

THE EFFECT OF DEAFFERENTATION UPON THE LOCOMOTORY ACTIVITY OF AMPHIBIAN LIMBS

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(Received 1 December 1939)

(With One Plate and One Text-figure)

IN 1912-13 Graham Brown and Sherrington showed, independently, that rhythmical activity could be maintained in a pair of deafferentated antagonistic mammalian muscles; since then it has been customary to regard locomotory rhythms as the expression of intrinsic activity within the nerve cord. On the other hand, a study of the responses of vertebrate limbs to suitable reflex stimulation makes it difficult to believe that impulses coming from peripheral sense organs do not play a very important role in the co-ordination of normal locomotory movements. The present paper constitutes an attempt to decide how far locomotion can occur in animals whose limbs have been completely desensitized by cutting the roots of their afferent nerves.

The work of Mott & Sherrington (1895), Bickel (1897) and Ranson (1931) shows that, after section of all its dorsal roots, a limb of a monkey, dog or cat fails to take part in subsequent locomotion. Such a profound effect of deafferentation in the limbs of otherwise intact mammals is in striking contrast to the observations of both Graham Brown (1912*a, b*, 1913) and Sherrington (1913) on isolated preparations of mammalian muscles; both these authors found it possible to elicit rhythmical stepping in a pair of deafferentated muscles so long as the nerve cord was exposed to an appropriate bilateral stream of afferent impulses.

The behaviour of deafferentated limbs in lower vertebrates has been described by various investigators. Trendelenburg (1906) found that the power of flight of a pigeon was not seriously disturbed by deafferentation of one wing; if, however, both wings were desensitized the bird could not fly. In amphibia, both Hering (1897) and Bickel (1897) found that deafferentation of one or both hindlimbs of the frog did not seriously impede swimming or jumping. Deafferentation of all four limbs reduced locomotory activity to a very low level (Bickel, 1897), and after total deafferentation of all spinal nerves no co-ordinated movements were observed by Hering (1893). Within recent years, Weiss¹ (1936) has confirmed the fact that deafferentation of both hindlimbs of a toad (*Bufo americana* or *B. fowleri*) does not prevent these limbs from displaying their normal locomotory activity. The description, given by Weiss, of a toad deafferentated in all four limbs does not differ substantially from that given by Hering (1897), but, nevertheless, Weiss concluded

¹ Our attention was only drawn to this paper after our own observations were complete.

that: "However serious the effect of deafferentation may be with respect to the total behaviour of the animals, it certainly cannot be said to involve a disintegration of the basic patterns of motor co-ordination as such; once made to appear, these manifest themselves in—qualitatively speaking—full integrity."

It is important to note that none of the above authors gives more than somewhat casual reference to the effect of deafferentation on ambulation: they were chiefly concerned with jumping and swimming, wherein the forelimbs play only a minor role and in which the movements of the hindlimbs are simultaneous.

During a study of the flexor reflexes of the limbs of spinal amphibia we have found it difficult to avoid the conclusion that peripheral events play a very important role in the co-ordination of the terrestrial locomotion of these animals, and it therefore seemed of interest to consider in some detail how far typical terrestrial movements can be displayed after deafferentation of the limbs. For these experiments we have used the toad (*B. bufo*). This animal seldom jumps unless violently stimulated; it walks with a characteristic diagonal sequence of limb movement (see Gray, 1939). It is with this pattern of movement that the present paper is concerned.

The nerve supply to the limbs of *Bufo* is essentially the same as that of *Rana*. The main supply to the forelimbs is from spinal nerves III and IV; V, VI and VII supply the trunk of the body; VIII, IX and X supply the hindlimb; and nerve XI supplies a small area in the neighbourhood of the anus. A small branch of the hypoglossal is also associated with the forelimb. Severance of this nerve caused respiratory disturbances, and animals, so operated upon, did not survive well. For this reason, the hypoglossal was usually left intact. Its sensory fibres did not appear to be associated with sense organs in the forelimb, since severance of nerves III and IV caused complete loss of sensitivity—both superficial and deep seated—in the limb. All operations were performed under ether, and the animals were kept under observation for periods varying from 2 days to 3 weeks. In no case did the pattern of behaviour change after the lapse of time.

