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# SPONTANEOUS LOCOMOTOR ACTIVITY IN ARCTIC CHARR MEASURED BY A COMPUTERIZED IMAGING TECHNIQUE: ROLE OF BRAIN SEROTONERGIC ACTIVITY

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## Summary

Using a computerized video-image analysis system, spontaneous locomotor activity was measured in dominant and subordinate individuals of Arctic charr (*Salvelinus alpinus*) and in individuals treated with inhibitors of serotonin (5-HT) synthesis and re-uptake. Arctic charr were put together in pairs. After 1 week, subordinate individuals were found to have elevated brain levels of 5-hydroxyindoleacetic acid, a major 5-HT metabolite, suggesting an increase in serotonergic activity. The subordinate individuals had significantly lower spontaneous locomotor activity than the dominant fish. Similarly, Arctic charr displayed a significantly reduced locomotor activity when their serotonergic activity was stimulated by the specific 5-HT re-uptake inhibitor zimeldine. In contrast, fish treated with the 5-HT synthesis inhibitor *p*-chlorophenylalanine showed a significant increase in locomotor activity. Dominant, subordinate and pharmacologically treated fish all had very similar activity rhythms for the 18h test period. Thus, neither the previous social experience nor the pharmacological treatment seemed to affect the diurnal activity rhythm *per se*. Taken together, these results suggest that the brain serotonergic system inhibits locomotor activity and support the possibility that 5-HT is involved in the inhibition of locomotor activity displayed by subordinate fish.

## Introduction

Brain serotonergic activity, measured by the concentration of 5-hydroxyindoleacetic acid (5-HIAA), the main metabolite of serotonin (5-hydroxytryptamine, 5-HT), or as the 5-HIAA/5-HT ratio, is increased in subordinate Arctic charr (*Salvelinus alpinus*) (Winberg *et al.* 1991). This elevation of serotonergic activity, which develops during social interactions (Winberg *et al.* 1992*a*), is probably a response to the stress experienced by the subordinate fish (Winberg *et al.* 1992*b*). In previous studies we have observed that subordinate as well as artificially stressed fish have reduced locomotor

Key words: Arctic charr, locomotor activity, serotonin, *p*-CPA, zimeldine, social experience, *Salvelinus alpinus*.

activity. The dominant fish in a pair of Arctic charr is the most active individual while the subordinate fish spends most of its time motionless close to the surface, often in a corner of the aquarium.

The reduced locomotor activity in subordinate fish could, at least partly, be an effect of increased serotonergic activity. The central serotonergic system is thought to be involved in the regulation of spontaneous locomotor activity in mammals (Gerson and Baldessarini, 1980). In rodents, it has generally been found that decreased availability of 5-HT increases locomotor activity, whereas increased 5-HT availability has the opposite effect (Gerson and Baldessarini, 1980). Available data on fish indicate a similar role for 5-HT in the regulation of locomotor activity, although the data are ambiguous. Thus, while Fingerman (1976) reported that 5-HT has an inhibitory effect on locomotor activity in Gulf killifish (*Fundulus grandis*), Genot *et al.* (1984) claimed that 5-HT had the opposite effect in eels (*Anguilla anguilla*). In the study by Fingerman (1976), 5-HT was administered by intraperitoneal injections. At least in mammals, 5-HT does not normally penetrate the blood–brain barrier (Cooper *et al.* 1986). Fingerman (1976) did not analyze brain levels of 5-HT or 5-HIAA, and it is uncertain whether intraperitoneal injections of 5-HT have any direct effect on brain serotonergic activity in fish.

Measurements of spontaneous locomotor activity in fish have at best been semi-quantitative and subtle changes in activity have probably been overlooked. Fingerman (1976) measured activity by counting the number of lines, marked on the bottom of the chamber, that a fish crossed during 10min observations. In the study by Genot *et al.* (1984), activity was measured by flexible metal rods dipping vertically into the aquarium. When these rods were displaced by a swimming fish, an electrical circuit was closed. Recent developments in the field of computerized video-image analysis mean that locomotor activity can now be readily quantified with much greater accuracy. Nevertheless, such systems have, to our knowledge, never been used for studies of fish behaviour.

The aim of the present study was to investigate the role of the serotonergic system in locomotor activity of Arctic charr. This was done by quantifying locomotor activity either in fish in which the serotonergic system had been stimulated or inhibited pharmacologically or in dominant and subordinate individuals, in which 5-HT activity appears to be altered by endogenous mechanisms. Spontaneous locomotor activity (swimming distance) was continuously recorded using a video camera connected to a newly developed computerized image-analysis system. Serotonergic activity was stimulated by the specific 5-HT re-uptake blocker zimeldine or inhibited by the irreversible 5-HT synthesis inhibitor *p*-chlorophenylalanine (*p*-CPA).

## Materials and methods

### *Fish*

The fish were 2-year-old offspring of Arctic charr (*Salvelinus alpinus* L.) caught in Lake Hornavan, Lapland, Sweden. They were kept indoors at the Department of Zoophysiology, in a 1000l holding tank containing approximately 250 fish, for more than 1 year before the experiment. The holding tank was continuously supplied with aerated

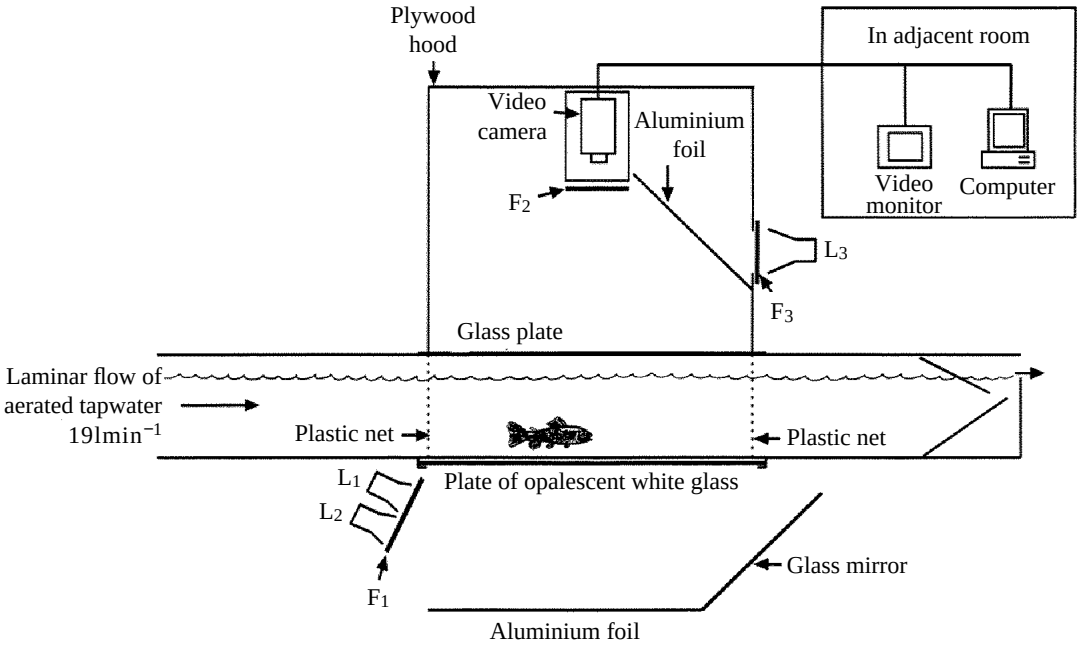


Fig. 1. The experimental arrangement. The test area, measuring 46cm×33cm×11.5cm (length×width×deep), was limited upstream and downstream by two fine-mesh plastic nets and covered by a glass plate. The video camera was placed in the plywood hood 33cm above the test area. $L_1$ and $L_2$ are 20W halogen lamps. The light from these two lamps was filtered through $F_1$, a glass filter which allowed no transmission of wavelengths shorter than 750nm, and reflected through the white opalescent glass plate. $L_3$ is a 75W bulb used to provide the light/dark regime. This light was filtered through $F_3$, a glass filter with transmission of less than 10% for wavelengths longer than 760nm. $F_2$ is a red plastic filter (Kodak WRATTEN 89B) which allowed no transmission of wavelengths shorter than 670nm.

