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# THE ROLE OF THE LATERAL-LINE EFFERENT SYSTEM IN *XENOPUS LAEVIS*

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## INTRODUCTION

Analyses of lateral-line receptors in fishes and amphibia have disregarded the possible influence of efferent innervation. Efferent fibres, however, have been reported to synapse with the membranes of lateral-line hair-cells (Hama, 1962; Flock, 1967) and it is clearly of interest to discover their function.

Observations already made on sense organs which receive efferent innervation indicate that in many cases the efferent fibres are sensitive to the excitatory state of the sensory epithelium. Sensory stimulation (for example, tone pips or clicks) delivered to the ipsilateral or contralateral ear evoke impulses in efferent fibres of the ipsilateral auditory nerve (Fex, 1962). In the olfactory bulb of fishes (Döving & Gemne, 1966) it is not the primary receptors but the secondary neurones which are centrifugally innervated, and electrical stimulation of either the ipsilateral or contralateral olfactory nerve activates efferent impulses in the ipsilateral nerve.

A clear interaction between the afferent and efferent systems has not been demonstrated in the lateral-line system. In *Xenopus* (Görner, 1967) and in *Necturus* (Schmidt, 1965) movements by the animal and mechanical stimulation of the skin evoke efferent impulses in the lateral-line nerves. Mechanical stimulation of the skin is, however, an unspecific stimulus and it is impossible to observe whether or not lateral-line impulses are evoked in response to the excitation of lateral-line receptors or other cutaneous sense organs. One object of this paper is to extend the observations of Schmidt and Görner to see whether the lateral-line efferent nerves are sensitive to lateral-line stimulation.

The lateral-line organs in *Xenopus* are not distributed singly over the surface of the body, but in small groups arranged in linear array. Because of their appearance they have been referred to as 'stitches' (Harris & Milne, 1966) and this term will be used here. Each lateral-line stitch is typically supplied by a nerve bundle comprising two afferent myelinated fibres (8–15 $\mu$m) up to three myelinated fibres (3–5 $\mu$m) and at least one unmyelinated fibre (0·25–0·8 $\mu$m). Stimulation of the myelinated efferent fibres inhibits impulses in the lateral-line afferent fibres (Russell, 1968). This function is shared by the efferent innervation of other acoustico-lateralis receptors including the cochlea (Fex, 1962), the vestibular system of amphibia (Llinás & Precht, 1969) and the lateral-line system of the Japanese sea eel (Katsuki, Hashimoto & Yanagisawa, 1968). In this paper the inhibitory action of the myelinated efferent fibres on afferent impulses in the lateral-line nerve of *Xenopus* is described and their possible role is discussed.

## METHODS

The animals used in these experiments were adult specimens of the South African clawed toad *Xenopus laevis*. They were kept in large aquaria at 23 °C and fed on raw liver and earthworms until required.

The animals were anaesthetized by immersion in tricaine methanesulphonate (M.S. 222, Sandoz), made up as a 0·01 % solution in tap water, for 10–15 min until all respiratory movements ceased. They were then removed to an operating bench where they were carefully kept moist. Excessive handling of the animals was avoided in order to minimize damage to the lateral-line organs. The surface of the skull was exposed and thinned by a dental burr, and finally cut away with scissors to reveal the brain. The brain anterior to the cerebellum was separated from the remaining part by an incision with a fine scalpel, and removed by suction. In some cases the nerve cord was transected behind the medulla and then pithed caudally.

Following this operation the animal was secured to the recording stand. This consisted of a cork board covered in water-saturated tissue paper and mounted on a heavy metal stand. A median incision was made in the skin of the dorsal surface. The dorso-lateral skin of one side of the animal was reflected to that side and pinned out on to the moist surface of the tissue paper. This was done in such a way that a pool of water was trapped between the skin surface and the tissue paper in order to minimize damage to the lateral-line cupulae. The lateral-line nerves innervating the upper and medial lateral rows of stitches were thus revealed embedded in the connective tissue of the exposed underside of the skin. The nerves were frequently irrigated with a saline solution having the following composition: sodium chloride, 111·1 mM; potassium chloride, 1·91 mM; sodium hydrogen carbonate, 2·35 mM; calcium chloride, 2·3 mM.

Action potentials were recorded from the branches of the lateral-line nerves by lifting the nerves on bipolar platinum hook electrodes in a pool of mineral oil. Signals were amplified and simultaneously displayed and stored on a Tektronix 502A dual-beam oscilloscope and a Sony stereo tape-recorder respectively. Selected signals were played back at a later date and filmed.

The lateral-line nerves were stimulated electrically by pulses delivered through bipolar platinum hook electrodes.

In order to investigate the effects of passing hyperpolarizing and depolarizing currents across individual stitches, the skin containing the stitch to be examined was placed outer surface downwards with the stitch adjacent to a 1 cm square silver plate. A silver ball electrode was placed on the exposed undersurface of the stitch. Current was then passed between the silver ball electrode and the silver plate.

Preliminary observations of the influence of efferent fibres on afferent impulses from stitches subjected to hyperpolarizing and depolarizing currents were compared with similar observations in which the frequency of afferent impulses was altered by mechanical stimulation. There was no significant difference between stitches subjected to electrical stimulation and those subjected to mechanical stimulation. Electrical stimulation was therefore mostly used because it is easy to control.

In one experiment it was necessary to stimulate mechanically a number of stitches simultaneously. The apparatus used to achieve this consisted of a 1 cm diameter glass coverslip cemented to the end of a short Perspex rod. The rod was driven back and

forth in a plane at right angles to its long axis by a Goodman vibrator which was supplied with amplified sinusoidal current from a 'Level' oscillator (Fig. 1 *b*). A thin film of water was trapped between the stitches and the coverslip, and the cupulae of the stitches were tangentially displaced by the viscous drag of the water film as it followed the movements of the coverslip.

Voluntary movements of the restrained animal were monitored by means of a gramophone pick-up in which the stylus was replaced by a light metal lever. Signals produced by the pick-up were amplified and displayed on the oscilloscope. Each animal was secured to the recording stand by threads tied to its limbs and the light metal lever of the pick-up was placed on the base of its skull. The base of the skull was chosen because all movements made by the animal were transmitted to this point. This method gives only a qualitative indication of movement but is sensitive enough, with suitable amplification, to measure heart beat.

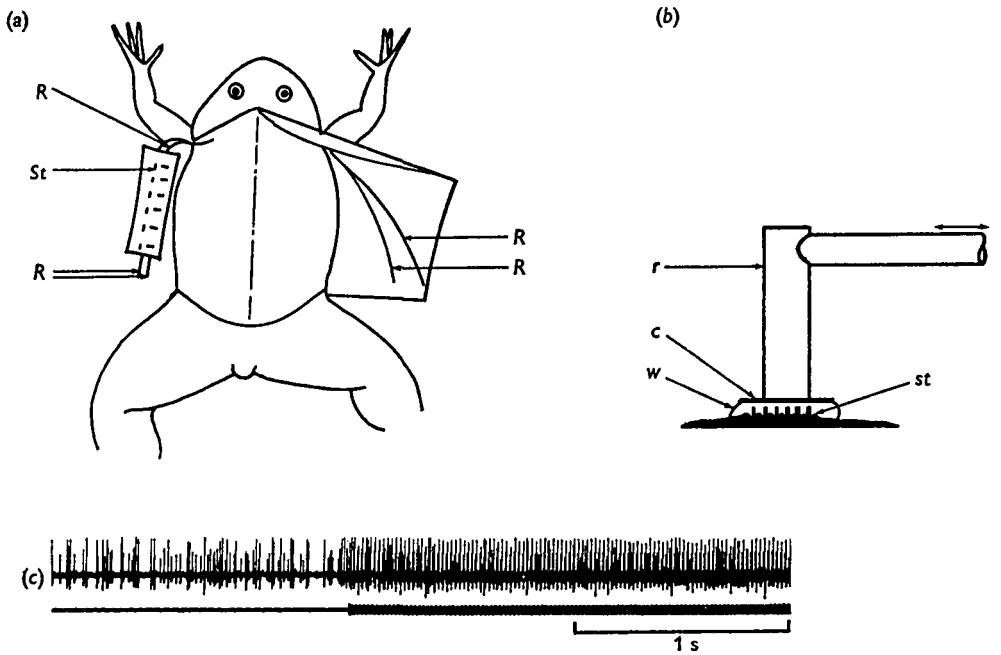


Fig. 1. (*a*) Preparation used for simultaneous recording of efferent impulses from branches of the posterior lateral-line nerves and for mechanical stimulation of stitches in the lateral rows. The skin on the left-hand side of the animal is pinned outer surface upwards to reveal the stitches, *St*. Impulses are recorded from points, *R*, on branches of the posterior lateral-line nerve. (*b*) Arrangement used for mechanical stimulation of stitches. (*c*) Coverslip; *r*, Perspex rod; *st*, stitch; *w*, water film. (*c*) Response of afferent fibres in a branch of the posterior lateral-line nerve to mechanical stimulation of the stitches. Upper trace, afferent impulses; lower trace, wave-form of displacement.

