THE INFLUENCE OF CEREBELLAR LESIONS ON THE SWIMMING PERFORMANCE OF THE TROUT

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Summary

The influence of partial cerebellar ablation on the performance of rainbow trout, *Oncorhynchus mykiss*, swimming in a water tunnel was studied. Before surgery, all fish maintained a steady position in the water tunnel at all speeds tested. A linear relationship was found between the specific velocity (body lengths^{-1}) and the tail-beat frequency. After partial cerebellectomy, the fish swam well in the tunnel at low speeds, retaining the relationship between tail-beat frequency and specific velocity, but they were unable to maintain a steady position at water speeds requiring tail-beat frequencies above $3.5 s^{-1}$ and were swept backwards. Two sham-operated fish swam at all water speeds tested. *Post mortem* histological investigation showed that the lesions were restricted to the cerebellar corpus. We conclude that the cerebellum plays no role in the generation of motor programmes but may be essential for their selection and implementation.

Introduction

As a consequence of the extensive research that has been carried out during the last two decades, a great deal of information is now available about how cerebellar neurones are arranged and interconnected, how they operate and what transmitters they produce (e.g. Ito, 1984). However, what the cerebellum actually contributes to the control of movement is still poorly understood. Most studies on the role of the cerebellum have been made on mammals making simple, single, rapid movements and there have been few studies on continuous sequences of movement. We report here on the changes in continuous locomotory performance that are seen after cerebellectomy in the rainbow trout. This fish, if placed in flowing water in a laboratory setting, readily swims continuously so as to maintain a steady position even when the water speed changes (Bainbridge, 1958; Webb, 1971; Hudson, 1973). It thus offers appropriate experimental material for the study of the involvement of the cerebellum in the smooth coordination of sustained locomotion.

The cerebellum is a brain structure present in most vertebrates (Nieuwenhuys, 1967). The fact that its internal circuitry is similar in different species has led to the

concept of a basic cerebellar circuit (Llinas, 1970) and to the idea that the cerebellum probably performs similar tasks in different animals even though they may locomote in different ways. In fish, for example, the cerebellum is constructed essentially as in mammals, with a cortical structure of molecular, Purkinje cell and granular layers, yet it supervises simple movements that are produced by relatively few muscle sets. Moreover, fish move in a medium that supports the body and simplifies postural control; this may be why no dramatic locomotory changes follow cerebellar ablation (the older literature is reviewed by Healey, 1957). Careful testing, however, has established that the cerebellum is involved in motor control in fish (Paul and Roberts, 1979; Schairer and Bennett, 1981).

The theoretical basis for the present study is provided by a previous investigation of the effect of cerebellectomy on a movement performed by the dogfish (*Scyliorhinus canicula*) (Paul and Roberts, 1979). In that study we found that the gain of a spinal reflex (a fin movement) was markedly changed following cerebellectomy and we suggested that the cerebellum regulated movement by varying the balance between excitatory and inhibitory brain stem systems projecting to the programming neurones of the spinal cord.

Materials and methods

Animals

The experiments were performed on 11 rainbow trout, *Oncorhynchus mykiss* (Walbaum), that had been obtained as fry from a commercial hatchery and reared in the laboratory under 12 h/12 h day/night conditions. Their body lengths ranged from 18 to 26 cm.

Water tunnel

Each fish was placed in a water tunnel, similar in design to the respirometer introduced by Brett (1964), which produced water velocities up to $50\,\mathrm{cm\,s^{-1}}$. The fish swam in a Perspex observation chamber 14.5 cm in diameter and 50 cm long, the inlet and outlet of which were protected with metal grids. Water movement was generated by a pump, and the flow was regulated by a valve and measured by a meter. Fresh aerated water was slowly added to the reservoir to compensate for the warming introduced by the pump. The experiments were performed in a temperature-controlled room at a water temperature of 15°C.

When the fish was swimming steadily and maintaining a relatively stable position in the water stream, the time taken to complete 10 tail beats was measured using a stopwatch. The mean of ten such measurements gave a measure of the tail-beat frequency. The water speed was changed (by $7\,\mathrm{cm\,s^{-1}}$) every 45 min and the tail-beat frequency was again measured after the fish had been swimming steadily for 15 min at this new speed. If the fish remained 'on station' its velocity equalled that of the water; if its velocity was less than that of the water the fish was swept back against the second grid in the observation chamber, a situation which, if irreversible, indicated that the 'critical velocity' had been reached (Webb, 1971).