Uppsala tapwater (8–11˚C). The light/dark regime was continuously and automatically adjusted to conditions at the latitude 51˚N. The fish were fed daily with commercial trout pellets (EWOS ST40, Astra-EWOS Sweden) at 2–4% of body weight.

### *Testing apparatus*

The experimental arrangement is shown in Fig. 1. All activity measurements were carried out on one fish at a time. The locomotor activity (horizontal movement) was measured in the fluviarium constructed by Höglund (1961). The fluviarium can be described as an artificial stream with a laminar flow of aerated water (Uppsala tapwater, $19\,l\,min^{-1}$, $0.008\,m\,s^{-1}$). The test area of the fluviarium, which measured 46cm×33cm×11.5cm (length×width×depth), was limited upstream and downstream by two fine-mesh plastic nets and covered by a glass plate. A plywood hood, painted white on the inside, was placed over the test area.

Two 20W halogen lamps ($L_1$ and $L_2$, Fig. 1) equipped with a red glass filter (RG 780, Melles Griot; $F_1$, Fig. 1), which allowed no transmission of wavelengths shorter than 750nm, was used as the light source for the video camera. This infrared light was

reflected by a glass mirror and a sheet of aluminium foil through a plate of opalescent white glass situated immediately below the bottom of the test area. On the video monitor, this arrangement gave a light background, without reflections, against which the fish was readily detected. The light:dark regime was provided by another light source (75W bulb) connected to a timer; the light was switched on between 06:30 and 18:30h. To prevent disturbance of the video picture, this light was filtered through a glass filter (Schoot; $F_3$, Fig. 1) allowing less than 10% transmission of wavelengths longer than 760nm. Disturbance of the video picture was further prevented by a filter (Kodak WRATTEN 89B) allowing no transmission of wavelengths shorter than 670nm, mounted in front of the video camera.

The video camera (4.8mm lens) was placed in the plywood hood, 33cm above the test area. The camera was connected to a monitor and a computer in an adjacent room.

### *The video-computer-based image-analysis system*

#### *Hardware*

The hardware consisted of an IBM-compatible video digitizer, PV VISION PLUS board (Imaging Tech. Inc., Woburn, Massachusetts, USA), connected to an IBM-compatible computer (386 DX, 20MHz). A TEA image manager TIM 3.30 (Difa Measuring Systems, Breda, The Netherlands) was used as a driver program for the card. Any standard video signal, either from a camera or from a video recorder, can be connected to the digitizer. We used a CCD black and white video camera (Panasonic WV-BL200, light sensitivity 0.5lx) from which the infrared filter had been removed.

#### *Software*

The motion-analysis program MOTION 0.13β (made by Jacob Rosseau, Institute of Molecular Biology and Medical Biotechnology, University of Utrecht, The Netherlands) has previously been described by Spruijt *et al.* (1992) for behavioural studies on rats.

In this system, the data acquisition procedure is based on the following algorithm. A picture of the empty test area is digitized. A picture of the test area with the fish is then made. These two images are subtracted from each other, leaving the fish and the noise. The noise is filtered by a thresholding operation, followed by transformation to a binary image. Irrelevant objects (e.g. faeces) are deleted, as the objects are tested against a criterion of size. The maximum sampling rate is 1.4 samples per second. All data were stored in RAM memory during an experimental run. The sampling storage capacity of the system was limited to 32000 samples, which corresponds to 18h at a sampling rate of one sample every 2s.

In the present study, we let the computer calculate the distance travelled for 1 and 30min periods. Distances travelled summed for 1min periods were only calculated for the first 4h of the experiment and were used to construct activity frequency diagrams. The computer also calculated the cumulative distance travelled by adding the distance between successive observations. Before each test, the computer was calibrated by placing a ruler in the test area. The smallest movement recorded by the computer was 1 cm.

### *Experimental protocol*

During all experiments the photoperiod was 12h:12h L:D with light on between 06:30 and 18:30h. The fish were kept in this photoperiod for at least 1 week prior to the experiments. They were fed daily with commercial trout pellets, at 2–4% of body weight.

#### *Experiment 1. Activity pattern over 48h*

One week prior to the experiment, the fish, weighing 29.2±2.6g (mean ± S.D., $N$=4), were isolated in individual 20l aquaria continuously supplied with aerated Uppsala tapwater (0.80l$\,min^{-1}$, 8–10˚C). Light was provided by fluorescent tubes (2×20W, warm white), placed 100mm above the water surface. Individual fish were transferred to the fluviarium 15min before the measurement of activity started and spontaneous locomotor activity was measured for 48h, starting at 16:00h. Because a maximum of 32000 samples could be stored in the computer RAM memory, the position of the fish was only recorded once every 6s. This experiment was carried out in November 1991.

#### *Experiment 2. Effect of dominant and subordinate experience*

Behavioural observations were made in four glass aquaria (1000mm×500mm× 500mm) continuously supplied with aerated Uppsala tapwater (0.80l$\,min^{-1}$, 8–10˚C). Each aquarium was divided into four 50l chambers by inserting black polyvinylchloride walls, and one fish, weighing 48.4±7.2g (mean ± S.D., $N$=15), was put into each of these chambers. In this way, each fish was kept visually isolated from the other fish for 7 days in an attempt to diminish the effect of previous hierarchic experience (Winberg *et al.* 1992*a*). A black nylon-mesh screen was attached to the front of the aquaria to minimize disturbance to the fish during observation. The remaining sides of the aquaria were covered with black plastic. Light was provided by fluorescent tubes (2×20W, warm white), placed 100mm above the water surface.

After the isolation period, fish were put together in pairs by removing the polyvinylchloride wall that had kept them separated. Thereafter, the fish were reared in pairs for another week and aggressive acts performed by the fish were counted for 4–7 sessions of 10min. The first observation was made 5–10min after the fish had been put together in pairs and the last immediately before activity measurements. The types of aggressive behaviour observed and recorded were attack (where the fish makes a rapid approach, often culminating in a bite), charge (a direct but slow approach towards the other individual) and nip (a bite without a prior approach). The dominance–subordinate relationship was usually apparent during the first observation session. After a short period of vigorous fighting, one individual in the pair, designated the subordinate, generally took up a position close to the surface. Thereafter, the subordinate fish spent most of the time in this position or in a corner of the aquarium. From this time onwards, aggressive acts were almost exclusively performed by the dominant individual, which moved freely around the aquarium, usually close to the bottom. The dominance relationship thus established seemed to be stable and no change in the relationship was observed during the experimental period.

Controls were isolated for 1 week, as described above, but were not allowed to interact with any other fish before activity measurements.

After 1 week of pair rearing, one of the fish in the pair was transferred to the fluviarium and spontaneous locomotor activity was measured for 18h, starting at 18:30h, with the light on from 06:30h. The position of the fish was recorded once every 2s. A dominant or subordinate individual was used for activity measurements on alternating days. The remaining fish in the pair was instantly killed by decapitation immediately after the other fish had been taken for activity measurements. The brain (excluding olfactory bulbs and the pituitary gland) was rapidly removed, wrapped in aluminium foil, frozen in liquid nitrogen (within 2min of decapitation) and kept at −80°C. The fish subjected to activity measurement was, at 18:00h, also decapitated and brain tissue was taken for analyses after the experiment. This experiment was carried out in January 1992.

