SPINAL REFLEXES OF THE DOGFISH, *SCYLLIUM CANICULA*

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(With Two Plates and Three Text-figures)

IN a previous paper (Gray and Sand, 1936) the persistent swimming rhythm of the spinal dogfish was described, and an account was given of experiments which demonstrate that the locomotory rhythm is determined by the intrinsic activity of the spinal cord without the participation of proprioceptor arcs. In the course of this work it became evident that the rhythmic activity of the spinal dogfish can be modified in various ways by external mechanical stimulation. These responses present a relatively simple pattern of reflex behaviour, their number is strictly limited, and they are uniformly reproducible. For these reasons, although our experiments raise a number of physiological questions which our data cannot answer, we have thought it worth while to record these phenomena in the present communication.

The activity of a spinal goldfish was recently investigated by von Holst (1934), who showed that a brief locomotory rhythm could be elicited by peripheral stimulation. The spinal cyprinoid, however, is a less suitable object for the study of reflex activity than the dogfish whose regular undulatory rhythm is more easily recorded, and whose remarkable persistent activity provides a "background" against which both inhibitory and excitatory responses stand out in clear relief. The reflex behaviour of the dogfish, moreover, is simpler than that of a teleost, in that the paired and median fins play little or no part in propulsion, and the responses of the locomotory mechanism to external stimuli are confined almost entirely to the musculature of the trunk.

When a dogfish whose cord has been transected behind the medulla is anchored by its snout in a shallow trough of sea water in the normal horizontal position with the dorsal surface uppermost, the persistent muscular rhythm is maintained at a frequency of about 40 per minute. The frequency is very constant for any one fish, but varies in different fishes, and is more rapid in small fishes than in larger ones. The rhythm can be conveniently recorded on a horizontal drum with a vertical lever attached to the dorsal fin, or, more usefully, by cinematographic photography.

Mechanical stimuli of various kinds induce a modification of the undulatory rhythm in three ways: (*a*) increase in frequency (acceleration), (*b*) increase in amplitude (augmentation), (*c*) cessation of rhythm (inhibition). Depending on the nature of the stimulus applied, an augmented rhythm may be accelerated, or it may show a slower rate of beat.

Augmentation and acceleration occur when gentle tactile stimulation is applied to any part of the body behind the level of the transection. In Text-fig. 1 the touch was applied to the dorsal surface, behind the anterior median fin. The record shows a 50 per cent. increase in amplitude, and a 40 per cent. increase in frequency. There is an interval of about 2 sec. before the reflex response appears. Its disappearance is coincident with the removal of the stimulus, but it is followed by a period of several seconds during which the rhythmic muscular activity is markedly
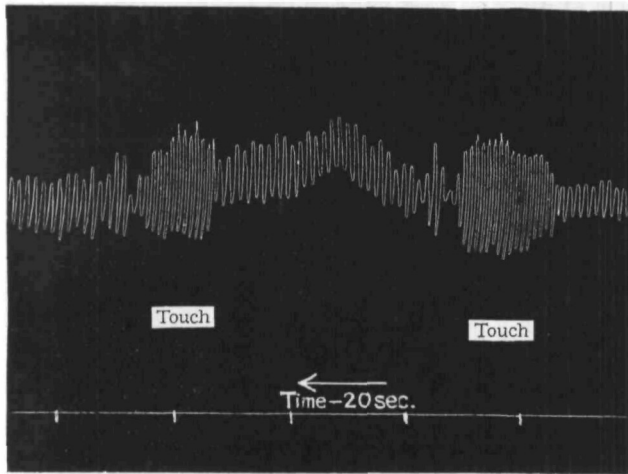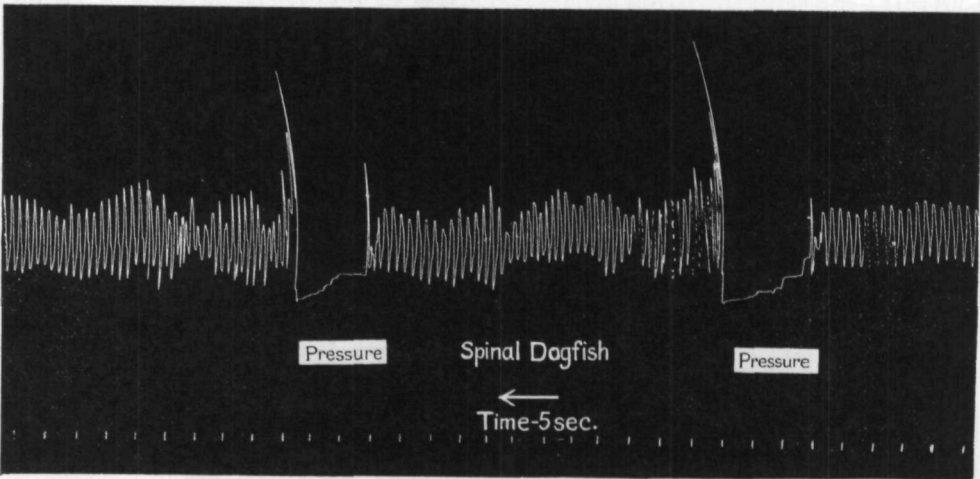

