FURTHER OBSERVATIONS ON THE EFFECT OF DE-AFFERENTATION ON THE LOCOMOTORY ACTIVITY OF AMPHIBIAN LIMBS

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(With Four Text-figures)

The extent to which co-ordinated rhythmical patterns of locomotory movement are to be regarded as expressions of intrinsic activity within the central nervous system, and how far they are dependent upon the patterns of impulses received from peripheral sense organs, remains a fundamental problem in general physiology. In previous communications we have discussed this problem with reference to the ambulatory activity of Amphibia (Gray & Lissmann, 1940). We found that when the limbs and body of a toad (*Bufo bufo*) are desensitized by severance of their sensory nerves, the effect on the locomotory pattern depends to a very marked degree upon the extent to which the process of de-afferentation has been applied to the whole of the somatic musculature. In conformity with the results obtained by previous investigators (Hering, 1893; Weiss, 1936) we found that de-afferentation of either one or two limbs did not substantially affect the co-ordinated, diagonal pattern of limb movements. When three limbs were de-afferentated the ambulatory activity of the animal was substantially reduced; nevertheless, the diagonal co-ordination of the limbs was maintained whenever movement occurred. When four limbs were de-afferentated, ambulation was laboured and restricted to a few steps at a time. On the other hand, when de-afferentation was applied to all the spinal nerves II–XI (inclusive) no trace of ambulatory rhythm or of diagonal limb movements was observed, although non-ambulatory responses were obtained from the limbs by labyrinthine or optical stimulation. From these observations we were unable to derive any positive support for the theory, advocated by Weiss (1936), that the power to maintain rhythmical ambulatory limb movements is an intrinsic property of the central nervous system and fundamentally independent of the peripheral sense organs. We see no reason to amend our views, but further consideration is desirable in view of the subsequent publications by Weiss (1941 *a*, *b*) which not only stress the conception of an inherent central nervous control of ambulatory rhythms but quote our experiments in its support.

A. THE EFFECT OF COMPLETE SPINAL DE-AFFERENTATION ON AMBULATORY CO-ORDINATION

Although severance of the dorsal roots of spinal nerves II–XI on both sides of the body of a toad effectively desensitizes the whole of the normal ambulatory musculature, it is necessary to note that considerable variation exists in the number, size and position of the individual nerves, particularly of those associated with the posterior end of the spinal cord. Not only may the dorsal root of XI be enclosed for a variable distance in the same connective tissue sheath as that of X, but on some occasions a XIIth spinal nerve may be present. Both XI and XII may be very small, and the only satisfactory test of total de-afferentation in the posterior region of the body is provided by careful post-operative tests for local sensitivity.

In all our experiments de-afferentation has been effected by the following technique. The toad was anaesthetized by ether, firmly fixed in an operating dish, and the skin opened by a dorsal median incision extending from the back of the skull to the anterior end of the urostyle. The dorsal aspect of the vertebral column was exposed by dissecting away the vertebral region of the longissimus dorsi, and by detaching, in the shoulder region, muscle fibres inserted on the dorsal side of the vertebrae (rhomboideus posterior, latissimus dorsi, etc.). The neural arches were then cut on each side, with a fine dental separating saw, about 1 mm. laterally to the neural spines; the spinal cord was exposed by removing this bony roof and the membranes covering the cord. In some instances slight bleeding was unavoidable at this stage. By subjecting the cord to a fine jet of saline or Ringer solution the dorsal and ventral roots of the cauda equina could be spread out and severed without much difficulty. Least accessible are the ventral roots of the 'hypoglossal' (II) and of the anterior spinal nerves. In cases where the dorsal roots of these nerves had to be left intact, a fine, hooked insect needle was inserted into the neural canal, laterally to the cord and below the corresponding dorsal root. The ventral root was caught with the hook, gently pulled up, and cut with spring scissors. After the operation the skin was closely stitched up. The animals survived well, and in the later stages of this investigation very few casualties occurred.