*Experiment 3. Effect of zimeldine*

The fish, weighing 20.8±3.5g (mean ± S.D., $N$=12), were isolated for 1 week as in experiment 1. After the isolation period, each fish was given zimeldine (5mg$kg^{-1}$ intraperitoneally) (zimeldine dihydrochloride monohydrate, Astra Arcus AB, Södertälje, Sweden, 0.5mg$ml^{-1}$ in 0.9% saline) and immediately transferred to the test area. Controls were injected with an equivalent volume of saline. After 15min, the measurement of spontaneous locomotor activity started and continued for 18h as in experiment 2. After the experiment, at 18:00h, the brain was removed as described above. The experiment was carried out in October 1991.

*Experiment 4. Effect of* p-*CPA*

The fish, weighing 19.0±3.1g (mean ± S.D., $N$=11), were isolated for 1 week as in experiment 1. Four days before activity measurements, the fish were given *p*-CPA (200mg$kg^{-1}$ intraperitoneally) (parachlorophenylalanine methylester, Sigma Chemical Company, St Louis, Missouri, USA, 2.5mg$ml^{-1}$ in 0.9% saline). Controls were injected with an equivalent volume of saline. Each fish was transferred to the test area 15min before the start of activity measurements, and spontaneous locomotor activity was measured for 18h as in experiment 2. After the experiment, at 18:00h, the brain was removed as described above. This experiment was carried out in October 1991.

*Assay of dopamine, 5-HT and 5-HIAA*

The frozen brain samples (weighing 169.4±36.4mg, mean ± S.D., $N$=30) were homogenized in 4% (w/v) ice-cold perchloric acid containing 0.2% EDTA, 0.05% sodium bisulphite and 40ng$ml^{-1}$ epinine (deoxyepinephrine, the internal standard), using a Potter-Elvehjem homogenizer.

Dopamine (DA), 5-HT and 5-HIAA were measured 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 (Shannon *et al.* 1986; Winberg *et al.* 1991).

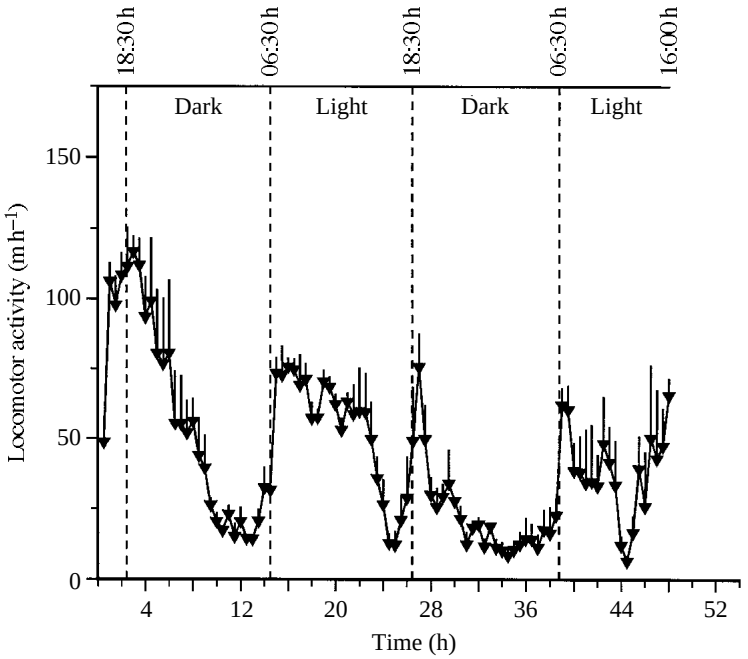


Fig. 2. Activity pattern over 48h of individual Arctic charr isolated for 1 week prior to the experiment. Activity measurements started at 16:00h and stopped at 16:00h, 48h later; the light was on between 06:30 and 18:30h. Values are means + S.E.M. for four individuals.

### *Statistics*

All values are given as mean ± standard error. When data from three or more groups were compared a Kruskal–Wallis analysis of variance (two-tailed) was used. If significant differences ($P<0.05$) were indicated with this test, or if data consisted of only two groups, individual groups were compared using a Mann–Whitney *U*-test (two-tailed).

## Results

### *Spontaneous locomotor activity*

*Experiment 1. Activity pattern over 48 h*

The fish showed the highest locomotor activity during the first 3h of the experiment (Fig. 2). Over the rest of the dark period, the activity gradually decreased. High activity was also seen at the beginning of the second dark period. However, the activity did not reach the same high level as in the initial part of the first night (Fig. 2). Furthermore, the duration of this high-activity period was much shorter than during the first night. A typical swimming pattern for a 1min period of the first high-activity period, 1h after the start of the experiment, is shown in Fig. 3A.

A high-activity period was also detected in the morning. When the light went on, the activity increased abruptly and stayed high during the morning hours, on both days (Fig. 2). The activity decreased in the early afternoon.

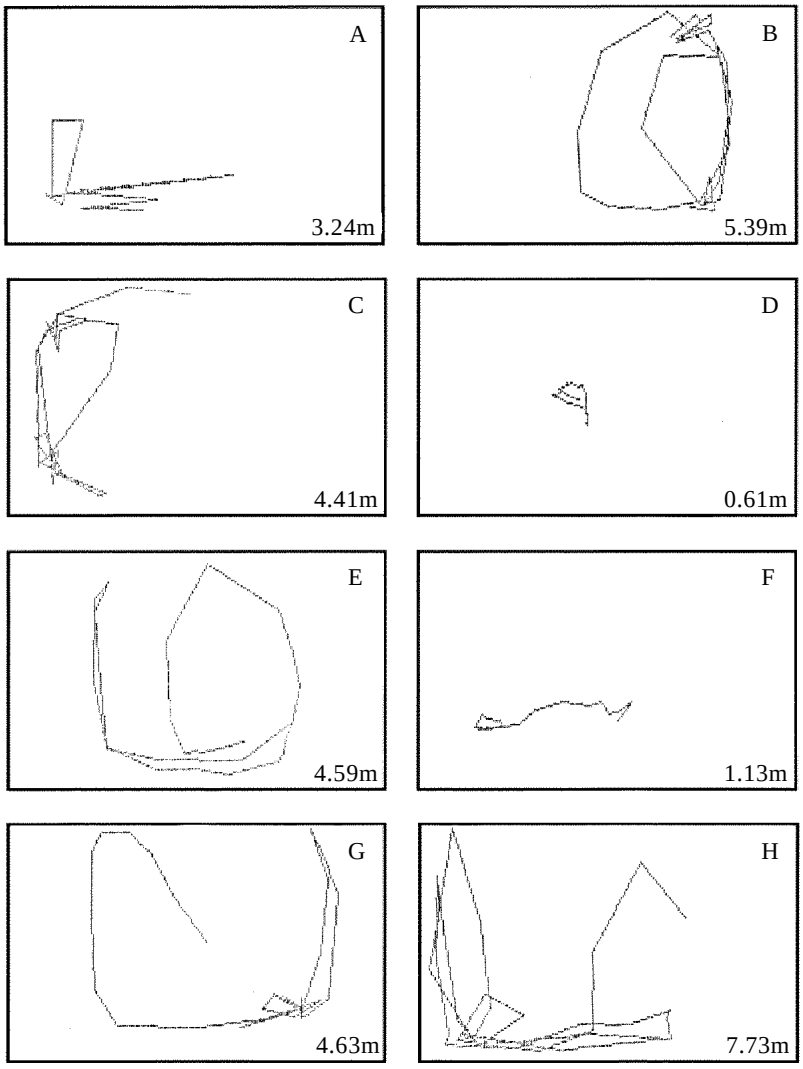


Fig. 3. (A–H) Tracking of movements over 1min (1h after the start of the experiment) in Arctic charr. The figure shows one fish, randomly chosen, from each experiment. Distance travelled (m) over the 1min period is also given. The position of the fish was recorded once every 2s except in experiment 1, when it was recorded only once every 6s. (A) experiment 1; (B) experiment 2, control; (C) experiment 2, dominant fish; (D) experiment 2, subordinate fish; (E) experiment 3, control; (F) experiment 3, zimeldine-treated fish; (G) experiment 4, control; (H) experiment 4, *p*-CPA-treated fish. See Materials and methods for further details.