## RESULTS

### *The responses of efferent fibres to electrical stimulation of the lateral-line nerve*

Efferent nerve impulses were recorded from the proximal portions of severed posterior lateral-line nerves which innervate individual stitches and groups of stitches in the upper and medial lateral rows. The efferent impulses may be distinguished from

the spontaneous, rapidly conducting (8–20 m $s^{-1}$) afferent impulses because they conducted at 4–7 m $s^{-1}$ and were never spontaneous.

An attempt was made to excite efferent fibres reflexly in a branch of the posterior lateral-line nerve by electrical stimulation. Two pairs of electrodes were placed beneath a branch of the posterior lateral-line nerve, and it was severed distally to the electrodes. Single pulses 0·05 ms long and trains of pulses were delivered through the posterior pair of electrodes, and compound action potentials were recorded in the anterior pair. The amplitude of the stimulating pulse was controlled so that only low-threshold afferent fibres were excited. The pulse amplitude was then increased until the pulses excited all the afferent nerve fibres, but this also failed to evoke impulses reflexly from the efferent fibres.

The recording electrodes were left in place, and the stimulating electrodes and another pair of recording electrodes were placed on a contralateral branch of the posterior lateral-line nerve. Neither threshold nor maximal electrical stimulation of the contralateral branch of the posterior lateral-line nerve evoked efferent impulses in the ipsilateral branch. On several occasions this stimulus caused the animal to move and the movement was accompanied by patterned discharges of impulses from the lateral-line efferent fibres which always preceded and lasted for the duration of the movement (Fig. 3).

*The response of efferent fibres to mechanical stimulation of the skin*

Görner (1963) has shown that the lateral-line nerve of *Xenopus* contains two types of afferent fibre, one of which responds with an increase in impulse frequency to displacement of the lateral-line cupulae in one direction, and the other to displacement in the opposite direction. Electrical stimulation of the lateral-line nerve therefore causes the simultaneous excitation of two groups of sensory fibres which respond to opposite stimuli. An explanation of the lack of response of efferent fibres to electrical stimulation of the lateral-line nerve might be that the information carried by the two types of afferent neurones interacts destructively in the medulla. This hypothesis can be tested by using natural mechanical stimulation of the lateral-line receptors.

A decerebrate animal was secured to a Perspex plate and a flap of skin containing the upper and medial lateral rows of lateral-line stitches and their innervation was detached from the underlying body wall and pinned out with its outer surface uppermost, beside the animal. Only the central connexions of the lateral-line nerves were kept intact (Fig. 1 *a*) and they were exposed proximally and distally to the flap of skin. Thus the only sense organs on the skin with intact innervation were lateral-line receptors. Efferent impulse activity was monitored from the proximal cut ends of other lateral-line nerves, including those innervating the upper and medial rows of stitches on the opposite side of the body, and from fibres innervating the circumorbital stitches (Fig. 1 *b*).

Efferent fibres innervating stitches in the lateral rows were insensitive to the mechanical stimulation of the stitches. Mechanical stimulation of the lateral rows of stitches failed to evoke efferent impulses in other branches of the posterior and anterior lateral-line nerves unless the stimulation caused the animals to make movements. Under these conditions efferent fibres responded with a train of impulses which preceded, and lasted for the duration of, each movement made by the animal.

During each experiment the afferent nerve fibres innervating the lateral rows were tested for their sensitivity to mechanical stimulation of the stitches. In every case they were found to be fully responsive (Fig. 1*c*).

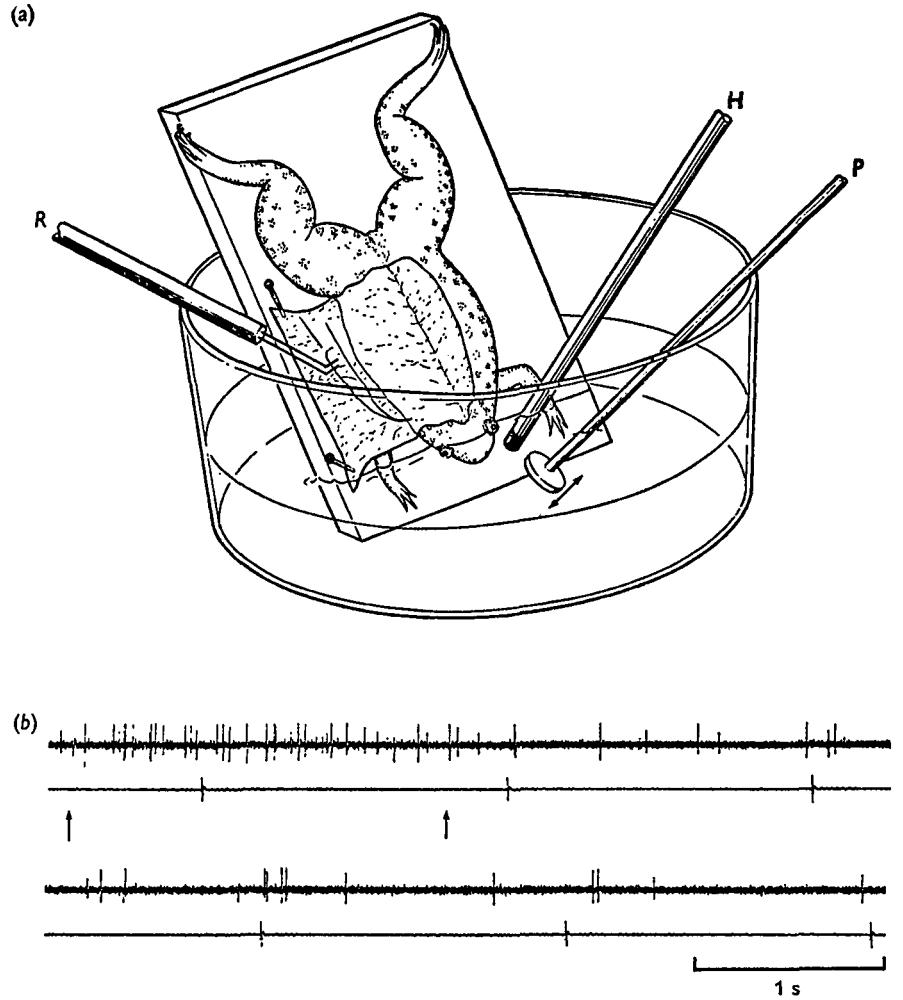


Fig. 2. (*a*) The method used for mechanical stimulation of the skin of the head and for the recording of efferent impulses from the posterior lateral-line nerve. *R*, recording electrode; *H*, hydrophone; *P*, plunger. (*b*) Response of efferent fibres to mechanical stimulation of the head. Upper trace, efferent impulses; lower trace, hydrophone signal. Between the arrows the animal attempted to move.

In another experiment an animal was decerebrated and secured on a sloping cork board (Fig. 2*a*) with its head immersed in a tank of water at room temperature. The posterior lateral-line nerve of one side was exposed. In order to permit the animal to respire the board was frequently raised. A branch of the posterior lateral-line nerve was transected and the proximal stump was placed over recording electrodes. Water pipetted, even vigorously, over the head and body usually failed to excite efferent fibres in the posterior lateral-line nerve. On the occasions when efferent impulses were

evoked, they occurred after a considerable latency (200–500 ms) and therefore could not be directly attributed to stimulation of the lateral-line receptors.