Text-fig. 1. Spinal dogfish. Persistent undulatory rhythm. At signals a light touch was applied to the dorsal surface. Record reads from right to left. Time, 20 sec.

depressed. The touch may be applied anywhere along the dorsal surface, or to the belly or fins, but the regions of the pelvic and the anal fins appear to be the most sensitive. In order to avoid the possibility of mechanical interference with the activity of the preparation we have employed a light **U**-shaped rider of soft metal placed on the fish's back behind the dorsal fin. It is to be supposed that the stimulus due to the rider involves principally the touch organs of the skin, but the possibility cannot be excluded that the rider also excites more deep seated receptors. This is a question of some importance, and we shall have occasion to return to it in connection with later observations.

Cessation of the rhythm is achieved by the application of strong pressure to the fish's body. For this purpose we had recourse to a strong wooden spring clamp of such a size that it could be firmly fixed to the trunk, behind the dorsal fin. The strength of the clamp was such that when one attached it to the palm of one's hand, one experienced a strong though not painful pressure. The application of this

clamp to a spinal dogfish in a vigorous condition evoked a violent writhing, which, however, was of short duration, and was soon superseded by a cessation of move-

Text-fig. 2. Spinal dogfish. Persistent undulatory rhythm. At signals strong pressure was applied to the fish behind the anterior dorsal fin. Record reads from right to left. Time, 5 sec.
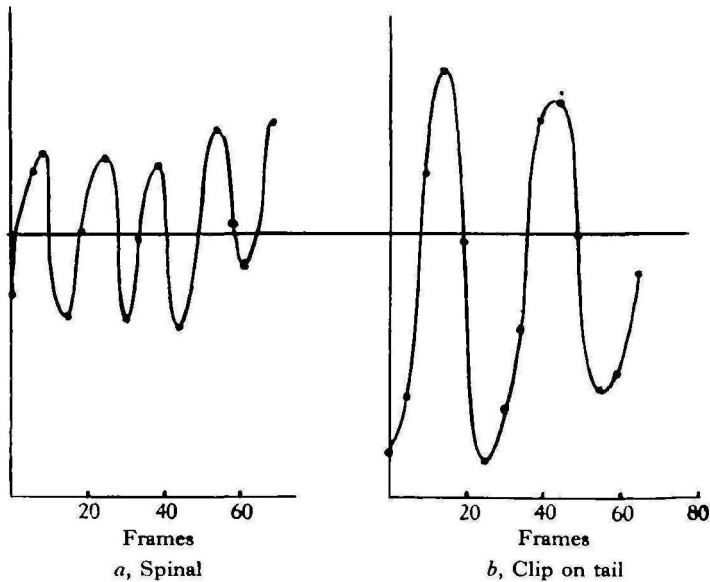
Text-fig. 3. Analysis of cinematographic records. Spinal dogfish, 7 days after transection of cord. *a*, the persistent rhythm, *b*, the response to a clip attached to the tail.

ment, the fish lying still, usually bent to one side or the other, with the muscles in a condition of fairly strong contracture. After some time the contracted muscles relaxed and the fish now lay limp, and could be easily bent to the right or to the left by moving it laterally backwards and forwards through the water. Text-fig. 2

is a record of this response. It is to be noted that again there is a latent period of about 3 sec. before the response occurs, and that the release of the clamp is instantly followed by resumption of the rhythm.