Thus, on both days of the experiment, two high-activity periods were obvious. One during the early night and another one during the morning (Fig. 2). Periods of low activity were seen during the later part of the night and during the early afternoon (Fig. 2).

It should be pointed out that the position of the fish in this experiment was recorded by the computer only once every 6s in order to allow a 48h sampling period. Because the fish cannot be expected to move in a straight line between recordings of its position, the

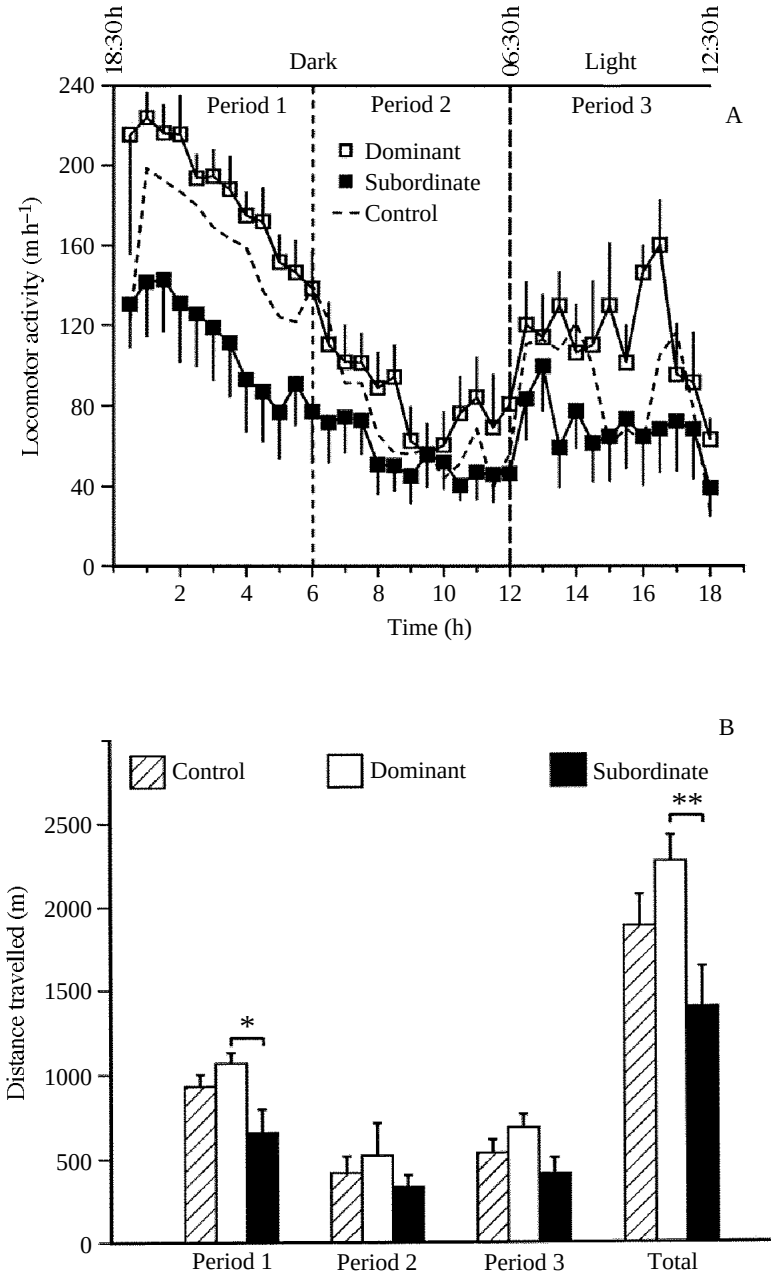


Fig. 4. (A,B) Locomotor activity of individual Arctic charr with a 1 week experience of dominant (*N*=7) or subordinate (*N*=8) position in a pair. Controls (*N*=6) are fish isolated for 1 week prior to the experiment. Activity measurements started at 18:30h and stopped at 12:30h; the light was on from 06:30h. (A) Locomotor activity was measured every 30min. Values are means ± S.E.M., except for controls, where only means are given. (B) Distance travelled over 6 h periods and total distance travelled. Period 1, 18:30–00:30h; period 2, 00:30–06:30h; period 3, 06:30–12:30h. Values are means + S.E.M. **P*<0.05, ***P*<0.01, Mann–Whitney *U*-test, two-tailed.

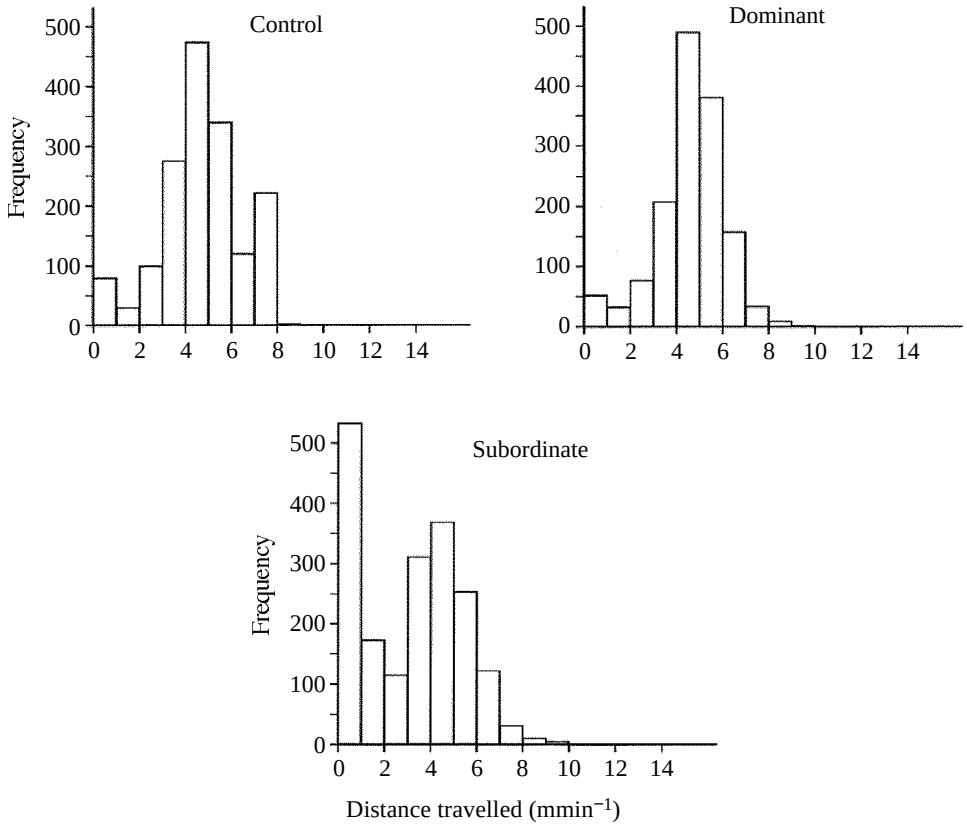


Fig. 5. Frequency diagrams of distance travelled over 1min periods by Arctic charr with a 1 week experience of dominant (*N*=6) or subordinate (*N*=8) position in a pair. Controls (*N*=6) were isolated 1 week prior to the experiment. Only the first 4h of the 18h experiment are included.

distance measured will be an underestimate of the actual distance travelled. Thus, the absolute values for distance travelled in experiment 1 cannot be directly compared with those of the following experiments, in which the position of the fish was measured once every 2s.