DEAFFERENTATION OF SINGLE LIMBS

After deafferentation of one hindlimb, it is not easy to differentiate between the normal and the operated limbs. Ambulation can be elicited by stimuli apparently no more intense than that required by a normal animal, and the characteristic diagonal pattern of movement is retained. When at rest, the animal adopts its customary squatting attitude, although in some cases there appeared to be a tendency to hold the plantar surface of the operated limb off the ground as was observed by Hering (1897). The operated limb is entirely anaesthetized and offers no resistance to external passive displacement, but as soon as the animal moves (in response to suitable visual, labyrinthine or mechanical stimulation), the limb quickly takes up its proper position and functions in a normal manner.

After deafferentation of one forelimb little or no disturbance of ambulatory behaviour can be detected when the animal is moving relatively rapidly. During slow movement, however, a loss of tone can be observed in the muscles of the

forearm and digits; the forearm may rest on the ground during the retractor¹ phase of the upper arm's movement, and the digits are not always extended. When the animal is at rest, the posture of a deafferentated limb is sometimes normal, but, not infrequently, the forearm rests on the ground, causing a downward displacement of the body towards the operated side. So far as could be seen, deafferentation of a single limb only disturbs the rhythm of locomotion in so far as loss of tone in the wrist involves purely mechanical effects.

Some of the features observed in the deafferentated forelimb of a toad, recall the observations of Mott & Sherrington (1895) on the monkey (*Macacus rhesus*), viz. the loss of muscular tone, and the impairment of movement in the distal joints of the limb. On the other hand, whereas the deafferentated limb of the monkey, dog (Bickel, 1897) or cat (Ranson, 1931) plays no part in ambulation, that of the toad continues, particularly in its upper arm or thigh, to play its normal role in diagonal ambulation.

It should be noted that the ability of a single deafferentated limb to take its normal part in ambulation does not constitute evidence in favour of the central nature of the ambulatory rhythm; ample evidence exists to show that stimulation of the peripheral sense organs in one limb can induce co-ordinated responses in neighbouring limbs.

DEAFFERENTATION OF TWO LIMBS

The effect of deafferentation of two limbs depends somewhat on the limbs selected for operation, but, in general, our observations confirm those of Weiss (1936) in demonstrating that, after deafferentation of two limbs, the locomotory pattern is singularly little disturbed.

If both hindlimbs are deafferentated, the animal walks with the usual diagonal sequence of limb movements but displays the following abnormalities: (i) the hind region of the body is not lifted off the ground to the normal extent, and (ii) the femur is not infrequently protracted to an abnormal extent. These features seem to be due to a loss of tone by the muscles rather than to a functional disturbance of the locomotory rhythm.

Deafferentation of one hind- and one forelimb did not seriously impede locomotory activity, but no records were kept of minor disturbances. After deafferentation of both forelimbs, all operated animals displayed characteristic features. In no case was the pattern of ambulation seriously disturbed, but the following minor abnormalities were present to a varying extent:

(i) Both at rest and during retraction of the humerus, the whole of the forearm was kept in contact with the ground, whereas in the normal animal the forearm is raised.

(ii) The degree of protraction of the humerus was abnormally great; at the moment of maximum protraction the upper arm faced obliquely forwards instead of lying at right angles to the axis of the body as in the normal animal.

¹ The term *retraction* is used to denote the movement of the whole limb during the propulsive phase of the step; *protraction* denotes the non-propulsive phase.

(iii) The amplitude of step of the hindlimbs appeared to be greater than normal.

The first two of these features prevent the forelimb from functioning as an efficient prop on which the body can be swung forward during its own retractor movement and that of the diagonal hindlimb; the animal thus tends to roll somewhat and the smoothness of progression is diminished. In no case, however, was the normal diagonal pattern of movement lost.