The possibility of arresting the undulatory rhythm appears to be confined to the receptors of the trunk; no such arrest follows the application of strong stimuli to the paired and median fins. When, for example, a strong clip is attached to a fin the rhythm is augmented, but it also tends to show a lowered frequency. This effect is especially marked when the clip is attached to the tip of the tail (Text-fig. 3). The amplitude of the movements is enormously increased, and, at the same time, the rhythm slows down very considerably (Table I). The application of the clip to one of the paired fins induces also a strong bending of the body to the stimulated side. The extreme form of this response is observed when the region of the gills is stimulated by pinching. The fish then bends its tail right round, and rubs it forward against the point of stimulation, thus executing a kind of scratching or rasping movement which is quite strong enough to remove any natural source of irritation, such as a small parasite (Plate I, fig. 1). On one occasion, when the experimenter was incautiously pinching the gills of a spinal fish with his fingers, the fish actually scraped the skin from his hand, and drew blood.

Table I. *Time in seconds for six consecutive groups of twenty-five complete swimming waves before and after attachment of tail clip*

Unstimulated	Clip on tail
40·8	56·4
42·0	66·6
41·3	68·0
36·4	63·4
42·2	67·6
43·0	65·0
Average 41·0	64·5

The responses may be summarised as follows:

(1) Tactile stimulation (touch or rider) causes augmentation and acceleration.

(2) Strong pressure stimulation of the trunk (clamp) causes arrest.

(3) Strong pressure, *i.e.* pinching of the fins (clip), causes augmentation and reduction in frequency.

(4) Unilateral tactile or clip stimulation is accompanied by bending to the stimulated side, and the undulatory rhythm becomes asymmetrical in consequence.

The reflex phenomena of augmentation, acceleration and arrest are not properties of any particular level of the spinal cord, for they may be demonstrated in the tail piece of a fish whose cord is transected some distance posterior to the level of the cloaca. Indeed, such a preparation even exhibits a spontaneous persistent rhythm (Plate I, figs. 2 and 3).

Since these reflex responses are all evoked by mechanical stimulation it must be concluded that the type of response is determined by the kind of receptors that are stimulated, by their anatomical situation, and, possibly, by the intensity of their

excitation. It would appear that afferent volleys from tactile receptors in any region of the skin excite the rhythmic centres of the cord. This conclusion follows from the augmented and accelerated rhythm that is evoked by touching or brushing any part of the trunk or fins. The tail clip and body clamp responses are more difficult to deal with, because they involve at least two and possibly more types of sense organs. The difference between them is certainly topographical rather than quantitative. Both clip and clamp apply a strong pressure to the part of the body to which they are attached; the stimuli are mechanically alike, and the only reason why two different types of appliances have to be used is that the shape and dimensions of the regions stimulated are different. Moreover, in the case of the clamp attached to the body, the compression of the body musculature must supply a strong stimulus to the proprioceptors situated within it—a stimulus which may interfere with the normal functioning of the rhythmic mechanism, and thus cause its arrest. It is significant in this connection to note that the inhibition is preceded by violent non-rhythmical muscular activity, and a phase of somewhat asymmetrical tetanic contracture of variable duration, but eventually a state of relaxed inactivity supervenes. That this is a true inhibition is confirmed by the behaviour of the fish when the clamp is again removed. However long the clamp has remained attached, its removal instantly releases the spontaneous motor rhythm (Plate II, fig. 1). The effect of the clamp is therefore a complex one in that it first excites a general contracture which replaces or suppresses the normal undulatory rhythm, and then, when the muscles relax again, presumably owing to rapid fatigue, the clamp maintains the inhibition of the normal rhythm.

It is particularly informative to consider the behaviour of the spinal dogfish when the body clamp (inhibitory) and the tail clip (excitatory) are applied together. If the tail clip is applied first, the augmented rhythm can be inhibited with the clamp in the same way as the normal spontaneous rhythm. But if the normal rhythm is first inhibited, and the tail clip is then attached to the inactive fish, the excitatory volley is adequate to break through the inhibition, and evokes several rhythmic waves before the inhibition is again established (Plate II, fig. 2).

It may be argued that the excitatory volley from the application of the tail clip is an affair of relatively short duration, and that sensory adaptation soon occurs. This is negatived, however, by the fact that when, in the case of a fish that has been allowed to lie still for some time with clamp and clip attached, the clamp is then released, the rhythm which instantly reappears is *not* the normal spontaneous rhythm, but has the low frequency and augmented amplitude typical of the tail clip response. Had sensory adaptation occurred, there would have been no reason why the presence of the attached tail clip should make any difference to the rhythm which emerged when the inhibitory clamp was removed from the body of the fish.