*Experiment 2. Effect of dominant and subordinate experience*

The dominant fish showed the highest locomotor activity (Fig. 4A) and there was a significant difference in distance travelled between dominant and subordinate fish during the first 6h of the experiment (Fig. 4B). Furthermore, the dominant fish travelled a significantly higher total distance than the subordinates (Fig. 4B).

The activity frequency diagrams (Fig. 5), based on distance travelled per minute during the first 4h of the experiment, also showed that subordinate fish (grand median 3.45 m min$^{-1}$, individual median range 0.61–5.45 m min$^{-1}$) generally moved more slowly than dominant individuals (grand median 4.72 m min$^{-1}$, individual median range 4.4–5.04 m min$^{-1}$) and controls (grand median 4.50 m min$^{-1}$, individual median range

3.61–5.53 m $min^{-1}$). However, there was a bimodal frequency distribution for the subordinate fish. This bimodality was caused by differences in median velocity between individuals, and no bimodality was seen in the frequency distributions of activity of the individual fish. Although three subordinate individuals had median velocities of 0.61, 0.88 and 0.93 m $min^{-1}$, the rest of the subordinate fish (five individuals) had median velocities of 3.88, 4.41, 4.59, 4.62 and 5.45 m $min^{-1}$.

Typical swimming patterns over a 1min period, 1h after the start of the experiment, are shown in Fig. 3. During the first hours of the experiment, when the activity was highest, the fish moved around in wide circles, seemingly exploring the unfamiliar environment. This behaviour was most pronounced in dominant individuals and controls (Fig. 3).

The pattern of diurnal activity was similar to that in experiment 1 and there were no noticeable differences between dominants, subordinates or controls (Fig. 4A). The highest activity was recorded during the first hours of the experiment. During the night, the locomotor activity gradually decreased and reached a minimum between 02:30 and 06:30h (Fig. 4A). When the light was turned on, the activity increased, only to fall again during the last hours of the experiment (Fig. 4A).

*Experiment 3. Effect of zimeldine*

The zimeldine-treated fish were less active than the controls (Figs 3 and 6A) and there was a significant difference in distance travelled during all three 6h periods of the experiment (Fig. 6B). The total distance travelled was also significantly lower in zimeldine-treated fish (Fig. 6B).

Furthermore, the frequency diagrams of activity (Fig. 7) showed that the zimeldine-treated fish generally moved more slowly than the controls. The median velocities over the first 4h of the experiment were 2.42 m $min^{-1}$ (individual median range 1.52–3.61 m $min^{-1}$) and 3.95 m $min^{-1}$ (individual median range 3.40–4.68 m $min^{-1}$) for zimeldine-treated fish and controls, respectively.

The movements of zimeldine-treated fish were quite similar to those of subordinate fish, while the controls swam around in large circles like the controls and dominant fish in experiment 2 (Fig. 3).

The diurnal activity pattern again resembled that of experiment 1 and no noticeable differences were seen between zimeldine-treated fish and controls (Fig. 6A). Initially, the fish showed high locomotor activity, which gradually decreased during the night (Fig. 6A). The activity then started to increase again about 1h before the light went on and a large increase in activity was seen when the light was turned on at 06:30 h (Fig. 6A).

*Experiment 4. Effect of* p-*CPA*

The *p*-CPA-treated fish showed a higher locomotor activity than controls (Fig. 8A). This difference in activity was most prominent during the first 6h of the experiment, when there was a significant difference in the distance travelled (Fig. 8B). Furthermore, the *p*-CPA-treated fish travelled a significantly higher total distance than the controls (Fig. 8B).

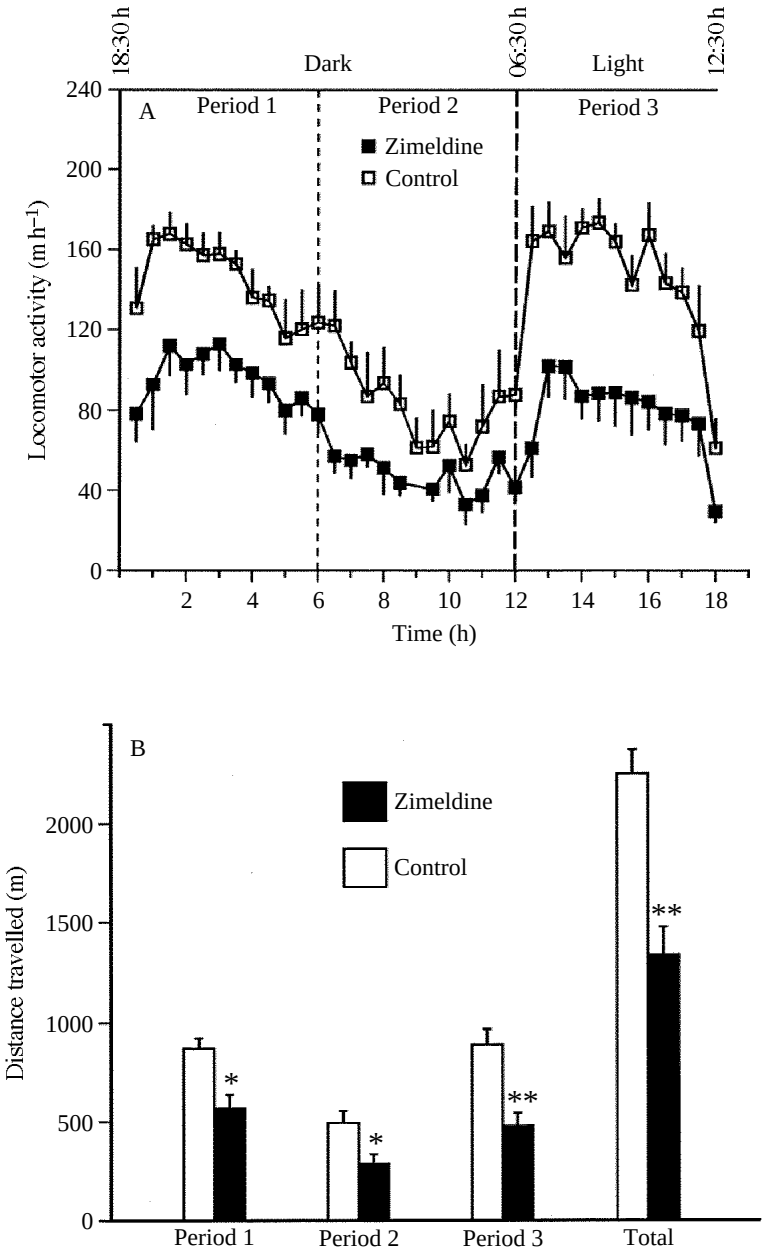


Fig. 6. (A,B) Locomotor activity of individual Arctic charr treated with zimeldine (*N*=6) and of controls (*N*=6). Activity measurements started at 18:30h and stopped at 12:30h; the light was on from 06:30h. (A) Locomotor activity was measured every 30min. Values are means ± S.E.M. (B) Distance travelled over 6h periods and total distance travelled. Period 1, 18:30–00:30h; period 2, 00:30–06:00h; period 3, 06:30–12:30h. Values are means + S.E.M. **P*<0.05, ***P*<0.01, Mann–Whitney *U*-test, two-tailed.