Lateral-line organs on the head were mechanically stimulated by the movement of a plunger driven by a Goodman oscillator (Fig. 2*a*). The oscillator was driven by an amplified square-wave voltage pulse which caused the oscillator to produce a transient oscillation. The pressure waves set up in the water by the transient oscillations of the plunger were monitored by a crystal hydrophone placed close to the animal's head.

Mechanical stimulation of the head by transient water displacements evoked single impulses, and occasionally groups of two or three impulses, from the lateral-line efferent nerves. The latency, however, was extremely variable (30–500 ms), and adaptation always took place (Fig. 2*b*). Bilateral transection of the eighth nerves did not abolish this response and it was concluded that the efferent neurones were perhaps responding to the activities of tactile receptors, because tonic and phasic water displacements failed to excite efferent fibres. Attempts by the animal to move its head during the experiment were accompanied by a train of impulses. After bilateral transection of the eighth nerves the animal often moved its head in a circular movement still preceded and accompanied by trains of lateral-line efferent impulses. The impulses were of a higher frequency and longer duration than could ever be achieved by mechanical stimulation of the skin.

### *Motor or sensory origins for lateral-line efferent fibres?*

As has been noted above, voluntary movements of the animal evoked lateral-line efferent activity. Passive movement of the head or limbs, however, failed to produce any response from the lateral-line efferent fibres. It is therefore possible that a correlation exists between activity in motor fibres to voluntary muscles and lateral-line activity.

Decerebrate animals were lightly restrained by elastic bands to a Perspex plate. The lateral-line nerves of one side were exposed and efferent activity was recorded from them in the usual way. Brief tactile stimulation of the limb extremities with a fine bristle often caused the leg to flex, followed by a crossed extensor reflex and an attempt by the animal to walk. Efferent impulse traffic was present in the lateral-line nerves just before and throughout the movement (Fig. 3). On the occasions when tactile stimulation failed to cause the leg to flex, efferent activity was not detected in the lateral-line nerves.

After this initial exploration the animals were immobilized by an injection of Flaxedil and the spinal nerves innervating the gastrocnemius and brachio-radialis muscles in the hind and forelimbs, respectively, were revealed and motor activity was recorded from their proximal cut ends.

A brief tactile stimulus to the plantar surface of a hind limb produced a long train of efferent impulses in the motor nerve innervating the gastrocnemius muscle of that limb and, simultaneously, a similar train in the lateral-line nerve fibre (Fig. 4). Usually the train of efferent impulses ended at the same time as the train of motor impulses in the nerve to the muscle. Occasionally, however, the lateral-line efferent impulses continued for a brief period after the cessation of the motor impulses.

Observations on lightly restrained animals demonstrated that trains of efferent

impulses accompanied all voluntary movements from the movement of whole limbs to the blinking of an eye.

A close scrutiny of the impulses recorded externally from motor nerves revealed the existence of two kinds of nerve fibre. One type of fibre accompanied voluntary movement with bursts of large impulses which were conducted at velocities of 15–30 m $s^{-1}$ whereas the other type fired with very much smaller impulses at conduction


Fig. 3. The response of efferent fibres to voluntary movements made by the animal. Upper trace, efferent impulses from a branch of the posterior lateral-line nerve; lower trace, voluntary movements made by the animal monitored by gramophone pick-up. Arrow indicates the moment when the animal gulps. This is followed by a struggling movement.

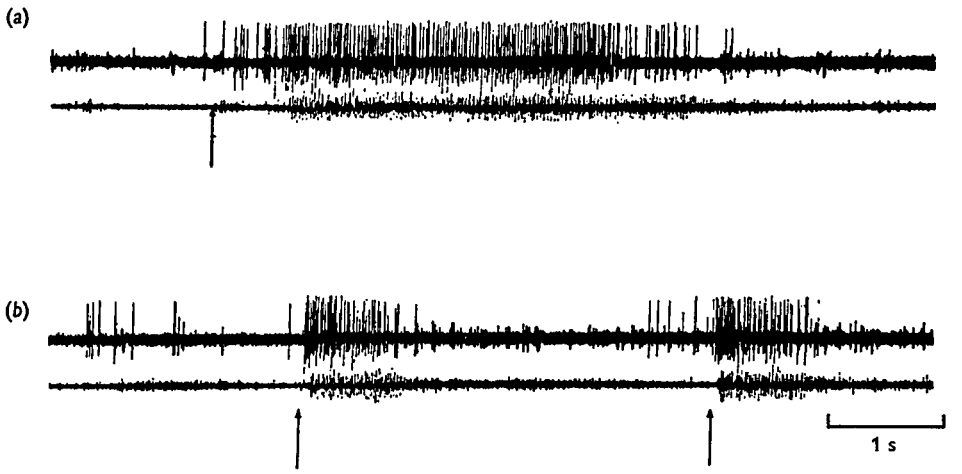


Fig. 4. The relationship between motor activity in spinal nerves and efferent activity in lateral-line nerves. (*a*) Upper trace, efferent impulse activity from the lateral-line nerve innervating the medial lateral row of stitches on the right-hand side; lower trace, motor activity from the nerve innervating the gastrocnemius muscle of the same side. Arrow represents the point at which a brief touch was delivered to the plantar surface of the right-hind limb. (*b*) Upper trace, in (*a*); lower trace, motor impulses from nerve innervating brachio-radialis muscles of the right-hand side. Arrows represent points at which the plantar surface of the right-hand side forelimb was briefly touched.

velocities of 3–8 m $s^{-1}$. This fibre was often quiet, but fired for prolonged periods immediately following trains of impulses from the larger fibres (Fig. 5). Experiments performed on restrained animals not immobilized with 'Flaxedil' revealed that impulse activity in the 'small' fibres was not associated with any form of voluntary movement in the limb which they innervated. The nerves conducting the small impulses were identified by their characteristics as the small motor innervation of the slow tonic muscle fibres (Kuffler, Laporte & Ransmeier, 1947), whereas those conducting large impulses were identified as the large motor innervation of twitch muscle fibres.

From the evidence already presented it seems that the lateral-line efferent neurones are responsive to the total motor outflow from that part of the central nervous system responsible for the contraction of twitch muscle fibres.

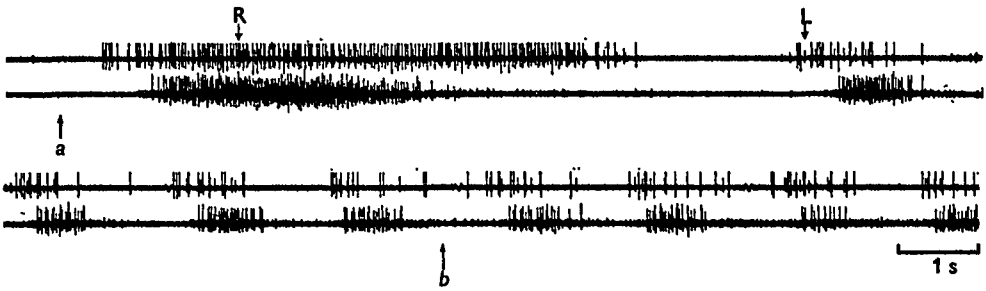


Fig. 5. A comparison between efferent impulses in a branch of the posterior lateral-line nerve and 'fast' and 'slow' motor activity in a branch of the sciatic nerve innervating the gastrocnemius muscle of the right-hind limb. The animal was given a rotational stimulus in the horizontal plane. R, rotation through 90° to the right, L, rotation through 90° to the left. Upper trace, efferent impulses; lower trace, motor activity in sciatic nerve. There is appreciable, 'slow' motor activity at (*b*) while it is absent at (*a*).