Altogether we have examined the behaviour of twenty-five animals after complete de-afferentation of their spinal nerves; in twelve of these, bilateral de-afferentation applied to spinal nerves III–XI abolished all signs of ambulatory co-ordination. In the other thirteen animals de-afferentation was applied to nerves II–XII; in twelve of these no sign of ambulatory co-ordination remained, but the thirteenth animal very occasionally displayed an alternation of limb movements which might be regarded as comparable to that displayed by the toad observed by Weiss. It is however significant that in our specimen the deeper tissues of the shoulder (supplied by cranial nerve X) showed an unusual degree of sensitivity to external stimulation; further, the movements of the limbs were always accompanied by marked movements of the head. For reasons to be given elsewhere it seems not improbable that the limb movements of this animal may also be related to stimulation of labyrinthine origin. Apart from this single, somewhat ambiguous exception, all our observations emphasize the dependence of the ambulatory rhythm on the integrity of a small but finite amount of the peripheral nervous fields.

The most striking feature of these experiments is the marked effect of the presence or absence of one, relatively small, region of the ambulatory musculature whose sensory and motor nerve supply is intact. Just as complete absence of true ambulatory movements is the characteristic feature of an animal which has been de-afferentated in respect to all its spinal nerves, so a definite though variable amount of diagonal limb movement is characteristic of an animal in which one segment of the body is intact in respect to both its sensory and motor nerves.

It is not possible to give a quantitative estimate of the intermediate levels of co-ordinated behaviour which lie between the two extremes of normal ambulation and complete absence of diagonal limb movements. The intermediate levels (characteristic of varying degrees of de-afferentation) have been described qualitatively elsewhere (Gray & Lissmann, 1940) and observation of a large number of animals has indicated very clearly the progressive loss of co-ordinated ambulatory activity with advancing stages of de-afferentation. Further, it is the extent and not the site of de-afferentation which is important. The number of fibres in dorsal roots V–VII collectively appears to be approximately equal to that in the dorsal roots of VIII. There is, however, no appreciable difference in the ambulatory activity of animals which retain roots V–VII from that displayed by animals retaining VIII; both types display clearly defined periods of diagonal ambulation. As reported in our previous paper (1940), one or more intact dorsal roots are only capable of sustaining ambulation provided that their associated motor roots are intact. A decisive demonstration of this fact is provided by a toad in which all the spinal dorsal roots were severed with the exception of IX and X supplying the left hindlimb; after this operation the animal exhibited well-defined diagonal ambulation in response to tactile stimulation applied to the intact limb. The ventral roots IX and X of the left hindlimb were then severed. After this second operation tactile stimulation of the left hindlimb invariably evoked a characteristic monophasic response in each of the three limbs; no sign of ambulation could be elicited. It therefore seems difficult to avoid the conclusion that ambulation depends on the existence of sensory impulses arising in the ambulatory musculature.

B. THE EFFECT OF DE-AFFERENTATION OF A SINGLE LIMB AFTER DE-EFFERENTATION OF THE REMAINING LIMBS AND BODY

Whilst the results of de-afferentation do not provide positive evidence in support of central determination of the ambulatory rhythm they do not disprove it. It is possible that extensive de-afferentation so depresses the excitability of the central nervous system as to render it incapable of displaying its normal activity. In order to explore this possibility, use has been made of preparations in which three of the limbs and the body of a toad are immobilized by severance of their motor roots whilst the fourth limb is, at first, intact but is subsequently de-afferentated. Preparations of this type were examined as follows.

After opening the spinal canal and severing the appropriate roots the animal was mounted on a moist glass plate in such a way as to permit freedom of movement for the intact limb. Subsequent to recovery from the anaesthetic, the application of relatively weak stimuli to any of the de-efferentated limbs elicited clearly defined ambulatory movements in the intact limb, the excitability being not substantially lower than that required to elicit a response in the intact animal. This observation shows that so far as the movements of the intact limb are concerned, the excitability of the central nervous system has not been seriously reduced by cutting off all the sensory impulses which may normally arise in the muscles of the other three limbs. Typical responses are illustrated in Figs. 1–3. In Figs. 1 and 2 the right posterior limb was initially intact, whilst the other hindlimb was immobilized by de-efferentation, and the body and forelimbs were totally denervated. On applying a series of condenser discharges (Fig. 1, tracing (*a*)) of appropriate strength to the plantar surface of the left posterior limb, the intact right posterior limb responded by a clearly defined rhythm of alternating retraction and protraction; similar responses were obtained in response to tactile stimulation (Fig. 2, tracing (*a*)). The dorsal roots (VIII–XI) of the right posterior limb were then severed and the same type of stimulation reapplied; in this case, all traces of a rhythmical response disappeared and could not be elicited by altering the duration or intensity of the stimulus; the right hindlimb invariably responded monophasically by retraction and remained in this position during the remainder of the stimulation and afterwards.