The fish's speed is expressed here as the specific velocity ($L s^{-1}$, where L is body length).

Cerebellar ablation

After it had been tested in the water tunnel at five or six different water speeds, each fish was anaesthetized by immersion in a freshwater solution of MS 222 ($60 \, mg \, l^{-1}$; Sandoz, Switzerland), placed in a plastic body holder and ventilated *via* a system that could be switched between anaesthetic-containing or pure fresh water, so as to maintain a low level of anaesthesia during surgery. The skin over the head was cut as a flap and the dorsal surface of the skull exposed, care being taken not to damage the rostral epaxial musculature. The skull was then opened and the fat overlying the brain was gently removed. Lesions were made in the cerebellar body by means of a small surgical knife. In each sham-operated fish the cerebellum was touched with the knife blade, but no lesion was made. Any bleeding during surgery was controlled by cautery. A small piece of sponge ('Steriospon': Allen & Hanburys, England) was placed over the wound and the skull flap was then replaced and sealed with 'Soft Oryl' denture filler (Teledyne Dental Products, USA). The skin flap was replaced using two stitches. Surgery took approximately 45 min and shortly after being returned to fresh water all fish recovered well. When placed in the aquarium, they began to swim spontaneously, usually with some problems of balance at first. After 24 h these difficulties were still present in three fish which were then killed by deep anaesthesia; *post mortem* dissection suggested that bleeding had occurred into the labyrinth (one fish) or that the lesion had extended into the vestibulocerebellum. The other eight fish swam and fed normally within 1 day of the surgery and were retested in the water tunnel.

After each fish had been retested in the tunnel it was reanaesthetized with MS 222 and injected with heparin prior to perfusion through the heart with Ringer's solution and then fixative (4 % formalin). The brain was removed, fixed for a further week in the same fixative, and sectioned sagitally on a freezing microtome at 12 μm. The sections were stained with Cresyl Violet and drawn with the aid of a drawing tube attached to the microscope.

Results

Swimming performance in the water tunnel

Within 15 min of being introduced into the observation chamber, each fish was able to swim steadily and maintain a stable position, neither accelerating into the front grid nor falling back against the rear grid. Fig. 1 shows graphically the relationship between specific velocity and tail-beat frequency for the fish at all water speeds tested. Each open circle is the mean of 10 values at each water speed for all fish prior to surgery. As the water speed was raised, so the tail-beat frequency increased linearly ($r=0.95$; $N=58$). All fish were able to maintain a
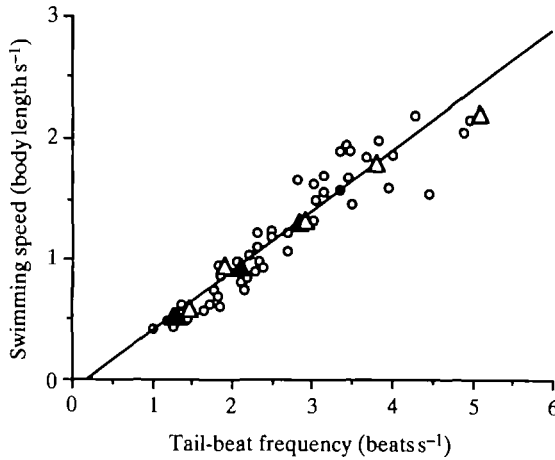
Fig. 1. Tail-beat frequency in relation to swimming speed (expressed as body length s^{-1}). Each small open circle is the mean of 10 measurements for each fish before surgery. The solid line shows the linear regression (least squares) for these points. All fish were able to stay 'on station' at all swimming speeds tested. The large open triangles show the presurgery values for one fish (fish 11) and the filled triangles are the values obtained for this fish after part of the cerebellar corpus had been ablated. The values obtained after surgery lie on the line at slow swimming speeds but the fish could not remain on station at speeds greater than 1.3 body length s^{-1}.

stable position for the full 45 min test period and a critical velocity was never reached.