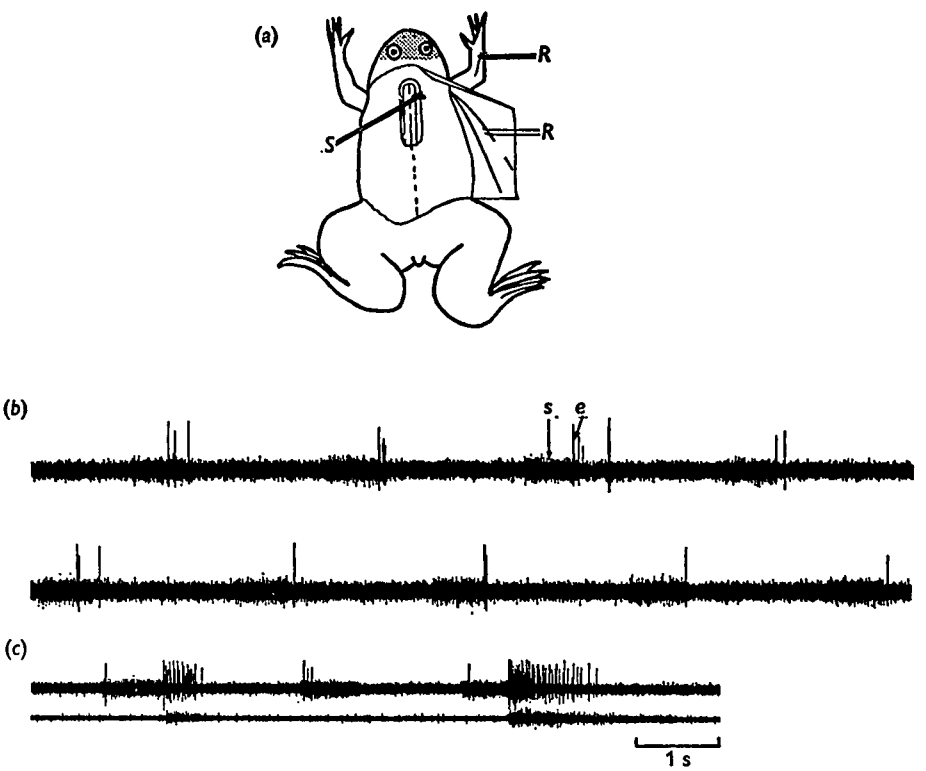


Fig. 6. (*a*) The preparation used to investigate the effect of stimulating dorsal roots on lateral-line efferent activity. The brain was removed anterior to the cerebellum (illustrated by stippling). *R*, recording electrodes; *S*, stimulating electrodes. (*b*) Efferent impulses, *e*, recorded from the upper lateral row of stitches in response to dorsal root stimulation. *s* = stimulus artifact. (*c*) Upper trace is the same as (*b*), lower trace is from motor fibres innervating muscles in the right forelimb.

*Dorsal root stimulation and lateral-line efferent activity*

A decerebrate animal was immobilized by injecting 'Flaxedil' into the dorsal lymph node. The trunk of the second spinal nerve on the right-hand side was then exposed and all distal connexions were severed. A dental saw was used to cut away the neural arch overlying the root and ganglion of the nerve and the dorsal root was transected immediately before it joined the ganglion (Fig. 6*a*). Stimulating electrodes were placed under the dorsal root while one pair of recording electrodes was placed under the second spinal nerve and another was placed under a branch of the posterior-lateral line nerve (Fig. 6*a*). Trains of electrical pulses at up to 100 $s^{-1}$ were delivered to the dorsal root. This form of stimulation produced bursts of efferent impulses in the

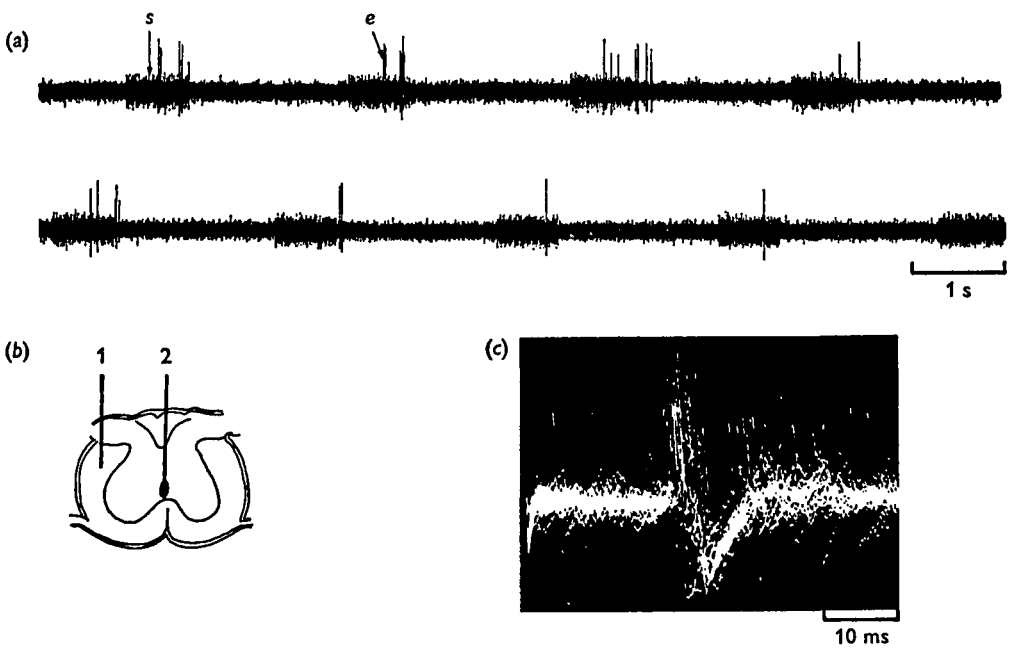


Fig. 7. Response of the lateral-line efferent impulses to spinal cord stimulation. (*a*) Efferent impulses, *e*, from the lateral-line nerve which innervates the right-hand side lateral row of stitches. Stimulating pulses were delivered to the dorso-lateral white matter. *s* = stimulus artifact. (*b*) The cross-section of the spinal cord of *Xenopus* in the region of the emergence of the second spinal nerves. 1 and 2 represent the positions of the stimulating electrodes in the dorso-lateral matter and in the region of the neural canal, respectively. (*c*) Fifty superimposed sweeps of efferent impulses recorded from the same nerve as in (*a*) but in response to repetitive stimulation at a frequency of 10 $s^{-1}$ in the region of the neural canal.

lateral-line nerve, but they occurred after variable latencies and always showed adaptation (Fig. 6*b*). Occasionally the lateral-line nerve would respond with a train of efferent impulses, and this was accompanied by a train of motor impulses in the second spinal nerve. These commenced simultaneously with the efferent impulses and continued for the same duration (Fig. 6*c*). This experiment was repeated on eight other spinal nerves with similar results.

*Spinal cord stimulation and lateral-line efferent activity*

A decerebrate animal was immobilized with 'Flaxedil' and the neural arches overlying the spinal cord in the region of the second spinal nerve were cut away. Bipolar stimulating electrodes were inserted into the cord. These consisted of a pair of fine stainless-steel needles separated by 0·3 mm and insulated with Araldite to within 30 $\mu$m of the 10 $\mu$m diameter tips. At the end of the experiment the nerve cord was perfused with 10% neutral formalin with the stimulating electrodes in place. After 2 h the electrodes were removed and frozen sections 8 $\mu$m thick were cut from the region penetrated by the electrodes. These were then examined by phase microscopy.

Trains of electrical pulses at up to 100 $s^{-1}$ were delivered to the lateral white matter of the spinal cord (Fig. 7*b*). The stimulus produced bursts of efferent impulses in the lateral-line nerve, but as with the stimulation of dorsal roots, they occurred with variable latencies and showed adaptation (Fig. 7*a*). When pulse trains were delivered to the central grey of the spinal cord near the neural canal (Fig. 7*b*), the efferent fibres responded differently. Efferent impulses followed stimuli at frequencies up to 20 $s^{-1}$ with a relatively constant latency (approximately 15 ms) and did not show adaptation (Fig. 7*c*). Furthermore, the lateral-line efferent fibres started to fire 'spontaneously'. Removal of the cerebellum had no apparent effect on the responses of the lateral-line efferent neurones either to electrical stimulation of the central grey matter or to voluntary movements made by the animal.