Precisely similar experiments were performed with an intact forelimb—the other three being de-efferentated. Here, again, a stimulus applied in the form of a series of gentle taps to any of the three de-efferentated limbs elicited a rhythm of movements in an intact right forelimb (Fig. 3, tracing (*a*)). As soon as the sensory roots (III and IV) of the intact limb were cut, all trace of rhythmical response ceased and was replaced by a monophasic response whose nature is described in the next section.

As is well known, Graham Brown (1912*a*, *b*; 1913) induced rhythmical movements in de-afferentated mammalian muscles by bilateral stimulation of sensory nerves. We have repeatedly attempted to induce stepping in the limbs of a toad by similar means. We have, however, been completely unsuccessful, although we have not infrequently observed biphasic movements in a limb by periodic variation in the intensity of persistent stimulation applied to the cut end of its own dorsal roots or those of the contralateral limb. The nature of these responses varies very greatly with the nature and intensity of the stimulus and it is premature to assume that they have any ambulatory significance.

C. THE MONOPHASIC RESPONSES OF LIMBS ISOLATED FROM ALL PROPRIOCEPTOR STIMULATION

As stated above, the response of a de-afferentated limb to tactile stimulation applied to the body or to one of the remaining limbs is invariably monophasic provided that the body and remaining limbs are de-efferentated. No proprioceptor activity can be displayed by such a preparation; the de-afferentated limb provides a motor field of response to the extensive sensory area provided by the body and remaining limbs.

(Legend for Fig. 1)

Fig. 1. Records showing the response of a right posterior limb of a toad (*a*) before, and (*b–e*) after its de-afferentation. Stimulation was applied to the de-efferentated left posterior limb; all other spinal nerves were cut. (*a*) Response to electrical stimulation (condenser discharges) of the left plantar surface. Note the rhythmic response. (*d*) After de-afferentation of the right posterior limb a similar stimulus produces a monophasic retraction (crossed extensor). Tactile stimulation, either short or prolonged (*b*, *c*, *e* 2), produces the same monophasic response, as does electrical stimulation of the dorsal roots of the left hindlimb (*e* 1).