Effect of fatigue. The responses hitherto described are characteristic of the fresh spinal dogfish, but when the responses to the rider, tail clip and clamp have been repeatedly evoked, the condition of the spinal fish is altered, and its behaviour becomes correspondingly modified. This occurred rather quickly in our experiments, but we were working in hot weather, and no doubt the high temperature of

the water (17–18° C.) facilitated a rapid development of fatigue. The general effect was that excitation produced less vigorous activity, and inhibition was more readily established.

The fatigued fish still showed a spontaneous rhythm, though this was feebler than in the fresh condition. The application of the tail clip now produced a less violent though very definite augmentation. Attaching the clamp to the body produced instantaneous cessation of movement in the relaxed condition. The initial violent muscular response and the phase of contracture did not appear. The most striking difference, however, between the behaviour of the fresh and the fatigued fish was in the response to gentle tactile stimulation of the body surface. The same rider which, in the fresh fish, evoked an augmented rhythm, now produced inhibition. In fact the rider could now be used in place of the clamp, and in combination with the tail clip gave the same series of responses as had been obtained with the clamp and tail clip in the fresh preparation. In a state of advanced fatigue a very small light rider resting on the fish's back was sufficient to cause inhibition, and its removal released a feeble rhythm.

The effect of fatigue is to depress the state of central excitation, and the weaker responses of the fatigued fish to excitatory stimuli with a clip on the tail or fins are therefore not difficult to interpret. In the same way it may be argued that the violent muscular response to the body clamp fails to appear in the fatigued fish, while the inhibition of the rhythmic motor discharge from the cord is more readily established. But the qualitative change which is implied in a stimulus (rider) which was originally excitatory becoming, in the fatigued state, inhibitory, is less easily accounted for. It is well known (Verzár, 1920, 1923) that fatigue can result in a reversal of reflex effect to one and the same afferent stimulus, and it is possible that we are dealing here with such a case of reflex reversal. The question to be decided is whether we are dealing with a case where a discharge of impulses in a certain afferent neurone produces, in the fresh fish, excitation, and in the fatigued fish, inhibition, or whether it is merely a balance between excitatory and inhibitory volleys in a group of neurones which is altered with the establishment of fatigue. The present evidence does not justify a definite answer to this question, but it may be pointed out that if the rider evokes an afferent discharge which is *qualitatively* the same as the discharge from the body clamp, and differs from it only in intensity, then one can interpret the inhibition of the fatigued rhythm by the rider in terms of a greatly depressed central excitatory state. But such an interpretation would imply that in the fresh and vigorous spinal fish there is a fairly sharp threshold of pressure excitation in the trunk, and that a stimulus which is below this threshold excites the rhythm, while one which is above it inhibits. Whether this reasoning does actually apply must await the verdict of experiment.

The undulatory rhythm becomes inhibited also when, as frequently happens, the spinal dogfish turns over and lies on its back. Since labyrinthine reflexes are absent, the normal righting reaction does not occur, and the fish may remain on its back indefinitely. It is sufficient to turn the fish the right way up for the rhythm to reappear. The occurrence of this phenomenon is somewhat variable. Some spinal

fishes, when turned over, come to rest after some half dozen rhythmic waves, others maintain the rhythm for several minutes before lying still, and a few show little tendency to come to rest at all. The nature of this response is at present obscure. One would expect that the contact of the inverted fish's back with the ground would excite the rhythm rather than inhibit it, and in some cases a temporary augmentation of the rhythm has actually been observed when the fish was turned over. Attempts to evoke inhibition by allowing a horizontal board to rest gently upon a fish in the normal position have failed. Contact with the board always increases the muscular activity. When a spinal fish is suspended in the inverted horizontal position in a deep tank, so that its body does not come into contact with the sides or bottom, the rhythm shows no signs of slackening. If, now, a board is brought up from underneath, so that the fish comes to lie upon it, the rhythm soon ceases. This indicates that the inhibition depends upon a complex pattern of stimuli resulting from the gravitational pressure of the inverted fish resting on a solid surface.