*A comparison of impulse activity in different efferent fibres*

A comparison was made between patterns of activity in efferent fibres to discover whether they behaved similarly or not in response to a stimulus.

Efferent activity in side branches of the lateral-line nerves to single stitches was examined in 21 different stitches in four animals. The animals were again decerebrated and immobilized with Flaxedil. The stimuli used were tactile and rotational. In the 21 stitches examined, all but four received paired efferent innervation, two apparently received none, one received one fibre, and one received three efferent fibres, as inferred from a study of recorded impulse heights. The patterns of impulses which were evoked in response to excitatory stimuli were almost identical in each of the efferent fibres to a single stitch (Fig. 8). Similarly, a comparison between patterns of activity in the efferent fibres of stitches in the same row revealed similar patterns of impulse activity in response to tactile and rotatory stimuli (Fig. 9). The study was finally extended to a comparison between the patterns of activity in efferent fibres innervating stitches in different rows. Fig. 10 is typical of the results obtained from these experiments. The patterns of efferent activity in different branches of the vagus nerve innervating the same and opposite sides are very similar.

*The controlled stimulation of myelinated efferent fibres*

In this investigation particular interest was concentrated on the similarity between efferent activity in each of the nerve trunks of the posterior lateral-line nerve innervating the medial and upper lateral rows of stitches. The patterns of efferent impulses in each trunk (Fig. 11) were so similar that it was tempting to suggest a common origin for them.

A train of square-wave pulses was delivered to the proximal cut end of the branch of the posterior lateral-line nerve which innervates the dorsal lateral row of stitches (Fig. 11*a*). Under these conditions recording electrodes placed beneath the proximal cut end of the branch innervating the middle lateral row of stitches of the same side

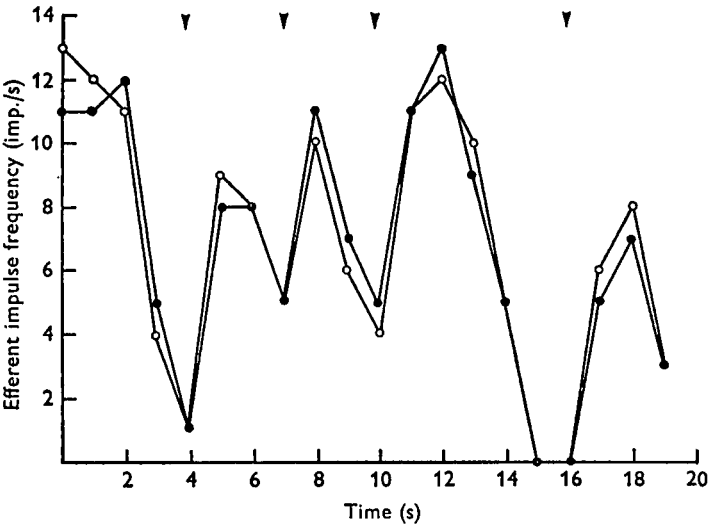


Fig. 8. A comparison of efferent impulse activity in two fibres innervating the same stitch. The figure is a plot of impulse activity in two efferent fibres (●—● and ○—○) innervating a single stitch in the right-hand side medial lateral row. Ordinate, efferent impulse frequency (F), $s^{-1}$. Arrows indicate times at which animal was briefly stimulated by touches to the plantar surface of the right hind limb.

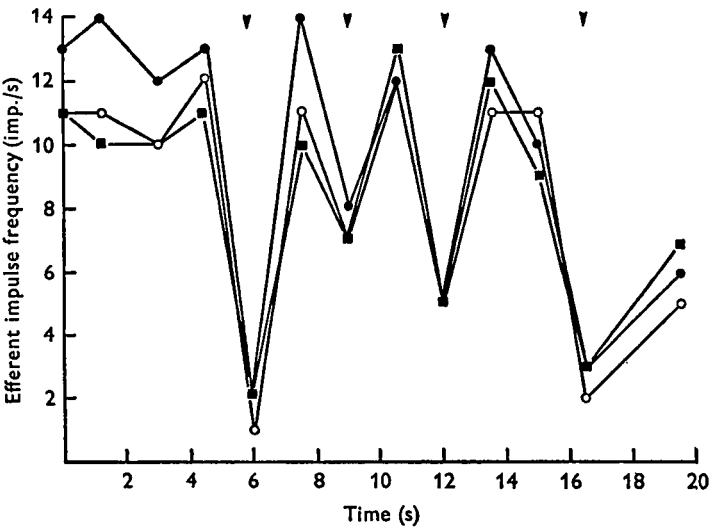


Fig. 9. A comparison of efferent impulse activity in fibres innervating stitches in the same row. The figure is a plot of impulse activity in three fibres, two (○—○, ●—●) innervating stitches in the hind region, and one (■—■) innervating a stitch in the head region of the left-hand side upper lateral row of stitches. Ordinate: efferent impulse frequency (F), $s^{-1}$. Arrows have the same meaning as in Fig. 8.

revealed a train of compound action potentials which followed the applied stimuli in a 1:1 relationship up to frequencies of 200 $s^{-1}$ (Fig. 11*b*). By inserting a second pair of recording electrodes beneath the nerve trunk, and a few centimetres proximal to *R* (Fig. 11*a*), the conduction velocity of the compound action potentials was found to be between 4–7 m $s^{-1}$, which is the conduction velocity of lateral-line efferent fibres. There are two other reasons for believing that the compound action potentials are conducted down myelinated efferent fibres. First, the compound action potentials are

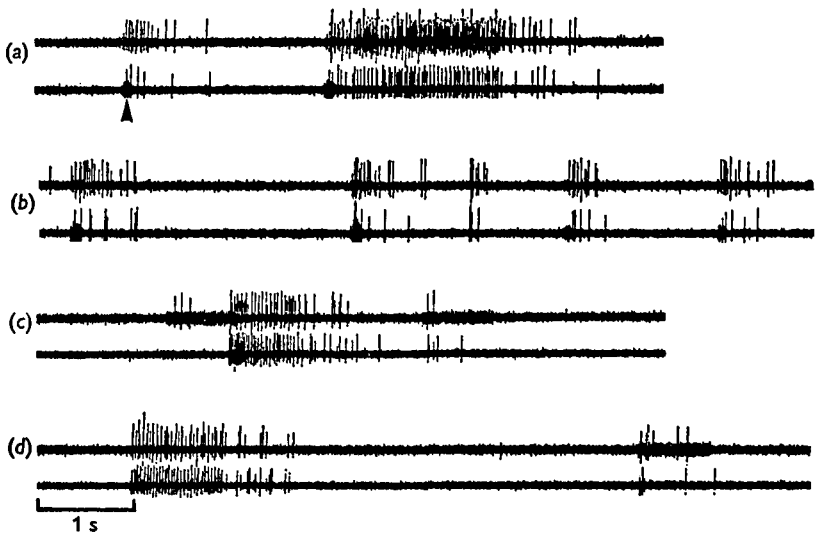


Fig. 10. A comparison of impulse activity in lateral-line nerves innervating different rows of stitches. (*a*) Upper trace, efferent impulses from nerve innervating left-hand side upper lateral row; lower trace, efferent impulse from nerve innervating left-hand side medial lateral row. Arrow indicates the point at which the plantar surface of the left-hand limb was touched. (*b*) Upper trace, efferent impulses from nerve innervating right-hand side medial lateral row; lower trace, same as (*a*). (*c*) Upper trace, same as (*b*); lower trace, efferent impulses from nerve innervating right-hand side upper lateral row. (*d*) Upper trace, efferent impulses from nerve innervating upper lateral row of stitches of left-hand side; lower trace, same as (*c*). The thickening in the base lines of the upper traces in (*c*) and (*d*) are the stimulus artifacts caused by pulses delivered to the dorsal roots of the second spinal nerve.

not conducted down afferent fibres because mechanical stimulation of stitches in one row does not evoke antidromic impulses in afferent fibres of another. Secondly, unmyelinated fibres in spinal nerves of the frog, with diameters similar to those found in the lateral-line nerves of *Xenopus* (0·25–0·8), conduct at velocities of less than 1 m $s^{-1}$ (Loewenstein, 1956). Action potentials conducting at velocities between 0·1 and 0·7 m $s^{-1}$ were evoked from lateral-line nerves in *Xenopus* (Fig. 12), but only by pulses of 1–2 msec. duration and of high voltage. By keeping the stimulating pulse length to less than 0·3 msec., and the stimulating voltage below 1·5 times threshold, only myelinated efferent fibres and not unmyelinated fibres were excited.