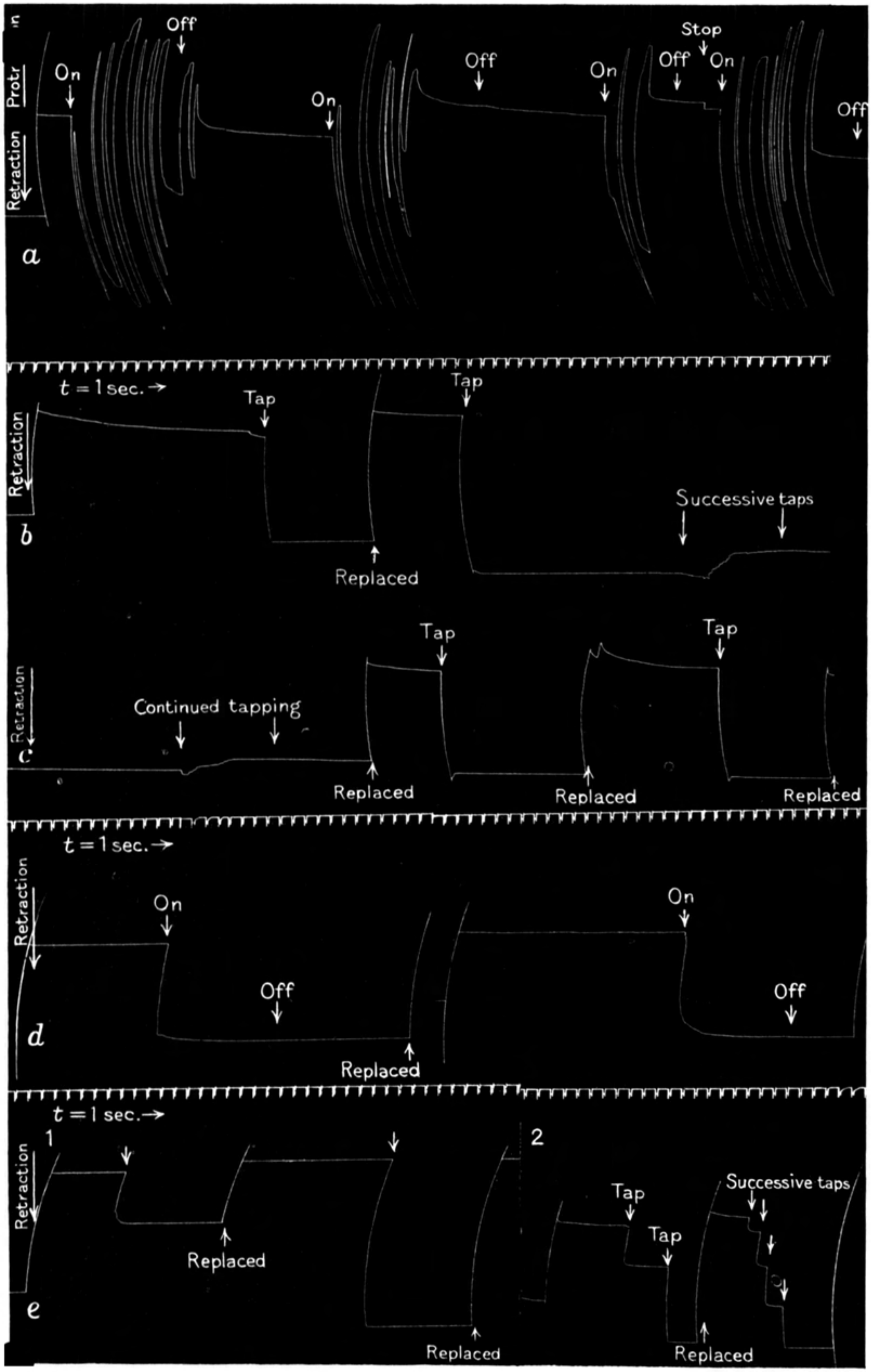
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Fig. 1.

The reflex behaviour of such preparations is extremely constant and has been examined in some detail. If the right forelimb is de-afferentated and the other three de-efferentated, the reflex picture is as follows: starting with the right forelimb passively flexed at the elbow (Fig. 4*a* 1), a gentle tap applied to the right hindlimb causes the right forelimb to be extended laterally from the body and to pronate (Fig. 4*a* 2); similar stimulation of the left hindlimb at once causes the right forelimb to