The day following its trial in the water tunnel, each fish underwent surgery and, after a further 18 h, was retested in the water tunnel. At low swimming speeds the performance of the fish before and after surgery was essentially the same and tail-beat frequency after surgery also increased linearly with increasing swimming speed ($r=0.91$), conforming to the relationship obtained before surgery. The regression lines for before and after surgery were considered to be sampled from populations with equal slopes [ANOVA, $F(1, 92)=0.104$, NS]. As the water velocity was increased over the same range of velocities as before, each cerebellectomized fish now reached a critical velocity at which it could no longer swim steadily, but was swept back against the second grid. To illustrate this, the results for one fish (fish 11) are also plotted in Fig. 1. Before surgery it swam at the highest water speed tested with a tail-beat frequency of $3.8 s^{-1}$, but after the cerebellum had been ablated (filled triangles) its highest tail-beat frequency was $2.8 s^{-1}$, and at sustained water speeds faster than $1.3 L s^{-1}$ it was swept back against the grid. This behaviour was typical of all the fish after cerebellar ablation: the highest tail-beat frequency observed averaged $4.70\pm0.5 s^{-1}$ before surgery and $3.21\pm0.4 s^{-1}$ after surgery (paired t-test, $P<0.001$); the critical velocity following surgery was $1.3\pm0.3 L s^{-1}$. Operated fish failed to swim at the higher speeds even after they had been rested for more than 1 h and even if they were first exposed to
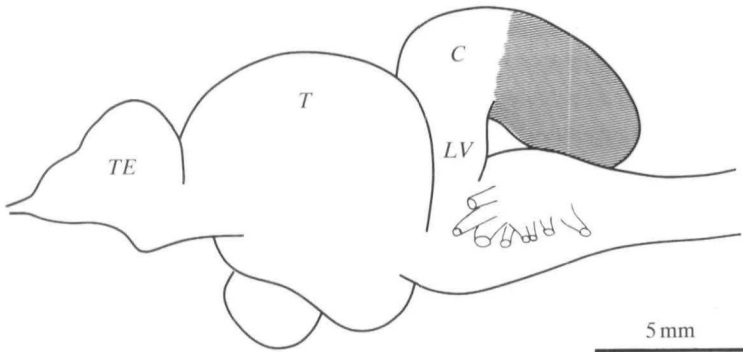
Fig. 2. Drawing of the brain of a trout in lateral view; the hatching indicates the approximate portion of the cerebellar body ablated in each experiment. *TE*, telencephalon; *T*, mesencephalic tectum; *C*, cerebellar body; *LV*, lobus vestibulolateralis. Anterior to left, dorsal at top.

high water speeds rather than to a gradually increasing series. The sham-operated fish swam well at all water speeds tested.

Extent of the lesion

The cerebellum of the trout is a large brain structure consisting of three major divisions (Nieuwenhuys, 1967): the well-developed cerebellar body, which overlies the fourth ventricle; the valvula cerebelli, a unique feature of the teleost brain, which extends into the third ventricle and is covered dorsally by the mesencephalic tectum; and the lobus vestibulo-lateralis, which is a primary projection site of the octavolateralis sensory system. Study of the *post mortem* histological material from each fish confirmed that the experimental lesion was restricted to the cerebellar body, approximately 50 % of which had been damaged or removed (Fig. 2; hatched pattern), and that the valvula and vestibulolateral lobe were undamaged.

Discussion

Our main finding in this study is that trout that were able to swim continuously at all water speeds tested before surgery were unable to do so after much of the cerebellum had been ablated. Webb (1971) suggested that the condition of the fish may be important in determining the critical velocity (his lowest value was $2.0 L s^{-1}$) and so the low critical velocities of our operated fish (around $1.3 L s^{-1}$) could be merely the consequence of a deleterious impact of the surgery on the fish's condition. However, we do not believe that this is so because the sham-operated fish performed normally at all water speeds tested and because the tested fish appeared to be in good condition following surgery; they swam well in the holding tanks, in some cases for many days, until they were prepared histologi-

cally. We consider, therefore, that the changes in locomotory performance seen after cerebellectomy result from the loss of cerebellar coordination. Before we can discuss what form this coordination takes, we first need to consider what is known about the control of sustained locomotion in fishes.