An examination of the two branches of the posterior lateral-line nerve innervating the upper and medial lateral rows of stitches shows that they become confluent just before entering the vagus ganglion (Fig.11*a*). By transecting the vagus immediately distal and proximal to the ganglion it was established that the trains of stimulating

pulses caused the efferent impulses to travel up one branch of the lateral-line nerve, through the vagus ganglion and down the other branch. There is no appreciable delay at the vagus ganglion. It is possible that the cell bodies of the efferent neurones

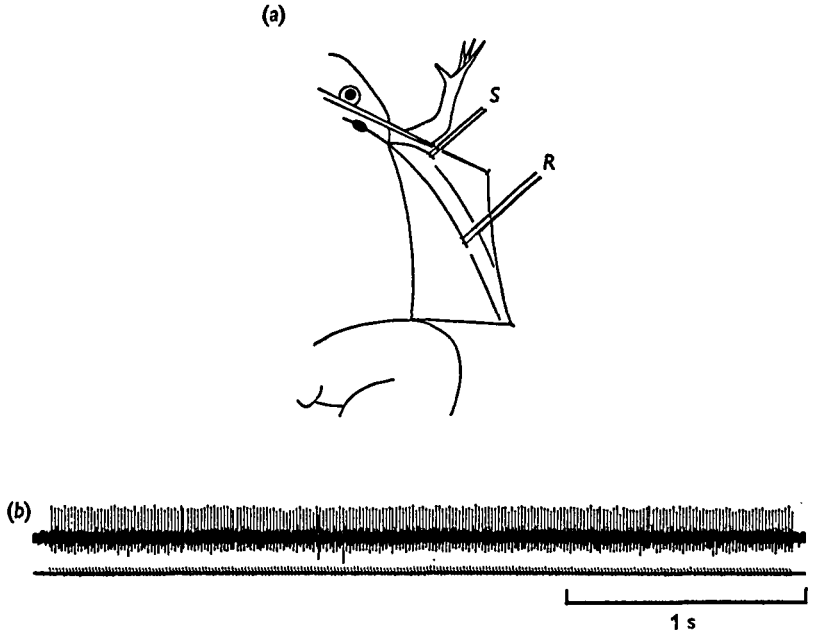


Fig. 11. (*a*) A means of controlled stimulation of efferent fibres; Schematic diagram of the preparation. *S*, Stimulating electrodes placed under the proximal cut end of the vagus nerve innervating the upper lateral row of stitches; *R*, recording electrodes placed under the proximal cut end of the vagus nerve innervating the medial lateral row of stitches. (*b*) Upper trace, compound efferent action potentials recorded from the vagus nerve innervating the medial lateral row of stitches; lower trace, stimulating pulses delivered at 80 $s^{-1}$ to the vagus nerve innervating the upper lateral row of stitches.

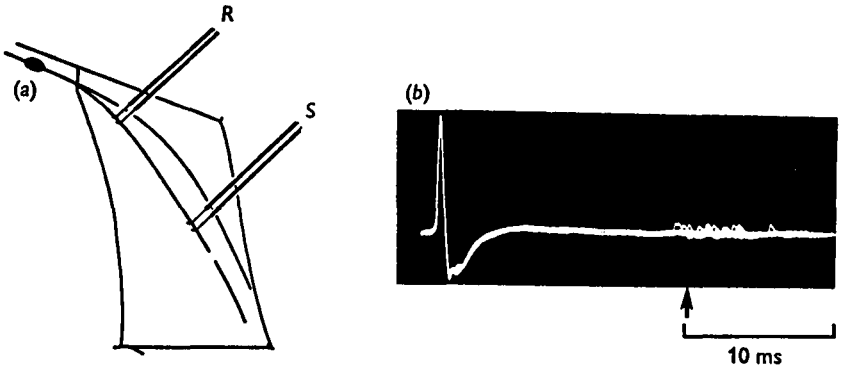


Fig. 12. The compound action potential of fibres in the posterior lateral-line nerves of *Xenopus*. (*a*) Stimulating electrode, *S*, and recording electrode, *R*, placed under a length of the vagus innervating stitches in the medial lateral row. (*b*) Compound action potentials from the vagus innervating the medial lateral row of stitches: the trace was produced by superimposing 30 triggered sweeps. Arrow indicates action potentials from unmyelinated fibres.

are in the medulla and they each send out axons which branch in the ganglion. Alternatively, the cell bodies may be in the ganglion and they send out axon collaterals into each of the branches of the posterior lateral-line nerves.

*The influence of efferent fibres on afferent impulses*

Electrical stimulation of efferent fibres innervating individual stitches caused impulse traffic in afferent fibres from the stitches to be inhibited.

An example of this effect is illustrated in Fig. 13. In this case afferent impulses were recorded from a single stitch in the medial lateral row of one side of one side of a pithed animal. This was done by placing recording electrodes under the main nerve trunk

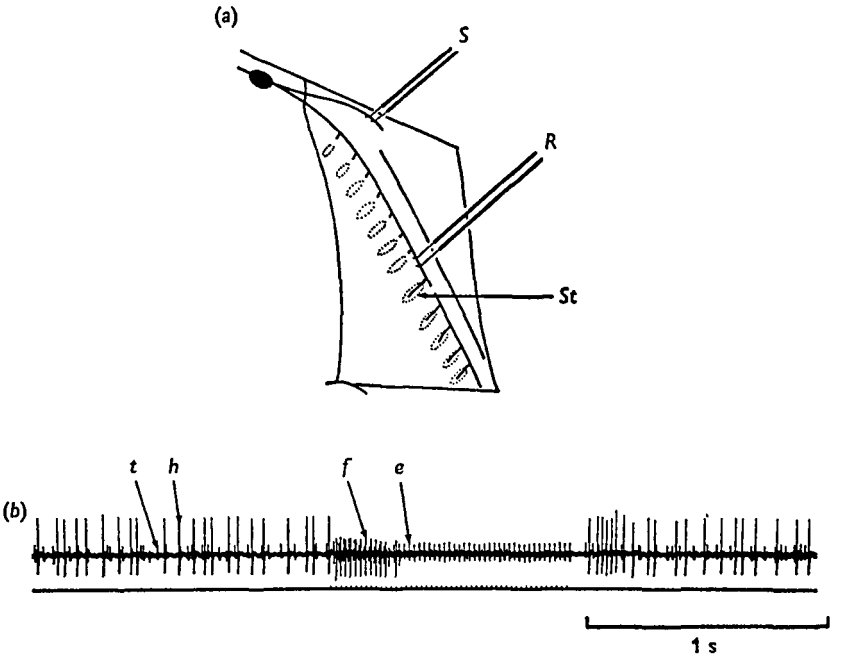


Fig. 13. The influence of efferent impulses on a lateral-line stitch. (*a*) Diagram of preparation. *S*, Stimulating electrodes under proximal cut end of vagus innervating upper lateral row of stitches. *R*, Recording electrode placed just proximal to a stitch, *St*; all other sensory input to the vagus innervating the medial lateral row of stitches has been removed. (*b*) Upper trace, impulses recorded through *R*. *t*, Afferent impulses from hair cells sensitive to tailward displacement; *h*, Afferent impulses from hair cells sensitive to headward displacement; *e*, *f*, efferent compound action potentials. Lower trace, Stimulating pulses delivered through *S* at frequency of 60 $s^{-1}$.

immediately anterior to the branch innervating the stitch, and by sectioning the trunk immediately posterior to the branch. Thus the electrodes were in a position which permitted the recording not only of spontaneous afferent impulses from the stitch, but also efferent impulses travelling to the stitch (Fig. 13*a*). Stimulating electrodes were placed under the nerve trunk innervating the dorsal lateral row of stitches of the same side (Fig. 13*a*).

