A Method for Complete and Selective Surgical Elimination of the Lateral-Line System in the Codfish, *Gadus morhua*

Surgical elimination of the lateral-line system represents 'a challenging task, since the system encompasses large areas of the head and body of fishes, arranged as canal, pit and naked organs'[1]. Mainly 3 methods have been applied: 1. permanent destruction of the sense organs by extirpation or cauterization; 2. temporal elimination by painting the skin with cocain or other pharmaca, filling the canals with paste, etc.; 3. severance of the afferent nerves. The first method can only be applied to part of the sense organs, the second method has usually been injurious to the animal's general state of health; neither of these methods has ever lead to a lasting, reliable elimination of all lateral-line sense organs, let alone without the causation of disturbing behavioural side-effects. The third method yielded much better results. The nerve branches supplying the lateral sense organs were cut somewhere along their course from the brain towards the periphery, for instance the nerves for the head organs at the point of entering the orbit, the trunk lateral-line nerve after its passage through the shoulder girdle[2]. In doing so, however, afferent fibres supplying other sensory systems, such as the general cutaneous sensitivity, taste buds, etc. are severed as well, because these fibres are intermingled with those innervating the lateral end-organs. Furthermore, to attain in this way a really complete elimination of the lateral-line system, additional destruction of several free neuromasts and canal organs in the temporal region is required, since the nerve fibres supplying these organs form separate small bundles that branch off intracranially from the main lateralis roots[3].

All such difficulties could be avoided if one could cut the lateral nerve fibres right at their origin from the brain. Here, on leaving the medulla oblongata, all lateralis fibres are bundled together in 1 or 2 'lateralis anterior' roots (facialis or VII roots), directed forward and innervating the neuromasts of the head region, and one backward-directed 'lateralis posterior' root (vagus or X root) supplying the trunk organs. These roots, all arising from the common 'lobus posterior' in the dorso-lateral wall of the medulla oblongata[4], contain all nerve fibres supplying the lateral-line system. In most fishes they are at their origin purely composed of lateral-line fibres[5], to become, after a short distance, mingled with fibres coming from the general cutaneous (V) and communis or taste bud (VII) roots[6]. This operation: severance of the lateralis nerve roots at their emergence from the medulla oblongata, has indeed been performed with success in the minnow, *Phoxinus phoxinus* s. *laevis*, at least with regard to the anterior roots. Combined with severance of the ramus lateralis vagi underneath the shoulder girdle and removal of the few remaining lateral-line organs in the temporal region (together with skin and canals), a complete, lasting and fairly selective elimination of the lateral-line system (only a number of taste buds being affected inevitably) has been achieved in this fish species long ago, without causation of any general disturbance[3,7].

Recently, on behalf of studies about directional hearing in the codfish (*Gadus morhua*)[8], a method was needed to eliminate the lateral-line system completely in this species, yet leaving all other sensory abilities unimpaired. The performance of this operation – involving bilateral severance of the lateralis anterior and posterior roots at their emergence from the brain – turned out to be practicable. It was carried out as follows[9].

A codfish of about 30 cm length was narcotized in a 0.01% solution of MS *222* (Sandoz, Basel) in sea-water. After a few min, when the animal had turned side-down and no longer responded to gentle pinching of the caudal fin, it was wrapped in moistened filter paper and put in the normal position on an operation table. It was fixed in this position by means of 2 lateral adjustable clamps, and provided with a respiration tube put into the mouth. From 2 overflow bottles the animal could be supplied either with a 0.005% solution of MS *222* in sea-water, or with pure sea-water. According to the requirements of the animal's state of anaesthesia, an assistant repeatedly switched from one solution to the other during the operation by means of a 2-way cock: towards sea-water when a stop of the ventilation flow failed to cause a spreading movement of the gill-covers; towards the MS *222* solution when weak flipping movements of the caudal fin set in.

