
Fig. 2. 5-HIAA/5-HT ratios in telencephalon (A) and brain stem (B). Values are mean and s.e.m. from eight individuals. Experimental details are given in Fig. 1. * $P<0.05$, *** $P<0.001$ Mann–Whitney U-test (two-tailed) indicate significant differences from control groups.
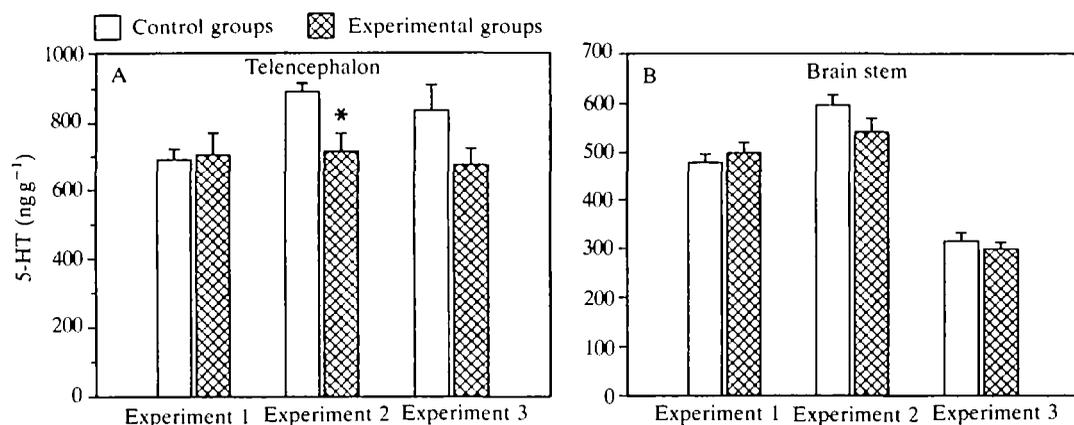

Fig. 3. Concentrations of 5-HT in telencephalon (A) and brain stem (B). Values are mean and s.e.m. from eight individuals. Experimental details are given in Fig. 1. * $P<0.05$ Mann–Whitney U-test (two-tailed) denotes a significant difference from the control group.

telencephalon or brain stem (Fig. 1) and starvation did not significantly affect 5-HIAA/5-HT ratios (Fig. 2).

The 5-HT concentration was significantly decreased in the telencephalon of starved fish (expt 2, Fig. 3). However, in brain stem no significant differences in 5-HT concentrations were seen between starved fish and controls (expt 2, Fig. 3).

There were no significant differences in brain 5-HT concentrations between stressed fish and controls when both groups were given food (expt 1, Fig. 3).
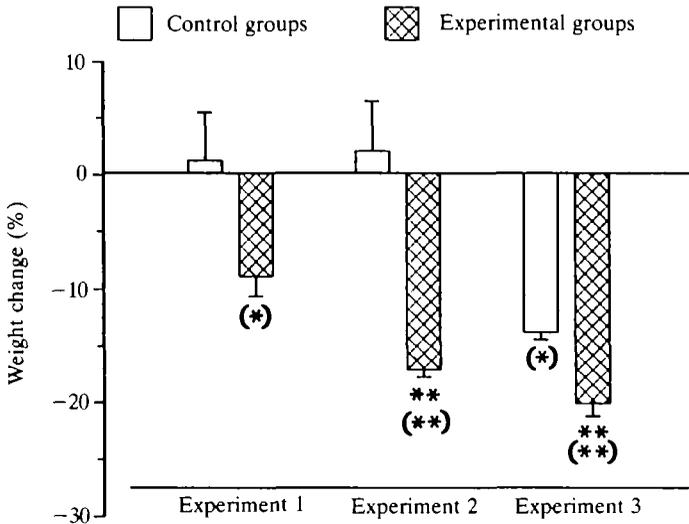
Fig. 4. Weight change during the 4-week experimental period. Weight changes are given as a percentage of starting weight. Values are mean and s.e.m. from eight individuals. Experimental details are given in Fig. 1. A significant difference between control and experimental groups is denoted by asterisks (Mann–Whitney U-test, two-tailed) while a significant weight change within a group (i.e. compared to the starting weight) is shown by asterisks in parentheses (Wilcoxon's rank sum test, paired, two-tailed). * $P<0.05$, ** $P<0.01$.