Spontaneous activity (*h* and *t*) of the two afferent nerve fibres from the stitch increased in response to headward and tailward displacements respectively of the cupulae.

When the myelinated efferent fibres to the stitch were stimulated by a train of pulses at a frequency of 60 $s^{-1}$ delivered to the posterior lateral-line nerve innervating the dorsal-lateral row of stitches, spontaneous activity in both afferent fibres was inhibited after a variable latency (10–30 ms). The inhibition often continued for the duration of the stimulus, providing it was no longer than 1 s, and for a period of 60 ms beyond. Immediately following the inhibition, afferent impulses reappeared at a frequency which was about 1·5 times their resting rate. This post-inhibitory after-discharge of the afferent fibres continued for a further 200–400 ms before returning to a resting level of activity.

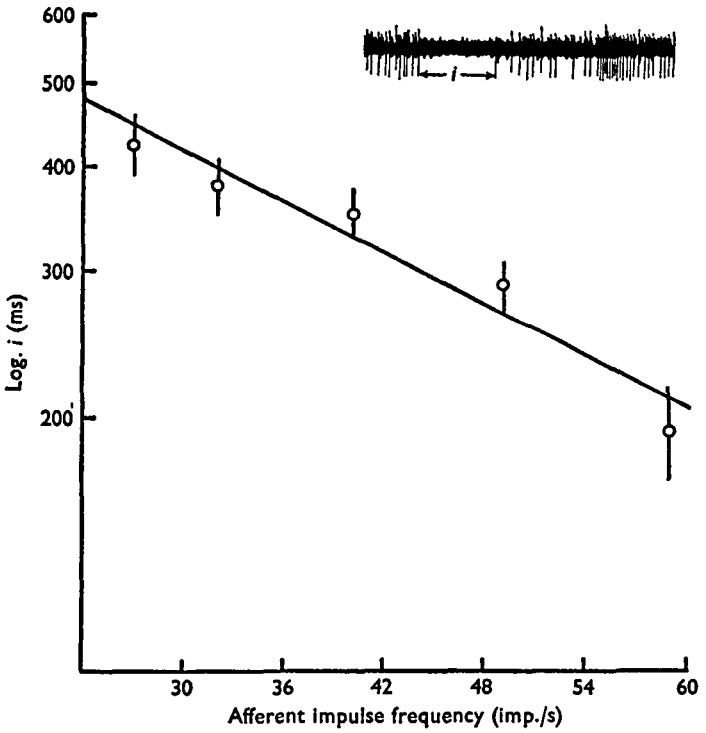


Fig. 14. The relationship between the inhibitory period (*i*) and the frequency of impulses in a single afferent fibre. Abscissa: resting frequency of impulses in a single afferent fibre ($s^{-1}$). Each point represents 15 observations. The vertical bars are twice the standard error.

In other cases inhibition was continued for several seconds and afferent impulses were observed to return during the inhibiting period, but were never observed to reach the resting frequency of spontaneous afferent activity. The disappearance of inhibition which occurs during prolonged stimulation of efferent fibres is a short-term effect. Recovery seems to occur during the after-discharge since the inhibition produced by a train of efferent impulses is not influenced by any disappearance of inhibition which may occur in trains which shortly precede it.

Attempts were made, by controlling the voltage-amplitude of pulses delivered through the stimulating electrode on all stitches examined, to inhibit spontaneous activity in one afferent fibre without inhibiting activity in the other. All attempts to achieve this have so far proved unsuccessful.

*The relationship between the length of inhibition and afferent impulse frequency*

For any lateral-line stitch, providing the frequency of stimulation of the efferent fibres and the resting frequency of afferent impulses remained constant, the period *i* (Fig. 14) between arrival of the first of a train of efferent impulses and the appearance at the recording electrode of the first afferent impulse to return during inhibition, was relatively constant. On the other hand, changes in the frequency of impulses in the afferent fibres had a profound influence on the time of reappearance of afferent impulses during periods of efferent inhibition.

This experiment was performed on six individual stitches. In each case the frequency of afferent impulses from a stitch was changed by applying a depolarizing current across it. At each frequency of afferent impulses ten successive trains, each containing efferent impulses at a frequency of 110 ips $10^{-1}$ with a duration of 1 s and separated from

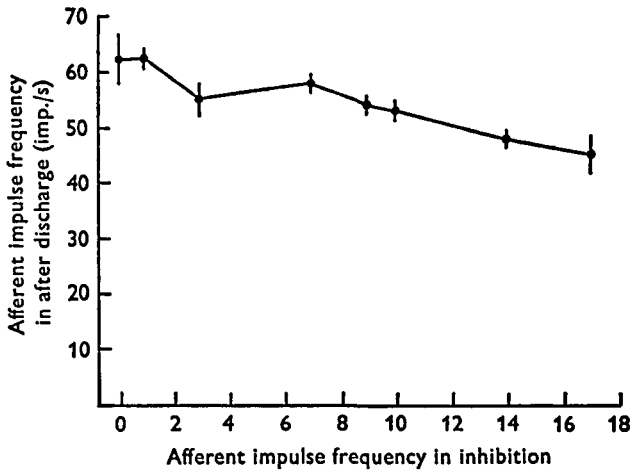


Fig. 15. The relationship between the frequency of afferent impulses returning during the period of inhibition, and the frequency of afferent impulses occurring during the after-discharge. Ordinate: the frequency, *F*, of afferent impulses occurring during the first 200 ms of the after-discharge. Abscissa: the frequency, *R*, of afferent impulses returning during the period of inhibition. Each point represents the mean of ten observations and the vertical bars represent the standard errors.

each other by 2·5 s, were delivered to each stitch. The length of the period *i* was then measured. Observations made at five different frequencies of afferent activity for a single stitch are shown in Fig. 14. These results together with those from the other stitches suggested a logarithmic relationship between the frequency of impulses in the afferent fibres and the period *i*. This was true provided the frequency of stimulation of the efferent fibres remained constant.

*After effects of inhibition*

Immediately following a train of inhibitory efferent impulses afferent activity from a stitch continues to be inhibited for a further 40–60 ms and then abruptly returns as a rapid discharge of afferent impulses. The post-inhibitory discharge (after-discharge)

continues for a period of 200–400 ms during which the frequency of afferent impulses is about 1·5 times resting frequency.

In an initial group of experiments impulses in afferent fibres innervating individual stitches were inhibited by trains of efferent impulses each with a duration of 1 s but with impulse frequencies varying between 10 and 150 $s^{-1}$. Trains of low frequencies gave incomplete inhibition and, consequently, a higher frequency of afferent impulses returning during inhibition. In every stitch examined the frequency of impulses occurring during the period of after-discharge was independent of the frequency of afferent impulses returning during the period of inhibition, even when this frequency was half that of the resting frequency of afferent activity (Fig. 15).

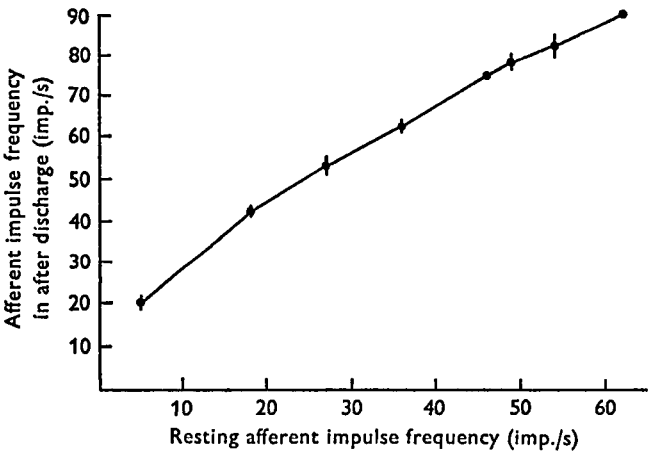


Fig. 16. The relationship between the resting frequency of afferent impulse activity and the frequency of impulses occurring during after-discharge of a single nerve fibre. Ordinate: the frequency, *F*, of afferent impulses occurring during the first 200 ms of after-discharge. Each point represents the mean of ten observations, and the vertical bars represent the standard errors.