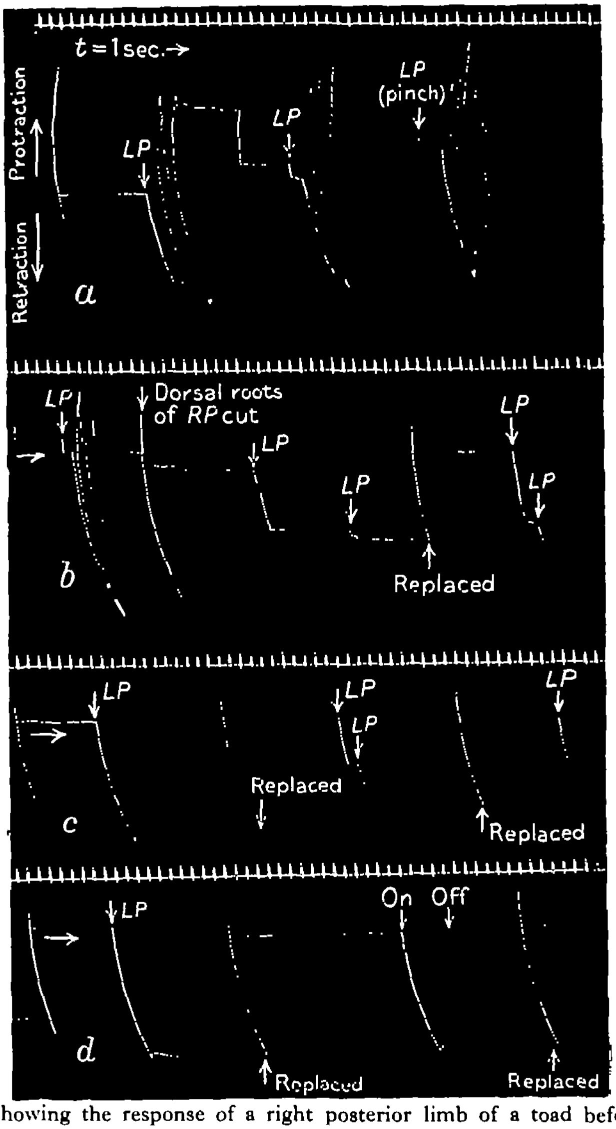

Fig. 2. Records showing the response of a right posterior limb of a toad before and after de-afferentation. Stimulation was applied to the de-efferentated left posterior limb. Forelimbs and body were denervated. (*a*) Rhythmic response of the intact limb after mechanical stimulation. (*b*) and (*c*) Identical stimulation elicits a monophasic extensor reaction after the dorsal roots of the right posterior limb are severed. (*d*) Electrical stimulation of the dorsal roots of the left hindleg produces similar monophasic reactions.