At this point, it is of interest to note the results obtained by deafferentation of both forelimbs of the newt (*Triton cristatus*). Such animals, when at rest, held the forelimbs in a partially retracted position not observable in intact animals; the plantar surfaces were held off the ground and the elbows pointed obliquely backwards. When the animals walked slowly, the forelimbs showed no movements—the body being pressed forwards by the hindlimbs and body muscles only. When, however, the animals were stimulated into rapid movement, by tapping on the tail, the forelimbs at once took their usual part in locomotion—and showed typical diagonal sequence with the hindlimbs. As soon as progression ceased, the retracted posture of the forelimbs was resumed. The behaviour of these limbs is interesting in view of the observations of Detwiler & Vandyke (1934); these authors found that no locomotory movements occurred in the forelimbs of a larval *Amblystoma* if the development of afferent nerves had been prevented by destruction of the dorsal crest of the nerve cord in an early stage of development.

The mobility of the deafferentated forelimbs of these newts when exposed to relatively strong stimulation recalls a similar phenomenon observed by Mott & Sherrington (1895); forcible and rapid movements occurred in the anaesthetized limb of a monkey when the animal was induced to “struggle”.

DEAFFERENTATION OF THREE LIMBS

Deafferentation of three limbs involves a still further loss of muscular tone and a marked decrease in the excitability of the animal. In such animals the body is not lifted from the ground either during periods of rest or during locomotion; the movements of the limbs are spasmodic in the sense that the retractor phase sets in abruptly. The diagonal sequence of limb movement, although not invariably preserved, is nevertheless clearly present, particularly in more active preparations. The animals usually only walked in response to fairly strong tactile stimuli, but if left in a lighted tank they walked spontaneously, after a fairly long latent period, towards a shaded area. Periods of active locomotion were brief, the animal fatigued quickly, movements were often confined to the shoulder in the fore-arm and to the thigh and knee in the hindlimb.

DEAFFERENTATION OF FOUR LIMBS

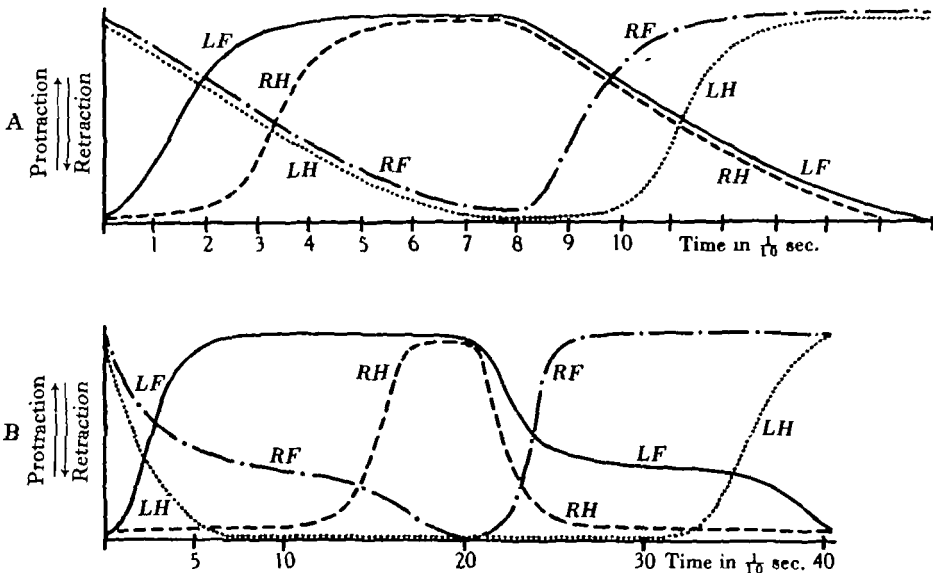
Deafferentation of all four limbs involves a marked decline in the excitability of the animal, and relatively strong stimuli, applied to the skin of the body, are required for the elicitation of limb movements; in many cases these movements

are unco-ordinated. The ability of the animals to walk normally was best observed by placing the animal in a lighted tank and allowing it to walk spontaneously towards a darkened area. Under such conditions, clearly defined diagonal co-ordination was observed, but in every case the limb movements were ungainly, slow and few in number. The chief abnormalities observed were:

(i) Considerable slip occurred during the retractor phase. The limbs were quickly extended to their maximum extent, and there was often a long delay before the onset of contraction in the protractor muscles.