Where both groups were deprived of food during the experiment (expt 3), a small non-significant ($P=0.094$) decrease in 5-HT levels in the telencephalon occurred in the stressed individuals (Fig. 3).

Weight changes

In expt 1, where both controls and stressed fish were given food, the stressed fish showed a significant weight loss during the experiment compared with their starting weight. However, in this experiment, the difference in weight change between stressed fish and controls was not significant (Fig. 4).

As expected, starvation *per se* (expt 2) resulted in a significant weight loss. There was a significant difference in weight change between starved fish and controls (Fig. 4).

In expt 3, in which controls as well as stressed fish were deprived of food during the experiment, significant weight losses were seen in both groups. The weight loss was, however, significantly higher in stressed fish (Fig. 4).

Behaviour and general observations

During the first days of the stress experiments the fish made furious efforts to escape when pinched on the caudal fin. As the experiments continued, these flight attempts gradually decreased. Stressed fish were inactive at the end of the

experiment, spending most of their time close to the bottom of the aquarium. Strikingly, stressed fish made little effort to escape when netted at the end of the experiment. In contrast, control fish and fish subjected to starvation were active and moved around in their compartments throughout the experiment.

No mortality occurred in any of the groups during the experiments.

Discussion

The results from the present study clearly demonstrate that stress increases brain levels of 5-HIAA, the main 5-HT metabolite, without affecting 5-HT concentrations in Arctic charr. Thus, the 5-HIAA/5-HT ratio, which can been used as an index of serotonergic activity (Fuller, 1985; Winberg *et al.* 1991), was elevated in stressed fish, indicating that stress induces an increase in the activity of the central serotonergic system in this species. Starvation, in contrast, had no effect on either 5-HIAA concentrations or 5-HIAA/5-HT ratios.

The stress-induced increase in 5-HT activity was present in both the telencephalon and the brain stem, although it was somewhat more pronounced in the telencephalon. Thus, stress seems to have a general effect on 5-HT utilization in the brain of Arctic charr. It should be mentioned that stress in mammals has been reported to increase the concentration of tryptophan, the precursor of 5-HT, in blood as well as in brain (Curzon *et al.* 1972; Neckers and Sze, 1975; Dunn, 1988). The activity of tryptophan hydroxylase, the enzyme catalyzing the rate-limiting step in 5-HT synthesis, is normally restricted by substrate (i.e. tryptophan) availability. Consequently, increased brain tryptophan concentrations are likely to increase 5-HT synthesis and 5-HT turnover (Boadle-Biber, 1982). However, this mechanism could hardly explain the increased 5-HIAA levels seen in stressed fish, since the 5-HT concentration remained unchanged or even showed a tendency to decrease during stress. Hence, it is more likely that the increased 5-HIAA/5-HT ratios during stress were caused by an increased use (release) of 5-HT.

Interestingly, Dunn (1988) observed a decrease in the 5-HT concentration in prefrontal cortex and hypothalamus in mice after 15 min of footshock, although plasma as well as brain levels of tryptophan increased progressively during the stress treatment. The decrease in 5-HT concentration was, however, reversed after a further 15 min of footshock. The 5-HIAA/5-HT ratio was elevated in both these brain regions after 15 and 30 min of footshock. Dunn (1988) suggests that the transient decrease in 5-HT reflects a stress-induced increase in 5-HT release, depleting existing 5-HT stores. Using *in vivo* voltametry in rats, Joseph and Kennett (1983) found that hippocampal 5-HIAA levels increased immediately after initiation of immobilization stress, suggesting stress-induced increases in 5-HT release into the extracellular space. Tryptophan administration did not increase brain 5-HIAA release, as determined by *in vivo* voltametry, but increased tissue 5-HIAA levels determined by *post mortem* neurochemical analysis (De Simoni *et al.* 1987). Furthermore, Lookingland *et al.* (1986) showed that exogenous tryptophan, given to rats, produced a dose-dependent increase in both 5-HT

and 5-HIAA concentrations in several hypothalamic regions, without affecting 5-HIAA/5-HT ratios in these regions. Thus, it seems that both tryptophan administration and stress can increase 5-HT turnover but only the latter increases 5-HT release.