Comparison with intact dogfish. Transection of the cord abolishes labyrinthine reflexes and orientated swimming, and releases the persistent undulatory rhythm. Apart from this the behaviour of the spinal fish is similar to that of the intact animal, except that the responses of the latter tend to be far more violent. In particular, the body clamp does not at first inhibit the fish, but, on the contrary, excites it to violent activity. But if one seizes the fish firmly in the hand at the level of the cloaca, it is sometimes possible by strong pressure to induce complete muscular relaxation. When, however, the stimuli were applied again and again, the fish came to react less violently, until finally its responses approximated extraordinarily closely to those of the spinal animal. The chief difference was that a persistent spontaneous rhythm never appeared. The fish, when undisturbed, lay still on the bottom of the tank. It could be excited by tactile stimulation, or by attaching the clip to the tail, and this evoked the same slow wide excursion as in the spinal preparation. The combination of clamp and tail clip stimuli now gave responses which were identical in all respects with those of the spinal fish. Of special interest, however, was the response to the clamp alone. When the state of fatigue was sufficiently advanced, the fish ceased to respond altogether to the *application* of the clamp. The fish would be lying still on the bottom of the tank, and when the clamp was put on no muscular movement could be observed. Nevertheless, when the clamp was again released, *the fish immediately started swimming*, and presently came to rest again. This performance was repeated, with the same result, a large number of times (Plate II, fig. 3).

The significance of this phenomenon is that it strongly indicates the essential identity of the normal swimming mechanism with the persistent undulatory rhythm of the spinal fish. The fact that the intact fish starts swimming when an external stimulus (clamp) which, during its application, had no noticeable effect, is removed, implies that the stimulus was not merely ineffective, but that it did in fact modify the central state in such a way that, on its removal, a rhythmic motor discharge could occur. In the spinal fish the same stimulus inhibits the spontaneous activity which is already manifest, but on the removal of the inhibitory influence the rhythm

which emerges is significantly accelerated. Table II shows that after removal of the clamp the rhythm is accelerated over a period of about 100 complete swimming waves. This indicates that during the period of inhibition the level of the central excitatory state has been elevated, or stored up, as it were, and that the maintenance of a state of inhibition acts as an excitatory influence which reveals itself when the source of inhibition is removed. The response of the fatigued intact fish to the removal of the clamp thus finds its explanation in terms of the elevated central excitation which results from the application of an inhibiting influence. It is most improbable that there should be two separate central mechanisms which show such a fundamental similarity of behaviour in relation to external pressure stimulation as the spontaneous rhythm of the spinal dogfish, and the normal swimming apparatus of the intact animal. We conclude that they are one and the same, and that the persistent rhythm of the spinal fish is the expression of a basic rhythmic activity of the motor centres of the central nervous system of the dogfish.

Table II. *Time in seconds for five consecutive groups of twenty-five complete swimming waves before and after a 2-min. period of inhibition by means of the body clamp*

Normal rhythm	After removal of clamp
49·2	32·4
45·0	36·6
46·2	40·0
46·0	40·0
44·8	43·0

SUMMARY

1. The persistent undulatory rhythm of the spinal dogfish can be reversibly augmented, accelerated and arrested by peripheral stimulation.

2. These reflexes are a property of the whole spinal cord, and are not localised in any particular level.

3. In a state of fatigue the reflex responses become modified, in particular a stimulus which, in the fresh preparation, caused excitation, inhibits the rhythm in the fatigued fish. The significance of this is discussed.

4. The behaviour of the fatigued intact dogfish approximates to that of the spinal fish. A striking resemblance in the response to inhibitory stimuli is described, and the thesis is developed that the persistent rhythm of the spinal fish is the expression of a basic rhythmic activity of the spinal cord.

REFERENCES

GRAY, J. and SAND, A. (1936). *J. exp. Biol.* **13**, 200.
VON HOLST, E. (1934). *Z. vergl. Physiol.* **20**, 582.
VERZÁR, F. (1920). *Pflüg. Arch .ges. Physiol.* **183**, 210.
—— (1923). *Pflüg. Arch. ges. Physiol.* **199**, 109.

EXPLANATION OF PLATES

The numeral beside each photograph indicates the position of the particular photograph in a series taken at intervals of approximately 0·1 sec.

PLATE I

Fig. 1. Spinal dogfish, 7 days after operation. Response to pinching in the region of the gills.

Fig. 2. Spinal dogfish. Cord transected at two levels, behind medulla and in front of anterior dorsal fin. Seven days after operation. Fixed rigidly by snout and in front of posterior cut. The photographs show the extreme left and right positions of the persistent rhythm of the tail.