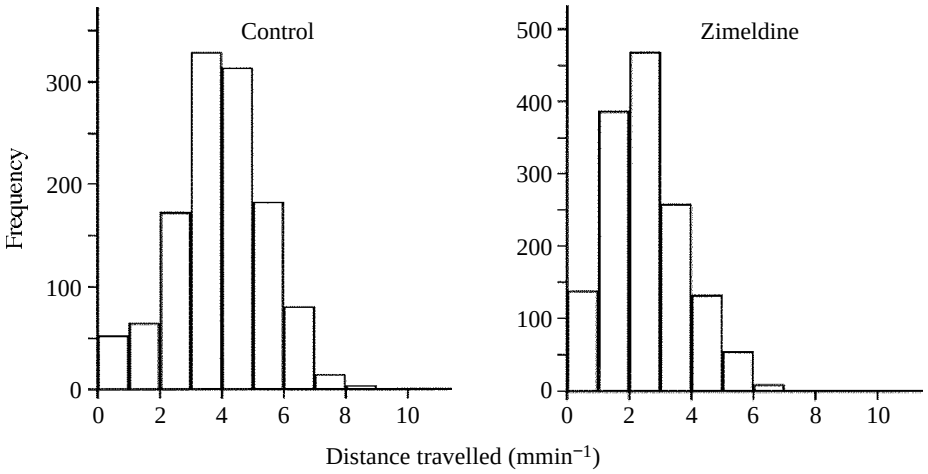


Fig. 7. Frequency diagrams of distance travelled over 1min periods by Arctic charr treated with zimeldine (*N*=6) and by controls (*N*=5). Only the first 4h of the 18h experiment are included.

The difference in locomotor activity between *p*-CPA-treated fish and controls was also reflected by a shift in the activity frequency distributions (Fig. 9). The median velocity during the first 4h of the experiment was 5.98 m min$^{-1}$ (individual median range 4.15–7.47 m min$^{-1}$) for *p*-CPA-treated fish and 3.85 m min$^{-1}$ (individual median range 3.08–4.78 m min$^{-1}$) for controls. Controls and *p*-CPA-treated fish showed similar swimming patterns, both moving around in wide circles during the first hours of the experiment (Fig. 3).

Both controls and *p*-CPA-treated fish showed a high locomotor activity initially, which gradually decreased during the night (Fig. 8A). The activity reached a minimum between 03:00 and 05:30h, whereupon it started to increase again. At 06:30h, when the light went on, the locomotor activity showed an abrupt increase (Fig. 8A). The activity then decreased again during the last hours of the experiment (Fig. 8A). The effect of *p*-CPA was only apparent during the first portion of the dark period of the experiment (Fig. 8A).

### *Brain levels of DA, 5-HT and 5-HIAA*

*Experiment 2. Effect of dominant and subordinate experience*

In fish taken directly from the aquarium (i.e. not tested for locomotor activity), the subordinate individuals had significantly higher brain levels of 5-HIAA than the dominant fish (Fig. 10). There was also a tendency for the 5-HIAA/5-HT ratio to increase in subordinate fish compared with the corresponding dominant individuals ($P$=0.082, Fig. 10). However, in fish that had been subjected to the activity measurements, no such differences were seen (Fig. 10). Dominant fish subjected to activity measurements had significantly higher brain levels of 5-HIAA and significantly higher 5-HIAA/5-HT ratios than untested dominant fish (Fig. 10). The brain concentration of 5-HT was the same in all groups (Fig. 10).

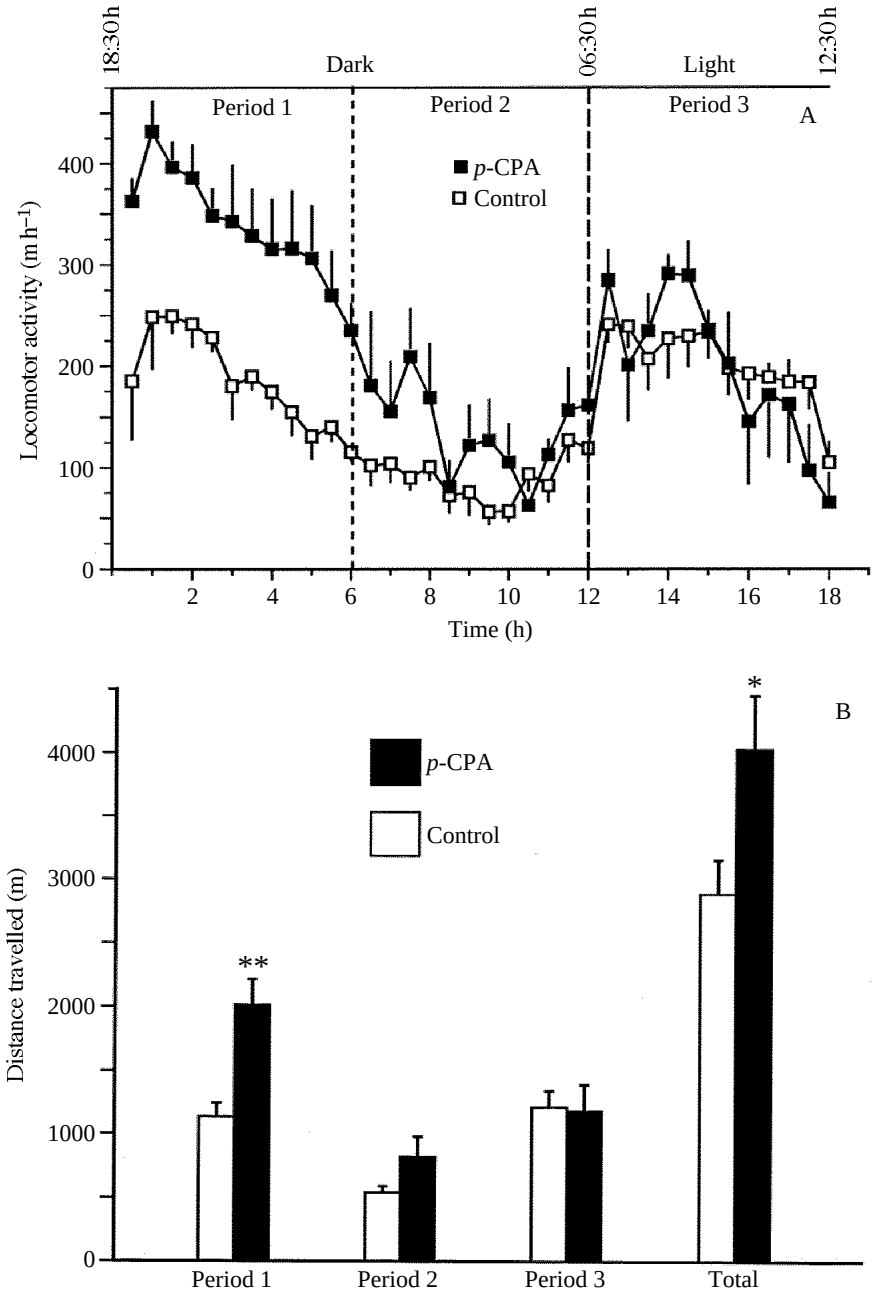


Fig. 8. (A,B) Locomotor activity of individual Arctic charr treated with *p*-CPA (*N*=5) and of controls (*N*=6). Activity measurements started at 18:30h and stopped at 12:30h; the light was on from 06:30h. (A) Locomotor activity was measured every 30min. Values are means ± S.E.M. (B) Distance travelled over 6h periods and total distance travelled. Period 1, 18:30–00:30h; period 2, 00:30–06:30h; period 3, 06:30–12:30h. Values are means + S.E.M. **P*<0.05, ***P*<0.01, Mann–Whitney *U*-test, two-tailed.

*Experiment 3. Effect of zimeldine*

The zimeldine-treated fish had significantly lower brain levels of 5-HIAA and significantly lower 5-HIAA/5-HT ratios than the controls (Fig. 11). No difference in 5-HT or DA levels was seen between zimeldine-treated fish and controls (Fig. 11).