(ii) The body was not lifted off the ground during the retractor phase, but tended to roll from side to side owing to weakness of the forelimbs.

(iii) The hindlimbs were not infrequently extended somewhat laterally instead of posteriorly as in the normal animal.



Text-fig. 1. Reconstructions (from cinematograph records) of a relatively rapid step by a normal toad (A) and of a toad (B) deafferentated in all four limbs. In the normal animal protraction of a forelimb is accompanied by simultaneous retraction of the ipsilateral hindlimb, and the period elapsing before protraction of the contralateral hindlimb is short. After deafferentation co-ordination is maintained with the ipsilateral hindlimb, but there is a long latent period before the contralateral hindlimb responds by retraction.

(iv) The smooth rhythm of movement of the normal animal is entirely absent; each limb movement being spasmodic.

(v) Abnormal limb postures were frequently observed, both hindlimbs exhibiting, at times, a state of maximum retraction (see Pl. I, fig. D 2, 4). This abnormality can be traced to a very long latent period between the protraction of a forelimb and the corresponding phase in the contralateral hindlimb (see Text-fig. 1 B).

Some, at least, of these characteristics, appear to be due to a lack of muscular tone particularly in the more distal regions of the limbs. Very rarely did such animals

progress for more than one or two co-ordinated steps at a time. Fatigue set in very rapidly.

The behaviour of the forelimbs of these animals only differed in minor respects from that of animals in which the forelimbs alone were deafferentated: the frequency of movement was much lower and the loss of muscular tone was perhaps more obvious. There could be no doubt, however, that deafferentation of the forelimbs had a profound effect upon the behaviour of deafferentated hindlimbs. The relatively smooth rhythm of movement is lost, the posture of the limbs is often abnormal, and the threshold stimulus for locomotion is very high. As noted by Weiss "the effect of deafferentating all limbs is immensely greater than one would expect from simply adding up the effect of fore- and hindlimb deafferentation".

At first sight, the ability of these toads to walk normally, even if only for a few slow and somewhat modified steps, tends to support the view that the locomotory rhythm is essentially of central origin, and that such irregularities as occur are due to general lack of muscular tone or lack of excitability in the motor centres of the limb muscles. On the other hand, the very marked difference in the activity of deafferentated hindlimbs before and after deafferentation of the forelimbs suggests that the hindlimbs are definitely influenced by peripheral events in the forelimbs and therefore also, possibly, by similar events in other parts of the body. Since the total ambulatory activity of an animal is reduced to such a low level by deafferentation of all four limbs, it is important to consider how far the remaining remnant of co-ordinated movement can be reasonably attributed to the presence of locomotory musculature not deafferentated by severance of the dorsal roots of nerves III, IV, VIII–XI (inclusive). No evidence of such musculature could be found in respect of the hindlimbs by direct stimulation of the body muscles, but it is possible, by such methods, to show that contraction of the anterior regions of the abdominal recti muscles causes retraction of the humerus by pulling on the posterior region of the large pectoralis muscle. Since the abdominal recti muscles are supplied by the Vth and VIth spinal nerves, it is of interest to consider how far complete deafferentation of all the spinal nerves still further reduces ambulatory activity.

TOTAL DEAFFERENTATION OF ALL SPINAL SEGMENTS

In six animals, all limbs and all the intervening segments were deafferentated by cutting the dorsal roots of spinal nerves III–XI (inclusive) on both sides. In no case did spontaneous co-ordinated ambulation occur, nor could it be elicited either through the eyes or through the labyrinth. If the body was rolled to the left, the left hindleg was actively extended: when placed on a turn-table and rotated to the right the right hindlimb was alternately flexed and extended—it failed to rotate the body owing to the inability of the planta to maintain contact with the ground. No trace of diagonal ambulatory co-ordination was ever observed. Apart from cleaning reflexes, only two types of co-ordination in the limb movements of these animals were observed:

(i) A few, but active, swimming movements were sometimes seen in the hind limbs when the animal was placed in water: both hindlimbs flexed simultaneously and extended simultaneously.