With a scalpel, a longitudinal incision of about 3 cm was made through the skin and the dorsal musculature along the median line on top of the head, behind the eye region. Skin and musculature were torn apart and pulled aside in order to expose part of the skull. The exposed part was extended until the ramus recurrens VII (the facial root of the r. lateralis accessorius) became clearly visible. This nerve branch, which contains mainly communis fibres (supplying external taste buds in the skin of the trunk), arises from the geniculate ganglion, runs dorsallly within the cerebral cavity, passes through a foramen in the top of the cranium, turns latero-caudally and runs backward between the musculatare.

Just before its emergence from the skull, it receives from behind a smaller branch, the vagal root of the r. lateralis accessorius, equally containing mainly communis fibres. This branch too can be seen through the translucent cranial bone. Skin and musculature are kept apart by 1 or 2 pairs of flat metal hooks connected to each other underneath the operation table by elastic strands. After loosening with a dental drill, an oblong, oval-shaped piece of the dorsal cranium is cautiously removed. The r. lateralis accessorius can (but must not) be left undamaged; the labyrinth and especially the vertical semicircular canals must remain untouched. Next, the connective tissue between brain and labyrinth is removed carefully in order to get free sight upon the lateralis anterior (VII) and

[1] P. H. Cahn, W. Siler and J. Wodinsky, J. acoust. Soc. Am. *46*, 1572 (1969).

[2] K. v. Frisch and H. Stetter, Z. vergl. Physiol. *17*, 686 (1932).

[3] S. Dijkgraaf, Z. vergl. Physiol. *20*, 162 (1933).

[4] H. Stannius, *Das peripherische Nervensystem der Fische* (Stiller'sche Hofbuchhandlung, Rostock 1849).

[5] Stannius describes, in his ancient but most valuable study of the peripheral nervous system of fishes, the lateralis fibres as 'breite Primitivröhren mit doppelten Contouren und deutlich gerinnendem Inhalte'.

[6] C. J. Herrick, J. comp. Neurol. *9*, 153 (1899); *10*, 265 (1900).

[7] According to Cahn et al.[1], surgical elimination of the lateral-line system had 'never been successful'. J. Moulton, in *Lateral Line Detectors* (Ed. P. H. Cahn; Indiana University Press, Bloomington/London 1967, p. 476) even writes that 'it has not been possible to eliminate the lateral line on the head of any schooling fishes'. The minnow being a schooling fish, both statements are obviously inaccurate.

[8] A. Schuijf, J. comp. Physiol, in press (1973).

[9] After preliminary studies at the Laboratory of Comparative Physiology (Utrecht), the work was mainly done during 2 short stays at the Havforskningsinstitutt of Bergen (Norway) in June 1971 and August 1972. The generous help offered by direction and staff of this institution, and in particular the invaluable support given by Mrs. Egidius, are gratefully acknowledged. Financial and was provided by the University of Utrecht.

posterior (X) roots. The disturbing effect of bleeding from small vessels can only be overcome by patient removal of clots of coagulated blood, and by the use of a permanent weak flow of sterile Ringer solution[10] from an overflow tube. The pipette must be mounted on an adjustable holder, and the amount of flow must be regulated by means of a glass cock. Bottle, solution, tubing, outflow pipette and cock were previously sterilized (kept 15 min in an autoclave at 120 °C). The pipette flow was indeed a most useful aid to make the deeply situated nerve roots clearly discernable under a binocular dissecting microscope (Bausch and Lomb) with 10–20-fold magnification and good illumination (Leitz Mignon lamp). Using fine-pointed watch-maker's tweezers, made of stainless steel (tips cleaned with alcohol and flame), first the lateralis posterior (X) root was severed, which causes no special difficulties. Then, the lateralis anterior (VII) root was cut through in several sections, starting from the most dorsal part. To cut this root completely without doing harm to either the neighbouring communis (VII) root or the ventrally adjoining motor VII and acusticus anterior (VIII) roots, requires a fair amount of experience. The communis root is tinted more white than the lateralis root and runs along its medial side; it can be followed within the brain towards the medially situated lobus vagi (= Stannius' 'lobus medullae oblongatae', or 'lobus impar' in cyprinids) and innervates only inner and outer taste buds. The motor VII root is a small bundle, originating from the medulla oblongata underneath the lateralis anterior root, next to and at the front side of the acusticus anterior root. It innervates important muscles. Since severance of this motor root causes respiratory and other troubles, it must be carefully spared[11]. A distinction between the most ventral part of the lateralis anterior root and the near-by anterior acoustical root (which innervates the utriculus and both anterior ampullae and should of course be left undamaged) can best be made when attention is paid to the fact the lateralis fibres run forward, following a latero-cranial direction, whereas the acoustical fibres go down and are directed latero-ventrally.