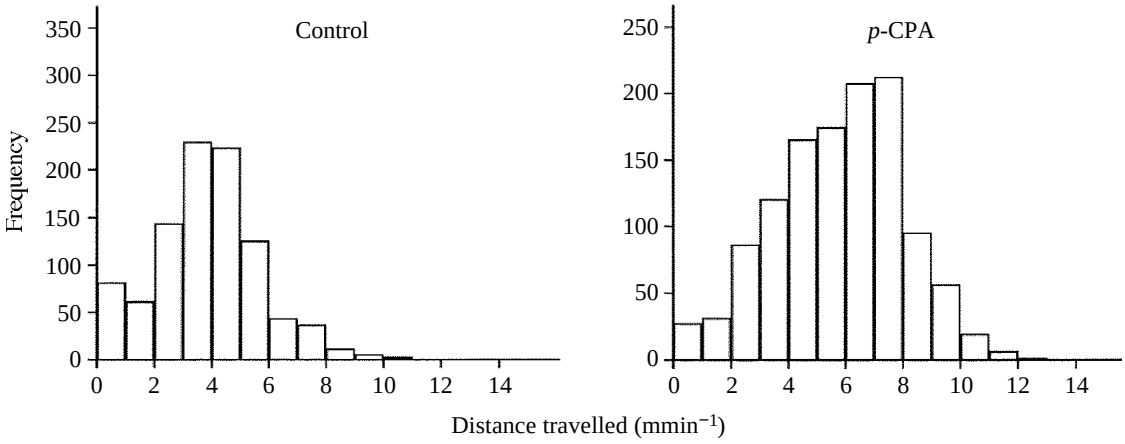


Fig. 9. Frequency diagrams of distance travelled over 1min periods by Arctic charr treated with *p*-CPA (*N*=4) and by controls (*N*=5). Only the first 4h of the 18h experiment are included.

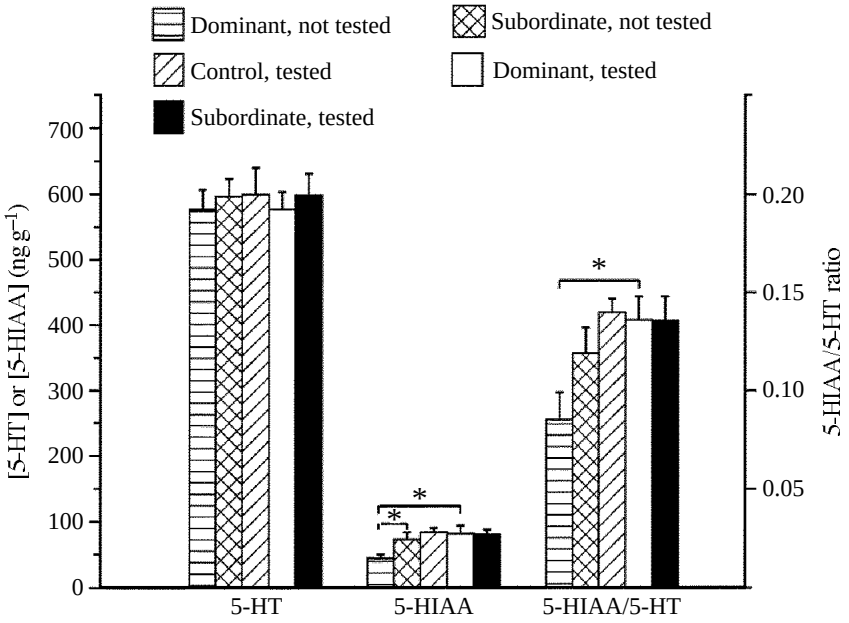


Fig. 10. Concentrations of 5-HT and 5-HIAA and the 5-HIAA/5-HT ratio in brains of dominant (*N*=5) and subordinate (*N*=6) Arctic charr not used for activity measurements (not tested) and in dominant (*N*=5), subordinate (*N*=8) and control (*N*=6) charr used for activity measurements (tested). Values are means + S.E.M. **P*<0.05, Mann–Whitney *U*-test, two-tailed.

*Experiment 4. Effect of* p-*CPA*

The *p*-CPA-treated fish had significantly lower brain levels of both 5-HT and 5-HIAA than the controls (Fig. 12). Furthermore, the 5-HIAA/5-HT ratio was significantly lower

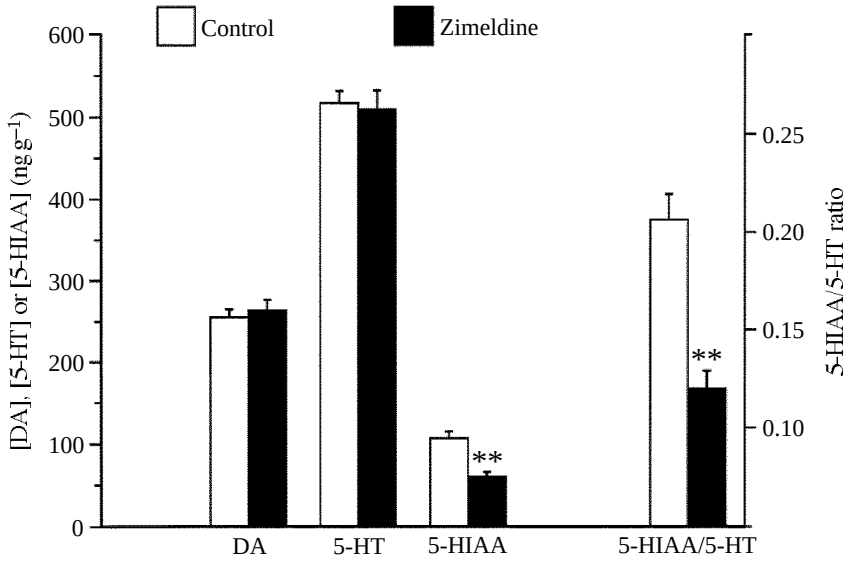


Fig. 11. Concentrations of DA, 5-HT and 5-HIAA and the 5-HIAA/5-HT ratio in brains of Arctic charr treated with zimeldine (*N*=6) and in controls (*N*=4). ***P*<0.01 Mann–Whitney *U*-test, two-tailed.

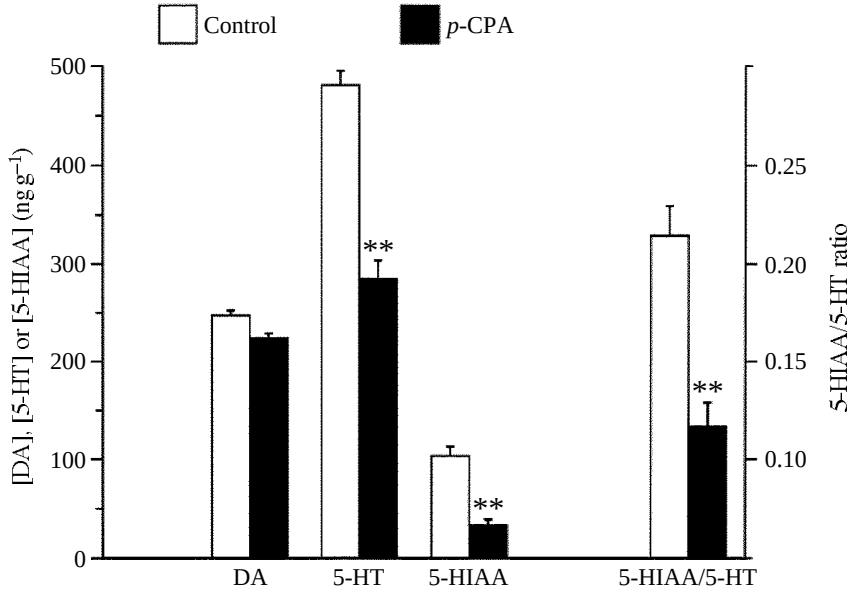


Fig. 12. Concentrations of DA, 5-HT and 5-HIAA and the 5-HIAA/5-HT ratio in brains of Arctic charr treated with *p*-CPA (*N*=5) and in controls (*N*=5). ***P*<0.01 Mann–Whitney *U*-test, two-tailed.

in *p*-CPA-treated fish (Fig. 12). No difference in the brain concentration of DA was found between *p*-CPA-treated fish and controls (Fig. 12).