In a second group of experiments the resting frequency of afferent impulses was changed by exposing the stitches to hyperpolarizing and depolarizing currents. The afferent activity was then inhibited with trains of efferent impulses, each 1 s in duration, but the impulse frequencies of the trains were adjusted so that only 1–2 afferent imp./s returned during the period of inhibition. It was seen that the frequency of impulses occurring during the after-discharge was very sensitive to the resting frequency of afferent activity (Fig. 16).

## DISCUSSION

Unlike the olivo-cochlear bundle, the lateral-line efferent system of *Xenopus* shows no direct response either to mechanical stimulation of the receptors or to electrical stimulation of the afferent fibres. If, however, the stimuli cause the animal to make a movement, impulses precede the movement by 10–40 ms and last for its duration. Similarly, in animals immobilized with Flaxedil, a correlation exists between efferent impulse activity in lateral-line nerves and impulses in large motor nerves which innervate twitch muscles. No correlation was found, however, between lateral-line efferent

activity and activity in small motor nerves. Small motor nerves innervate muscles which maintain the relatively large tensions produced by the twitch muscles, and are concerned with the maintenance of posture (Kuffler & Vaughan Williams, 1953). Furthermore, the reflex pathways involving the small and large motor systems are independent of each other (Kuffler *et al.* 1947). Thus the correlation which exists between impulses in lateral-line efferent nerves and large motor nerves, and the lack of correlation with the small nerve activity, is in agreement with the functions of the two motor systems.

In *Xenopus* mechanical stimulation of any sense organ may indirectly excite efferent fibres, providing it causes the animal to make a movement. An exception seems to be the stimulation of tactile receptors on the head. Relatively violent stimuli produced by large transient oscillations of water near the head (Fig. 2) may produce single or double impulses from lateral-line efferent fibres although the animal does not appear to move. These responses vary in latency from 30 to 500 ms and show adaptation. It is possible that the impulses accompany imperceptible movements made by the animal. The significance of these weak responses may be appreciated when the central connexions of the lateral-line efferent system are known.

The responses of the lateral-line efferent system of *Xenopus* to voluntary movement has two interesting features. First, the patterns of impulse activity are similar in all the lateral-line efferent fibres regardless of the position of the stitch they innervate. Secondly, the lateral-line efferent neurones exist as centres of convergence since they fire to all voluntary movement of any part of the body. In order to exhibit these features the neurones must all receive input from neurones responsible for the control of movement. Furthermore, the lateral-line efferent neurones are either closely coupled so that the firing of one neurone excites all the others, or they all receive similar neural input from the motor cells. The location of the motor cells is thought not to be in the cerebellum because lateral-line efferent responses are not dependent upon the integrity of the cerebellum. In this respect lateral-line efferent neurones in *Xenopus* are different from efferent neurones innervating the vestibular system of frogs. These have been identified as Purkinje cells in the auricular lobes of the cerebellum (Llinás & Precht, 1969). There is, instead, strong reason for believing that lateral-line efferent neurones receive neural input from the reticular cells of the medulla. Reticular cells are typically centres of convergence and interaction of sensory information (Shiebel *et al.* 1955; Restieaux & Satchell, 1958), and they co-ordinate motor activity. The descending axons of reticular cells travel, in urodele amphibia at least, in tracts beneath and on either side of the neural canal (Kappers, Huber & Crosby, 1936; Herrick, 1948). It was in this region that electrical stimulation caused efferent neurones to be excited and to follow pulses at frequencies up to 20 $s^{-1}$ with a latency which was relatively constant (Fig. 7*c*). An explanation of the observation might be that the spinal cord was massively stimulated and caused sensory and motor centres in the medulla to fire synchronously. Alternatively the brevity and constancy of the efferent responses to stimulation of the central grey matter might be explained by assuming that the descending reticular axons were excited antidromically and that collateral branches of these axons made contact with lateral-line efferent neurones directly or through interneurones. Support for this latter suggestion comes from experiments where stimulation of dorsal roots or of lateral white matter produced rapidly adapting responses of variable latency,

(Figs. 6, 7*b*). If lateral-line efferent neurones do receive neural input from the reticular system, then an interesting comparison might be made with the efferent innervation of the vestibular system in teleosts. Furukawa (1966) found that stimulation of Mauthner cells in goldfish inhibited afferent impulse activity in primary afferent fibres in the eighth nerve. From this and other experiments he proposed that collateral branches of the Mauthner cells made synaptic contact with efferent neurones via several interneurones.

In *Xenopus* the inhibition of sensory impulses from a lateral-line stitch, by a train of efferent impulses, is similar to the inhibition of sensory impulses in the cochlea, produced by stimulation of the olivo-cochlear bundle (Fex, 1962). In both cases inhibition takes a long and variable time to appear (11–30 ms), continues for 40–60 ms, and disappears with prolonged stimulation of the efferent fibres. Inhibition disappears more rapidly when the impulse frequencies of afferent impulses is high (Fig. 14). Disappearance of inhibition decreases, however, when the frequency of impulses in the efferent fibres is increased. In the lateral-line system inhibition is always followed by an after-discharge, during which the response of the lateral-line stitches to a given stimulus is 1·25–3·0 times greater than under resting conditions (Fig. 16). The reason for the after-discharge is not known but it may be important in understanding the function of the lateral-line efferent system.

The interpretation placed on the observation described in this paper is that whenever *Xenopus* makes a movement impulse traffic in the lateral-line afferent fibres is reduced by the action of the efferent fibres. Thus there is a reduction in the lateral-line sensory input to the central nervous system. Such a reduction could, however, be carried out centrally. In this respect it seems to be a general feature of vertebrate sensory systems that interaction with the reticular formation usually occurs at the secondary neurone level (Hernandez-Peon, 1961). Thus the efferent fibres may have some additional function which requires that the sense cell themselves be inhibited.

Lateral-line receptors are stimulated by water movements which tangentially displace the cupulae relative to the sensory epithelium. In common with other acousticolateralis receptors, e.g. the goldfish vestibular system (Furukawa & Ishii, 1967), the lateral-line system is fatigued by or adapts to prolonged stimulation (Sand, 1937). A source of excessive or prolonged stimulation to the lateral-line stitches in *Xenopus* are the water displacements produced each time the animal makes a movement. Simultaneously, during the movement, the lateral-line stitches are inhibited by the efferent fibres. During long periods of movement the sensory impulses will gradually reappear due to the disappearance of inhibition which occurs with long periods of efferent activity. The lateral-line stitches, however, are as sensitive to stimulation immediately following long periods of movement (and consequently long periods of efferent activity) as they are after short periods. This is because the after-discharge is relatively insensitive to the disappearance of inhibition (Fig. 15). The function of the efferent fibres therefore may be to ensure a high sensitivity of the receptors to mechanical stimulation immediately movement ceases.

SUMMARY

1. Efferent impulses have been recorded from branches of lateral-line nerves. The functional significance of the efferent innervation and its action on afferent impulse activity has been examined.

2. Neither mechanical stimulation of the lateral-line receptors nor electrical stimulation of afferent nerves excites lateral-line efferent activity.

3. Trains of efferent impulses accompany all active movements for their duration. In immobilized animals a close correlation exists between impulses in lateral-line efferent nerve fibres and motor impulses in 'large' nerves innervating 'twitch' muscles, but not with impulses in nerves innervating 'slow' muscles. A close similarity also exists between impulse activity in different lateral-line efferent fibres.

4. Whereas electrical stimulation of ascending tracts in the spinal cord fails to excite lateral-line efferent fibres, stimulation of the spinal cord in the region of descending reticular motor axons causes efferent impulses to follow each pulse after brief, constant, latencies. It is suggested that the efferent neurones may be innervated by axon collaterals from reticular cells.

5. Electrical stimulation of efferent fibres innervating a lateral-line receptor produces transitory inhibition of impulse activity in the afferent nerve fibres. The inhibition has a long variable latency (11–30 ms) and persists for 40–60 ms. Upon cessation of inhibition, caused by a train of efferent impulses, afferent impulses reappear at an accelerated frequency (after-discharge), and quickly return to resting frequency.

6. A role of the lateral-line efferent neurones during active movement is discussed.

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