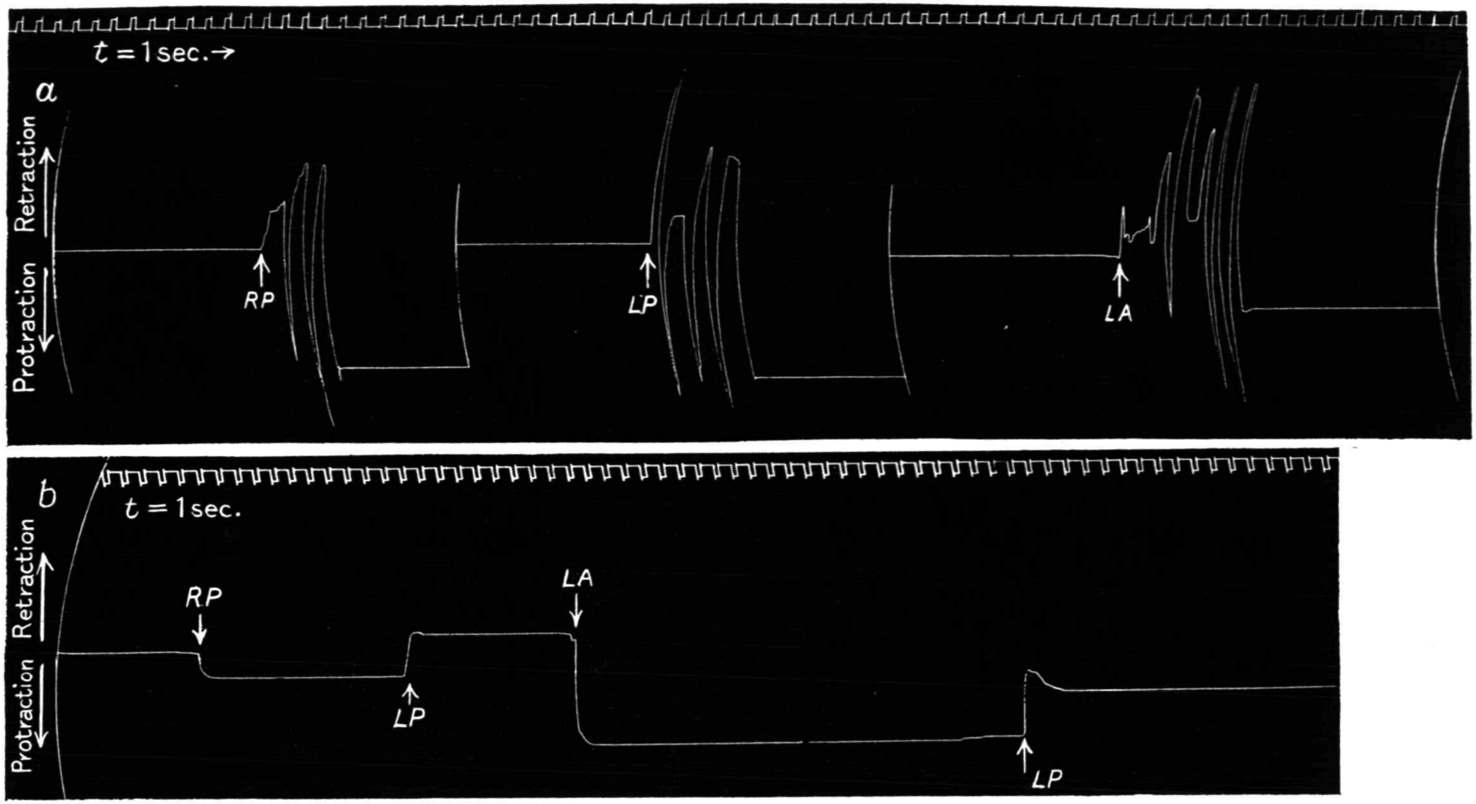

Fig. 3. Records showing the response of the right anterior limb of a toad before and after its own dorsal roots are severed. Stimulation was applied to the three remaining limbs, which were de-efferentated. The body was denervated. (*a*) Rhythmic responses before de-afferentation of the right anterior limb. (*b*) Monophasic responses after de-afferentation.

retract (Fig. 4*a* 3), whilst stimulation of the left forelimb causes the right forelimb to return to its original flexed position with the foot near the median line (Fig. 4*a* 4). It will be noted that all these monophasic responses are of such a nature as, under normal circumstances, would tend to move the animal away from the source of stimulation.

Precisely comparable responses are obtained from a de-afferentated hindlimb; in Fig. 4*b* the right hindlimb was de-afferentated whilst the motor roots were cut and the sensory roots left intact in the remaining three limbs. If the right forelimb is then stimulated the right femur is partially retracted whilst the knee remains extended (Fig. 4*b* 2), thus displacing the foot laterally to the right. If a sharp tap be applied to the left hindlimb or if this limb be seized by forceps, the right hindlimb sharply extends (Fig. 4*b* 3); this is the well-known *crossed extensor reflex*; if now the left forelimb be similarly stimulated, the right hindlimb at once flexes (*diagonal flexor reflex*) (Fig. 4*b* 4). Again, the response of each of the three limbs is such as would, if the plantar surface remained stationary relative to the ground, co-operate in a movement of the body away from the site of the stimulus.

By combining the appropriate phase of Figs 4 *a* and *b*, it is possible to reconstruct the simultaneous reactions of the right hind-, right fore- and left forelimbs in response to a stimulus applied to the left hindlimb, viz. the right hindlimb would extend (Fig. 4*b* 3), the left forelimb would be abducted and extended laterally (mirror image of Fig. 4*a* 2), and the right forelimb would be retracted (Fig. 4*a* 3). The body would be moved anteriorly and swung towards the right side, i.e. in a direction away from the stimulus which was applied posteriorly over the left side. In an exactly similar way a stimulus applied to a de-efferentated left forelimb would induce flexion of the right hindlimb (Fig. 4*b* 4), abduction of the left hindlimb (mirror image of Fig. 4*b* 2), and adduction of the right forelimb (Fig. 4*a* 4); the right fore- and left hindlimbs would co-operate to move the body to the right side away from the stimulus. This picture has been checked experimentally by using a de-efferentated left forelimb as the sensory field and the remaining three de-afferentated limbs as the motor field. A preparation of this type is shown in Fig. 4*c*; on stimulating the left forelimb, the right forelimb adducts, the right hindlimb flexes, and the left hindlimb extends. The only difference between this figure and the reconstructed pattern from Figs. 4*a* and *b* is the retraction of the thigh and extension of the ankle of the left hindlimb in Fig. 4*c*; in this case the body would move forward as well as to the right side.