(ii) If a moving object was suddenly brought near the eyes or if the head was gently tapped with a seeker, all four limbs moved in a purposive manner; the hindlimbs were brought into the fully flexed position; the head was lowered and the forelimbs brought close up to the sides of the head.

Weiss (1936) described the behaviour of one animal of this type and concluded that its behaviour was essentially the same as one in which the dorsal roots of the spinal nerves V, VI and VII were intact. "Occasionally the animals move for a few steps with the hindlimbs alternating as in walking" (Weiss). In our experiments, we failed to detect any pattern of limb movement which appeared to be of ambulatory significance unless at least one dorsal root was intact.

Since the integrity of one dorsal spinal root appears to be essential for the maintenance of the ambulatory pattern it is of interest to consider how far the sense organs associated with this nerve are acting as essential pacemakers for the rhythm or how far they are concerned with the maintenance of a stream of impulses which enables the nerve cord to generate its own efferent rhythm. If the presence of proprioceptor sense organs in the muscles or skin innervated by nerves V, VI and VII are acting as pacemakers for the ambulation of a toad (deafferentated in all four limbs) ambulation should not occur if the ventral roots of these nerves are cut—leaving their dorsal roots intact. If, on the other hand, the dorsal roots are maintaining the general level of excitability of the cord, it might be possible to cut these ventral roots and elicit ambulation by continuous tactile stimulation of the skin of the body. Four such preparations have been made, and in no case was ambulation observed; stimulation of the skin or body wall only elicited cleaning responses or completely unco-ordinated limb movements. Either the proprioceptor endings are acting as pacemakers or they alone provide the type of stimulation necessary to maintain the locomotory activities of the nerve cord.

DISCUSSION

Three facts are clearly established by the present series of experiments: (i) A comparatively large amount of the total locomotory musculature of the toad can be deafferentated without seriously disturbing the role which the deafferentated muscles play in the maintenance of the normal ambulatory pattern. (ii) Deafferentation involves a loss of muscular tone, and, if widespread, causes a marked increase in the threshold of stimulation which is required to elicit muscular responses in the limbs. (iii) If the whole of the locomotory musculature be deafferentated, the animal can no longer walk. The simplest interpretation of these facts is to assume that the ambulatory rhythm is essentially dependent upon the activity of peripheral sense organs in the muscles. The experiments provide no positive evidence in support of the central control of amphibian ambulation; on the other hand, it cannot be regarded as disproved. After severance of all afferent spinal nerves, III–XI

(inclusive), the limbs of a toad will still respond by alternating flexion and extension to labyrinthine stimulation; the animal can sometimes swim, but it cannot walk.

The ambulatory activity of a single totally deafferentated limb of an amphibian forms, at first sight, a marked contrast to the immobility of a similar limb in a mammal. It is, however, interesting to note that Mott & Sherrington found that quite extensive deafferentation could be carried out on a monkey's limb before marked impairment of movement was observable. To some extent this was attributed to an overlap of sensory areas, but even when a field of complete anaesthesia was established on the most sensitive parts of the limb the impairment was comparatively slight. Even in mammals, therefore, a deafferentated muscle may prove to be sustainable in its normal movements by the influence of neighbouring intact musculature and skin. In the amphibia peripheral control can be exercised by sense organs lying in other limbs; in the mammal such control may be limited to sense organs lying within the operated limb. In this case the difference between the two types is quantitative rather than essentially different in nature.