Regulation of swimming speed

A fish such as a trout swimming in a water stream maintains its position on station by undulating the body and tail so as to match its forward speed to that of the water flow. The amplitude of these undulations changes little at most swimming speeds, and the swimming speed is therefore primarily determined by their frequency (Bainbridge, 1958; Webb, 1971). Our own data for the trout confirm that as it swims faster the tail-beat frequency increases. The relationship that we determined between frequency and speed is similar to that obtained previously for this species (Bainbridge, 1958; Webb, 1971). The small discrepancies from the earlier studies may result from differences in the construction of the water tunnels (our own tunnel, for example, produced turbulent flow at the higher water speeds) and because we did not correct the water speeds for the influence of 'horizontal buoyancy' or 'solid blocking' (Webb, 1971).

Careful control of tail-beat frequency is essential if the trout is to remain on station in the water stream. As the water speed increases, the trout will initially be moved backwards and, to compensate, it will have to increase its tail-beat frequency. This process requires sensorimotor computations somewhere in the brain which transform a representation of spatial position in the water tunnel to a representation of the new movement required to maintain that position (i.e. the appropriate tail-beat frequency and recruitment of motoneuronal sets). The mechanism by which a trout detects its position is unknown but sensory information provided by the visual, vestibular, lateral-line and tactile systems would probably be important. Full details of the circuitry involved in sensory processing are not yet available, but regions such as the tectum, torus semicircularis, octavus and trigeminal centres are likely to be involved in the ultimate production of an output that would be relayed *via* brain stem descending systems to the spinal cord pattern generators.

The myotomes of fish are built of muscle fibres with different properties that relate to the tasks they perform: the outer (red) fibres are used for slow swimming and the inner (white) fibres are used for more rapid movement (Bone, 1978). In salmonids, the deep part of the myotome is considered to be a mosaic of fibres of different sizes and properties (Boddeke *et al.* 1959) and possibly consists of intermingled red and white fibres. According to Hudson's (1973) electromyographic study of trout, only the superficial red fibres are active at slow speeds, but electrical activity can be recorded from the deeper portions of the myotome when tail-beat frequencies begin to exceed $3-3.6\,s^{-1}$. This pattern of muscle use presumably reflects changes in the motor programming and is perhaps equivalent to the changes in gait shown by terrestrial animals moving at different speeds.

Changes in performance after cerebellectomy

Cerebellectomized trout feed and move normally in the aquarium and they also perform well in the water tunnel at slow water speeds, retaining the same linear relationship between tail-beat frequency and specific velocity as is seen before surgery. This is an important observation because it means that the transformation of sensory input into descending motor commands (i.e. the motor programming) is not occurring in the cerebellum but is taking place elsewhere in the brain. However, this transformation is inappropriately carried out without cerebellar participation, perhaps because of gain changes in the brain stem circuitry making the transformation.

The failure to remain on station at higher swimming speeds after cerebellectomy could result from an inability to increase further the tail-beat frequency, to discharge motoneurones at a sufficiently high frequency or to recruit specific motoneuronal sets so as to increase myotomal force. When considering these options, it is perhaps worth noting that the failure occurs at a tail-beat frequency of approximately $3.5\,\mathrm{s}^{-1}$, which is when the mosaic portion of the myotome normally becomes active (3.05–$3.6\,\mathrm{s}^{-1}$, Hudson, 1973). A delayed recruitment of the mosaic muscle motoneurones would, of course, result in an insufficiently forceful tail beat and loss of power, and would thus explain the locomotory incompetence. There is, however, no generalized failure of the white muscle system in cerebellectomized fish because it can still be activated for fast escape movements (but with errors in timing, Bosch and Roberts, 1991).

The role of the cerebellum

What could the cerebellum be doing as locomotory speed changes? It is known both from work on mammals (Udo *et al.* 1981; Armstrong and Edgley, 1988; Edgley and Lidierth, 1988; Apps and Lidierth, 1989) and from our own work on the dogfish (Paul and Roberts, 1984) that Purkinje neurones are rhythmically modulated during locomotion, but how this activity changes with changing locomotion has been little investigated. Armstrong and Edgley (1988) have made recordings from cats walking at different speeds and found little change in the activity of either Purkinje cells or nuclear neurones; they were, however, unable to obtain good data at faster speeds. The results of the present study indicate that it may be at just such times when smooth changes in motor programming (e.g. change of gait) are required that the role of the cerebellum is particularly significant.

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