The central serotonergic system is thought to be involved in the regulation of locomotor activity in fish (Fingerman, 1976; Genot et al. 1984). During the present stress experiments, the attempts of the fish to escape when pinched on the caudal fin gradually disappeared. Indeed, the locomotor activity of the stressed fish showed a general decrease during the experimental period. Similarly, subordinate Arctic charr in a dominance hierarchy show lower locomotor activity compared to dominant fish (Winberg et al. 1991). The observation that subordinate fish show physiological stress symptoms (Noakes and Leatherland, 1977; Ejike and Schreck, 1980; Peters et al. 1980; Scott and Currie, 1980) as well as elevated brain 5-HIAA/5-HT ratios (Winberg et al. 1991) makes it tempting to suggest a causal relationship between stress, increased serotonergic activity and reduced locomotor activity in these individuals.

Food deprivation has been reported to increase brain 5-HIAA levels in mammals (Curzon et al. 1972; Kantak et al. 1978; Fuenmayor and Garcia, 1984). In the present study, starvation had no effect on brain 5-HIAA concentrations in Arctic charr. Starvation did, however, significantly decrease the 5-HT concentration in the telencephalon. In the mammalian experiments, the animals were only deprived of food for 24–72 h, which may explain the discrepancy between these studies and our results. As tryptophan is an essential amino acid, the four-week starvation period used in our experiment may very well have reduced tryptophan availability, and thus 5-HT synthesis. Phylogenetic factors are, of course, also likely to explain these discrepancies.

As expected, fish subjected to four weeks of starvation lost weight. A weight loss was also seen in stressed fish given food during the experiment. The latter result could be related to a decreased food intake due to the stress. Indeed, the stressed fish often paid little attention to the food. However, most interestingly, starved stressed fish lost more weight than starved controls, even though they showed less locomotor activity. This could be related to a stress-induced increase in metabolic rate. Abbott and Dill (1989) showed that the dominant individual in a size-matched pair of steelhead trout (Oncorhynchus mykiss) had a higher growth rate than the subordinate, even when both fish were fed equal amounts. As subordinates were less active, Abbott and Dill (1989) suggested that the reduced growth in subordinate fish correlated with increased metabolic rate caused by stress.

Brain concentrations of 5-HT and 5-HIAA varied between experiments. This variation is likely to reflect seasonal variations in brain monoamine levels. Seasonal and daily variations in brain monoamine concentrations are well known and have previously been shown to occur in fish (Fingerman, 1976; Sauerbier and Meyer, 1977; Popek, 1983; Ehrenström and Johansson, 1987; Khan and Joy, 1988). Furthermore, there was an increase in the size of fish used in later experiments and it is possible that this growth affected brain 5-HT and 5-HIAA concentrations.

However, since a control group was included in each experiment, the significant changes seen within an experiment must reflect changes in serotonergic function that are responses to the experimental treatment, that is short-term changes that occur in addition to seasonal variations in serotonergic function. Indeed, our experiments clearly suggest that the seasonal and weight changes seen in the present study did not interfere with the main conclusion – that stress increases serotonin utilization in Arctic charr. Thus, in the first two experiments, which were run close together (May–July, fish weighing approximately 50 g), we show that stress in fish given food (expt 1) increases serotonergic activity and that this is not due to food deprivation (expt 2). In the third experiment, we show that stress also has this effect in September when on average the fish weighed 141 g.

In conclusion, the present results show that stress significantly increases brain 5-HIAA levels without affecting 5-HT concentrations in Arctic charr. This pattern of change appears to be identical to that displayed by subordinate Arctic charr in a dominance hierarchy (Winberg *et al.* 1991). Thus, increased stress, which subordinate individuals are likely to experience, could very well be the cause of the increased 5-HIAA/5-HT ratios seen in these fish. The stress regime used here could be regarded as a number of acute stresses. Apart from the acute stress caused by each attack by dominant fish, a subordinate fish is probably also exposed to a chronic challenge by the constant presence of the dominant fish. Such a constant stress is probably impossible to imitate experimentally. In spite of this difficulty, the experimental stress regime used caused changes in serotonergic activity that were very similar to those in a subordinate fish. In contrast, the food deprivation experienced by subordinate Arctic charr can probably be ruled out as a factor causing the increased 5-HIAA/5-HT ratios observed in low-ranking fish.

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