In one respect the totally deafferentated limb of a mammal differs from that of an amphibian. When such a limb of a toad is not actively engaged in ambulation, its muscles appear to be entirely inactive, it is only during active movements that the limb displays muscular activity. The deafferentated limb of a mammal usually shows definite signs of muscular tone (Mott & Sherrington, 1895; Ranson, 1931). Ranson has described the behaviour of the deafferentated forelimb of a decerebrate cat as follows: "In the periods of activity which would come on spontaneously after brief periods of rest, the three normal limbs would show rhythmically repeated and well-co-ordinated movements in which the deafferentated leg did not as a rule participate. Instead, it would be rigidly extended and often drawn back against the chest wall. This posture of rigid extension would continue throughout the period of activity. In the intervals between these periods of activity, the deafferentated legs would relax and become considerably less stiff than the normal legs" (Ranson, 1931, pp. 342-3). The impression given by this account is that the extensor activity in an intact walking limb is counteracted at the appropriate moment by flexor activity induced proprioceptively within the limb itself. If the rhythm of ambulation were of central origin one would have expected the rhythm to appear even more readily in a deafferentated limb than in a normal limb.

So far as is known, there is only one series of facts which provides positive evidence in favour of the view that the ambulatory rhythm is of central origin. Both Graham Brown (1912*a, b*, 1913) and Sherrington (1913) showed that deafferentated pairs of antagonistic muscles would step if subjected simultaneously to two well-balanced but opposing streams of reflex stimuli. The crucial case is that given by Graham Brown (1912*a*), in which stepping was elicited from the deafferentated tibialis anticus and gastrocnemius muscles of a cat by severance of the nerve cord, for in this particular preparation all afferent nerves in the preparation had been cut; in Sherrington's preparations, the isolation of the nerve cord from proprioceptor influence was apparently not quite so complete. These rhythms must be of central origin, as are the cleaning reflex or swimming rhythms of a frog or toad. If,

on the other hand, they represent true ambulatory movements, it is surprising that the deafferentated limb of the mammal does not participate in locomotion. Both Graham Brown and Sherrington noted that stepping only occurred in a pair of deafferentated muscles when the two opposing stimuli were very carefully balanced, any excess from one side resulted in sustained contraction of one muscle and sustained relaxation of the other; excess of stimulation from the other side reversed the picture. Since the deafferentated mammalian limb does not step but appears to show sustained extensor activity, it would seem that the balance of opposing stimuli is upset by deafferentation. In other words the balance is normally sustained by proprioceptor activity within the limb itself; in this case the gap between the central and peripheral conceptions of the ambulatory rhythm seems narrower.

The ability of a toad to walk after extensive, but not complete, deafferentation recalls a similar phenomenon in the swimming activities of a leech. So long as a few segments of *Hirudo medicinalis* retain their nerve supply and are performing active swimming movements, relatively distantly situated efferent nerves (previously severed from their own musculature) display an electrical rhythm whose frequency is identical to that of the mechanical rhythm of the intact segments (Gray, Lissmann & Pumphrey, 1938). The electrical rhythmicity in such a nerve is at once abolished by severance of the nerve cord between the recording nerve and the intact regions of the body. As in the toad, it is possible to assume that a rhythm can be maintained in all segments of the body so long as peripheral pacemakers are operative in a few intact segments.

SUMMARY

1. Deafferentation of not more than two limbs in the toad (*Bufo bufo*) only disturbs the normal diagonal pattern of ambulation in so far as a loss of muscular tone in the operated limbs introduces complications of a purely mechanical nature. In some cases the extent and smoothness of the protractor and retractor phases of the step may be modified.

2. Deafferentation of three or four limbs involves marked loss of muscular tone, and the excitability of the animal is greatly reduced. In some preparations the diagonal pattern of ambulation is preserved, in others it becomes irregular owing to a long latent period between the protraction of a forelimb and that of the contralateral hindlimb. Ambulation is ungainly, and fatigue rapidly sets in.

3. After total deafferentation of the whole body no ambulation has been observed. No ambulatory activity was observed in preparations in which all four limbs were deafferentated and in which the ventral, but not dorsal roots, of nerves V, VI, and VII were cut.