Fig. 3. Same as fig. 2. The photographs show the extreme left and right positions of the tail piece when a clip is attached to its tip.

PLATE II

Fig. 1. Spinal dogfish, 25 days after operation. The persistent rhythm had been inhibited by a clamp attached to the body. The photographs show the emergence of the rhythm when the clamp is released.

Fig. 2. Spinal dogfish, 25 days after operation. The persistent rhythm is inhibited with a clamp. The photographs show the excitation due to the attachment of a clip to the tail breaking through the inhibition.

Fig. 3. Intact dogfish in fatigued condition. The fish has been lying still with a clamp attached to its body. The photographs show the release of the clamp and the emergence of the swimming rhythm.
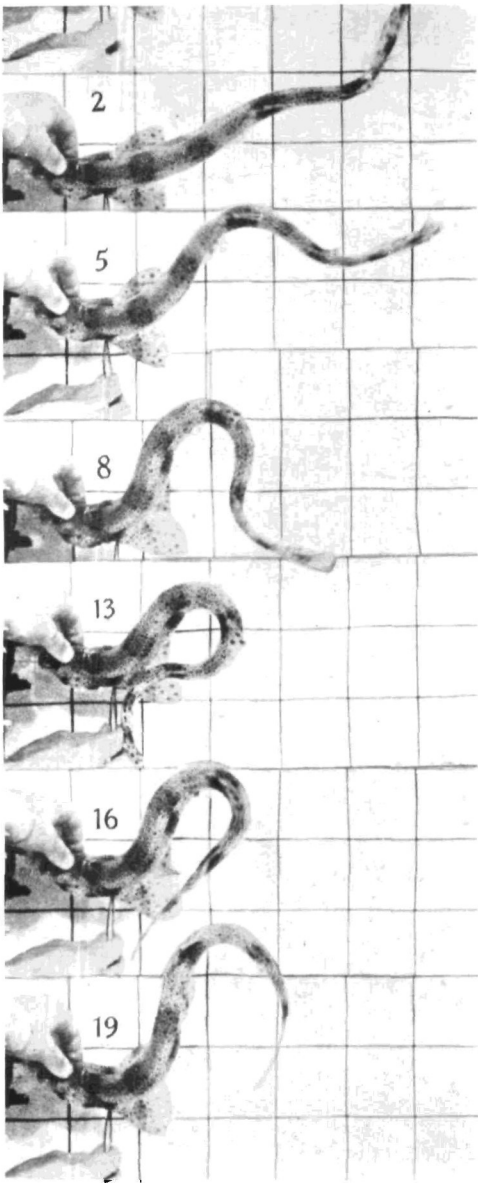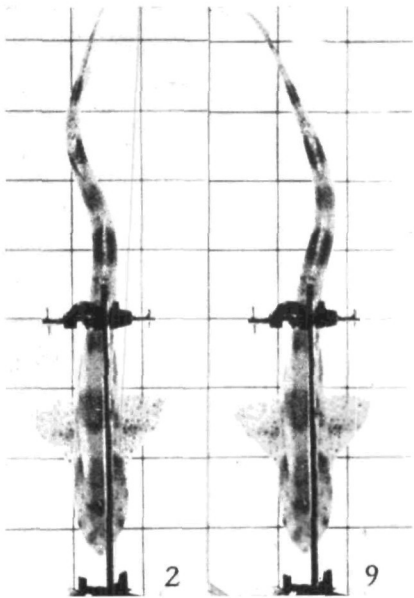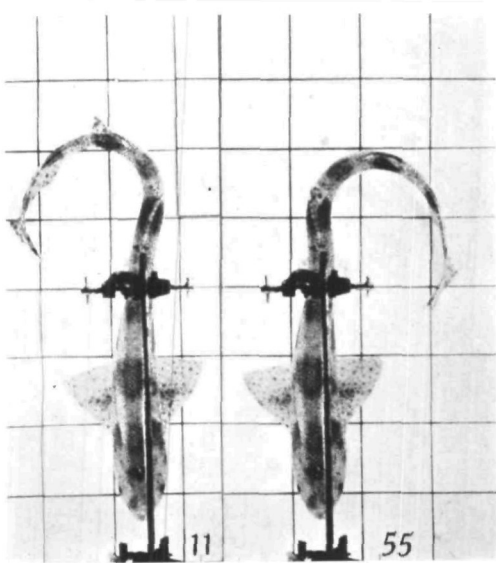
Fig. 1.