After this operation has been performed on both sides, the lateral-line system of the codfish is completely and lastingly put out of function, without deterioration of any other of the sensory abilities. The hooks that kept skin and musculature separated are removed and the tissue is brought back to its original place. The skin on both sides of the incision is sewed together, using a curved needle, with 7–8 separate knots of operation silk. The operated animals often took food again on the day following the intervention, and sometimes even after a few hours. However, a few animals did not stand the bilateral operation and died a couple of hours after recovery from the narcosis. Others showed first complete recovery, including normal locomotion and acceptance of food for several days, but this behaviour was then suddenly followed by incidental wild abnormal swimming movements, equilibrium disturbances and other signs of secondary consequences of the operation, presumably caused by infection of organs bordering the cerebral cavity (brain, labyrinth). To avoid these consequences, room and water temperature should be kept low (at about 10 °C) and Ringer fluid as well as instruments should be kept sufficiently sterile. Then the animals can be kept alive for weeks and even months. Dissection proved that after 3 months the wound was completely closed (except for the bony skull) and no visible nerve regeneration had occurred.

The same operation technique can be used to eliminate any desired part of the labyrinth, with or without simultaneous elimination of the lateral-line system. Severance of the anterior acoustical root puts the utriculus and both frontal ampullae out of function. The remaining posterior ampulla can be eliminated by severance of the ramus ampullae posterior. This nerve branch originates from the medulla oblongata below the lateralis posterior root and immediately above the origin of the ramus lagenae. R. ampullae posterior and r. lagenae run closely together backward beneath the lateralis posterior root, but they can be separated by the use of a fine hook (insect needle tip). In this way the pars superior labyrinthi has been eliminated completely, leaving the pars inferior (sacculus and lagena) untouched. In reverse, the sacculus and lagena nerves can be cut through separately so as to eliminate only the pars inferior of the labyrinth. The r. lagenae can be seized with a fine hook, followed by severance of the posterior branch of the r. sacculi. This latter root becomes visible when the medulla oblongata is pushed medially with a suitable instrument (dental spatula). The anterior branch of the r. sacculi is partly hidden by the anterior acoustical root, if seen from above. To grasp it completely with the hook, the acusticus anterior root must, rather cautiously, be pushed a bit forward. Whereas severance of the acusticus anterior roots causes explicit equilibrium disturbances, severance of the nerve roots supplying sacculus and lagena has no such effect. This is in accordance with previous experience from most other teleosts, though not from all[12].

Zusammenfassung. Es wird eine Methode beschrieben, nach der beim Kabeljau (*Gadus morhua*) das gesamte Seitenorgansystem operativ bleibend ausser Funktion gesetzt werden kann, ohne Schädigung anderer Sinnesfunktionen und ohne Beeinträchtigung des Allgemeinbefindens der Tiere. Mit ähnlicher Methodik kann, je nach Wahl, auch die pars superior oder die pars inferior des Labyrinths, oder auch bloss Sacculus oder Lagena, ausser Funktion gesetzt werden. Die Methode beruht auf Durchschneidung der entsprechenden Nervenwurzeln nahe ihrem Austritt aus dem Gehirn (medulla oblongata).

S. DIJKGRAAF

Laboratory of Comparative Physiology
Jan van Galenstraat 40
Utrecht (The Netherlands), 25 January 1973.

[10] 9 g NaCl + 0,42 g KCl + 0,25 g $CaCl_2$/l aqua dest.
[11] The motor VII root can best be observed after complete severance of the lateralis anterior root; on pulling at the motor root long varicose single fibres are torn out from the medulla oblongata.
[12] S. DIJKGRAAF, Proc. R. Soc. B *152*, 51 (1960).