## Discussion

The image-analysis system could only handle single fish so all recordings of locomotor activity were made on one fish at a time. However, this approach has the advantage of excluding the direct physical effects of social interactions on locomotor activity.

The 48h experiment (experiment 1) showed the daily activity pattern of Arctic charr. Two high-activity periods were detected, one during the first part of the dark period and another during the first part of the light period. Increasing or decreasing 5-HT availability, by treatment with zimeldine and *p*-CPA, respectively, did not cause any striking changes in the diurnal activity rhythm *per se* in Arctic charr. In contrast, the diurnal activity rhythms of eel (Genot *et al.* 1984) and Gulf killifish (Fingerman, 1976) appear to be synchronized with circadian rhythms in the brain 5-HT concentration, although causality has not been shown. Experimental studies on the effect of decreased 5-HT availability on the diurnal activity pattern of mammals are ambiguous. In rats, Fibiger and Campbell (1971) found that *p*-CPA treatment increased locomotor activity during the entire day–night cycle, without affecting the diurnal activity rhythm *per se*. In contrast, Marsden and Curzon (1976) reported that *p*-CPA increased locomotor activity in rats only during the light period of the experiment.

In the 48h experiment, higher activity was recorded during the first hours of the experiment than during the corresponding period 24h later. This period of high activity may have reflected exploratory behaviour or disorientation, as the test area was a novel environment to the fish. This possibility is supported by the behaviour of the fish, which during this period was characterized by large movements, generally in wide circles. Interestingly, the effect of *p*-CPA on locomotor activity and the difference in swimming activity between dominant and subordinate fish were most pronounced during the first 6 h of the experiment, suggesting a possible role of the serotonergic system in suppressing exploratory behaviour. Indeed, Geyer *et al.* (1976) found that rats that had been depleted of 5-HT by lesioning the median raphe nucleus became hyperactive when placed in a novel environment. Furthermore, these animals also showed an increase in startle response. Geyer *et al.* (1976) hypothesized that serotonergic pathways originating in the median raphe nucleus inhibit behavioural responsiveness in the rat. However, Marsden and Curzon (1976) obtained contradictory results and reported that *p*-CPA treatment, which increased locomotor activity of rats in their home cages, reduced locomotor activity when the animals where placed in a novel environment.

In contrast to our results, and to the results from most mammalian studies (Gerson and Baldessarini, 1980), Genot *et al.* (1984) found that *p*-CPA decreased locomotor activity in eels. However, in their study *p*-CPA was injected immediately before activity measurements. In mammals, the effect of *p*-CPA has a very slow onset and the 5-HT-depleting effect reaches a maximum 2–3 days after a single high dose (Vogt, 1982). Genot *et al.* (1984) did not analyze brain levels of 5-HT and 5-HIAA in their fish. Thus, it is possible that the effect of *p*-CPA on brain 5-HT levels was too small to affect the

swimming activity of the eels in their study. In the present study, *p*-CPA was administered 4 days before activity measurements. This made it possible to reduce brain 5-HT levels greatly and to minimize unspecific effects (i.e. effects on catecholamine synthesis). Our fish were killed after 24h in the test area, i.e. 5 days after *p*-CPA administration. At that time, the *p*-CPA-treated fish had significantly lower brain levels of 5-HT and 5-HIAA, as well as significantly lower 5-HIAA/5-HT ratios, while the *p*-CPA treatment did not affect brain DA levels (Fig. 12).

Zimeldine, a specific 5-HT re-uptake inhibitor (Ross *et al.* 1981), significantly decreased locomotor activity throughout the 18h experiment. The frequency distribution of swimming distance over 1min periods was calculated for the first 4h of the experiments. In zimeldine-treated fish, the frequency distribution indicated that zimeldine caused a general decrease in locomotor activity. There was no obvious bimodality in the frequency distribution of swimming distance in zimeldine-treated fish, indicating that zimeldine-treated fish were not still most of the time. The effect of zimeldine is in agreement with the results by Fingerman (1976), who found that 5-HT decreased swimming activity in the Gulf killifish. Furthermore, Fenwick (1970) found that blinded goldfish, which had depressed brain levels of 5-HT, exhibited higher swimming activity. He also found a negative correlation between brain 5-HT concentration and locomotor activity in the goldfish. Similarly, in mammals, increased 5-HT availability has generally been found to decrease locomotor activity (Gerson and Baldessarini, 1980) and intraventricular injections of 5-HT decrease locomotor activity in mice (Herman, 1975) and in rats (Green *et al.* 1976).

Zimeldine-treated Arctic charr had significantly lower brain 5-HIAA levels and 5-HIAA/5-HT ratios than controls. This can probably be explained by the fact that 5-HT is metabolized to 5-HIAA intracellularly by monoamine oxidase (Cooper *et al.* 1986). Thus, by inhibiting 5-HT re-uptake from the synaptic cleft, zimeldine also decreases 5-HT catabolism. In contrast, zimeldine had no effect on brain 5-HT levels. Both these results agree with previous experiments on rats showing that a single dose of zimeldine decreased brain 5-HIAA levels, whereas 5-HT levels were reduced only after repeated administrations (Ross *et al.* 1981).

In the present study, subordinate fish had significantly lower locomotor activity than dominant fish for the first 6h of the experiment. Furthermore, the subordinate fish showed a significantly lower total distance travelled for the 18h experimental period. In subordinate individuals, there appeared to be a bimodality in the frequency distribution of swimming distance for the first 4h. However, there was no bimodality in the frequency distribution of individual fish. Instead, the apparent bimodality was caused by differences in median activity between subordinate individuals. During this first 4h of the experiment, three subordinate individuals had a much lower median activity than the rest of the subordinates that were tested.

When the fish were killed, 24h after being released into the test area, there was no difference in brain 5-HIAA concentrations or 5-HIAA/5-HT ratios between dominant and subordinate fish. There were no differences in either brain 5-HIAA levels or 5-HIAA/5-HT ratios between the subordinate fish tested for locomotor activity in the fluviarium and the subordinate fish taken directly from the aquarium. Thus, during the test period in the

fluviarium, the subordinate fish did not appear to recover from the increase in serotonergic activity that they had undergone during their subordinate experience 24 h earlier. The dominant fish subjected to activity measurements had significantly higher brain 5-HIAA levels and 5-HIAA/5-HT ratios than the dominant fish taken directly from the aquarium. Hence, the activity measurements seemed to affect serotonergic activity, at least in dominant fish. This effect may be related to the experience of a novel environment or to stress caused by netting and transferring the fish from the aquarium to the test area. An increased serotonergic activity in tested dominant fish during the activity measurement may explain why the difference in activity between dominant and subordinate fish became less marked after several hours in the test apparatus.

In conclusion, the results of the present study provide evidence for an inhibitory role of 5-HT in the regulation of locomotor activity in Arctic charr. When the 5-HT concentration in the synaptic cleft was increased pharmacologically, or when brain 5-HT activity was increased as a result of subordinate experience, a significant decrease in locomotor activity was observed. Moreover, when 5-HT availability was decreased pharmacologically, the fish displayed a significant increase in spontaneous locomotor activity. Taken together, these results also provide circumstantial evidence for a role for 5-HT in the suppression of locomotor activity displayed by subordinate fish.

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