Fig. 2.

Fig. 3.

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Fig. 1.

Fig. 2.

Fig. 3.

GRAY AND SAND—SPINAL REFLEXES OF THE DOGFISH, *SCYLLIUM CANICULA*
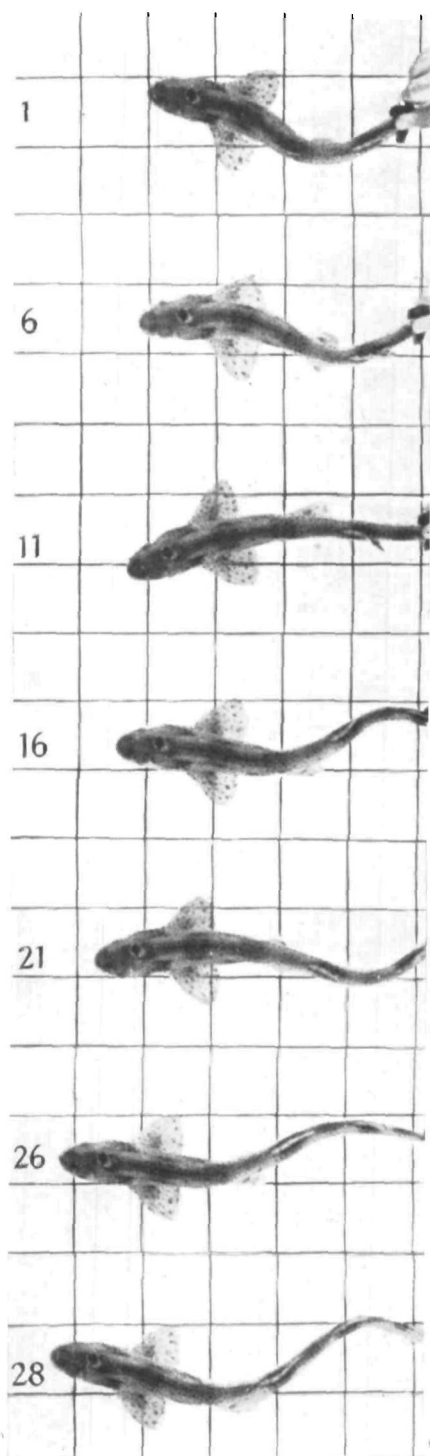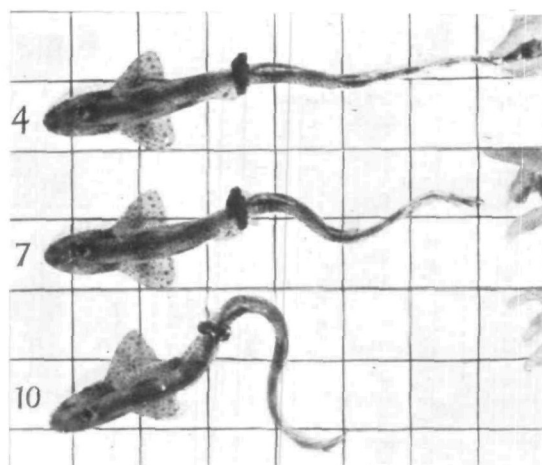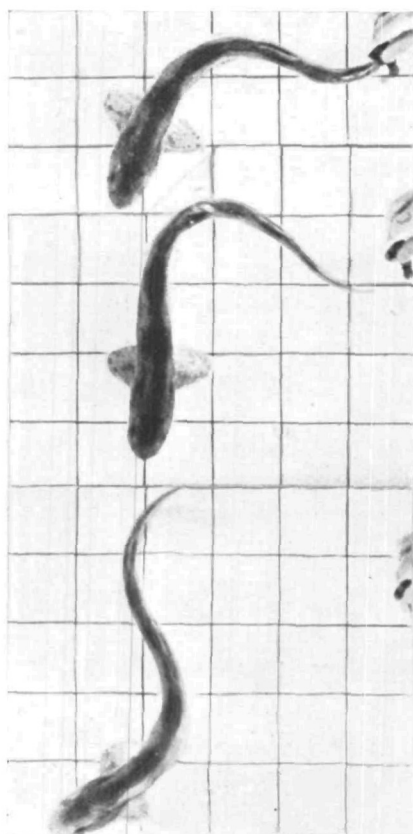(pp. 210—218).