It should be noted that in all the responses described in this section, the posture adopted by a limb in response to tactile stimulation is independent of its original posture before stimulation. In every case the response is monophasic and the complete absence of rhythmical ambulatory movements confirms our belief that the walking rhythm induced in an intact animal by tactile stimulation involves proprioceptor reflexes which arise either within the limbs themselves or within the muscles of the body. If tactile stimulation excites any intrinsic ambulatory rhythmicity within the central nervous system the latter is quite unable to express itself unless reinforced by stimuli of proprioceptor origin. On the other hand, the response of de-afferentated musculature to tactile stimulation is clearly of functional

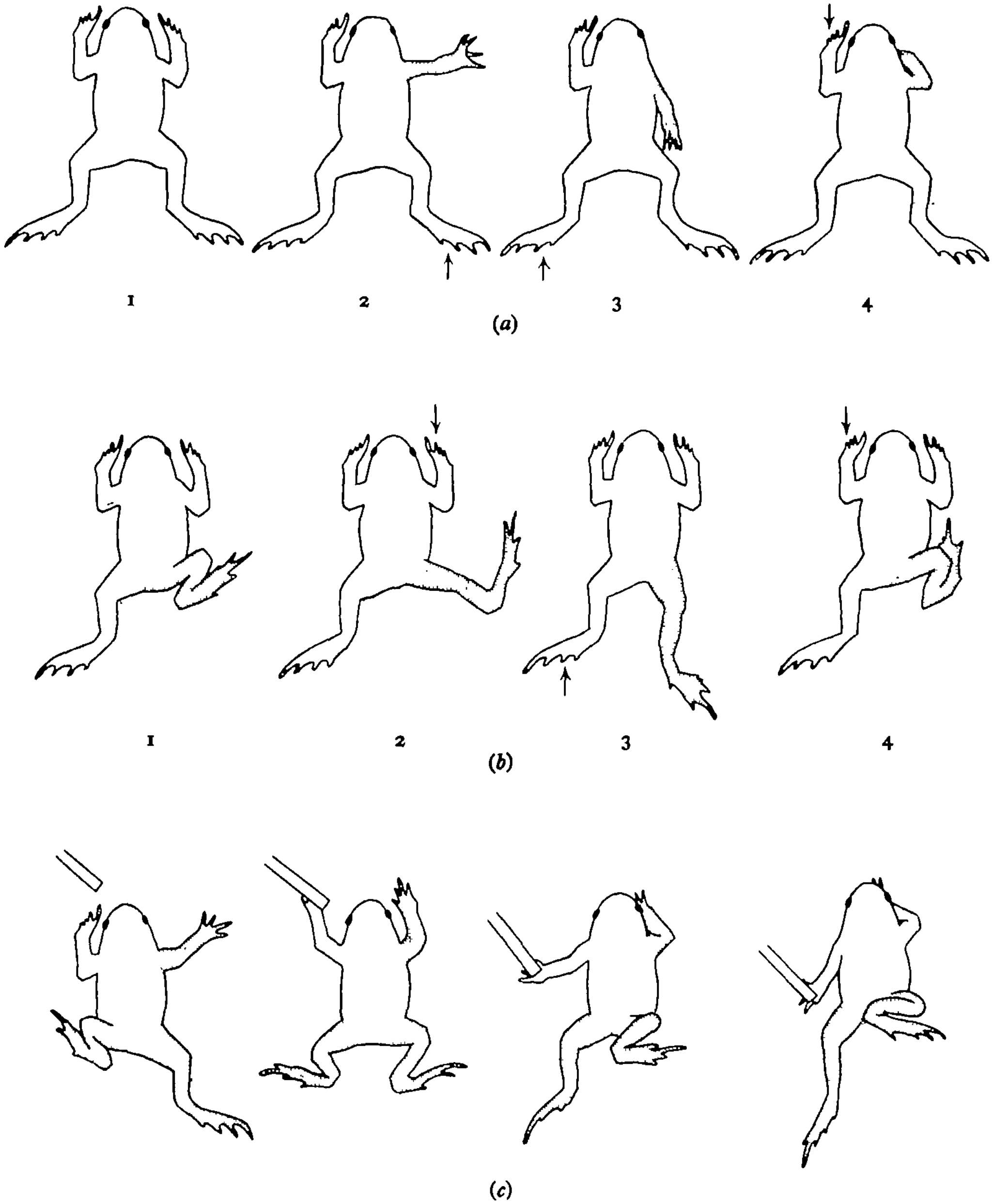

Fig. 4. Reflex postures of de-afferentated limbs. The shaded areas represent regions in which only the motor nerves are left intact, the white areas correspond to regions with only the sensory nerves intact. The arrows indicate the sites of stimulation. (*a*) Monophasic responses of the de-afferentated forelimb. (*b*) Monophasic responses of the de-afferentated hindlimb. (*c*) Monophasic responses of three de-afferentated limbs to pressure and passive retraction applied to a left, de-efferentated forelimb. The left figure in each series indicates the passive posture of the limbs before any stimulus was applied.

significance, displaying itself in an intact animal when the strength of an applied tactile stimulus is such as to elicit a response sufficiently powerful to mask the effect of proprioceptor controls.

DISCUSSION

The experiments described above provide strong evidence in support of the view that the co-ordinated limb movements which are characteristic of ambulation in the toad are dependent on the integrity of the sensory and motor elements of at least one spinal nerve. Two somewhat remarkable facts emerge. First, the ability to display normal ambulation varies with the number of segments possessing an intact nerve supply and not on the position of these segments. Secondly, the extent of the muscular field, over which the sensory equipment of a single spinal nerve can exert its influence, is surprisingly wide. As already stated, an intact XIth dorsal root on one side of the body can sustain a reasonable number of well co-ordinated steps; this nerve supplies only a very limited region of the body in the neighbourhood of the anus and according to Birge (1882) contains, in the case of the frog, only forty-one sensory fibres. As shown elsewhere (Gray, 1944), there are few if any of the somatic muscles which are not involved in the process of ambulation, but, nevertheless, it