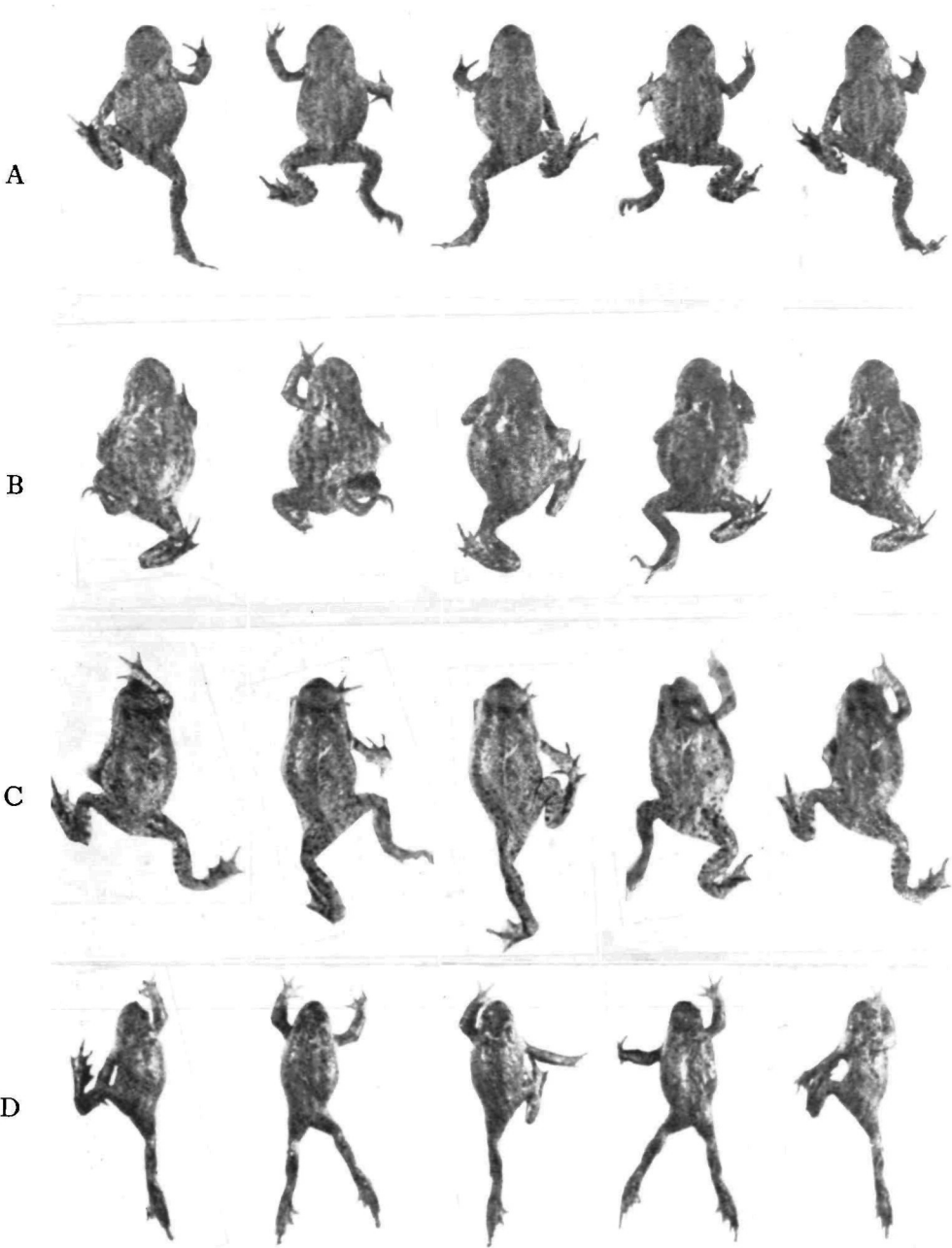
4. No positive evidence was found in favour of the view that the ambulatory rhythm originates in the central nervous system. Ambulation only occurs when the afferent and efferent nerve supply of at least one spinal segment is intact.

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EXPLANATION OF PLATE I

Corresponding phases in the step of A, intact toad; B, toad after deafferentation of both hindlimbs; C, toad after deafferentation of both forelimbs; D, toad after deafferentation of all four limbs. [This plate is republished by permission of the Royal Society.]



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