is remarkable that impulses arising in a very small fragment of the total musculature should be sufficient to control the activity of the whole. The effect of de-afferentation in the toad seems somewhat analogous to the effect of decortication in mammals where the amount rather than the site of the operation is the controlling factor. At the same time, the observations described in this paper show that a localized exteroceptive stimulus may spread over just as wide a field of de-afferentated musculature as that which participates in the ambulation of an animal whose proprioceptor endings are confined to one segment of the body. Experimental evidence of the wide muscular field which responds to a localized proprioceptor stimulus will be given in a subsequent paper.

Although the integrity of one segment of the peripheral nervous system is decisive for the maintenance of an ambulatory rhythm it must not be assumed that all rhythmical limb movements are dependent on the activity of the proprioceptor mechanism. As reported in our previous paper, de-afferentation of all the spinal nerves does not abolish the swimming rhythm in the toad. For reasons to be given later, it does not follow that the swimming rhythm is therefore of central origin, and it is not surprising that it should persist under circumstances which cannot support ambulation: an intact toad invariably swims when, by immersion in water, its limbs and body are deprived of exteroceptive stimulation: walking only occurs when contact with the ground is established by regions of the body whose sensory equipment is intact. We have had numerous instances of animals with four limbs and body de-afferentated performing well-defined synchronous swimming movements with their hindlegs when both body and limbs were in contact with the ground. As will be shown elsewhere, these movements are dependent on the presence of the membranous labyrinth; labyrinthectomy and de-afferentation together totally abolish all swimming movements.

In describing the decrease of spontaneity and smoothness of the ambulatory pattern which attends extensive de-afferentation in amphibia, Weiss (1941*b*, p. 71) states that 'neither of which defects, however, can obscure the fact that the typical co-ordination patterns of levels *O* and *S*, the former integrating muscles into limb movements and the latter integrating different limbs among one another, have not been abolished and cannot therefore be under sensory guidance'. We find it impossible to harmonize this conclusion with the experimental data. In his original paper, Weiss (1936) restricted his account of extensive de-afferentation in the toad to two animals—in one of which some of the dorsal roots of the vertebral segments remained intact; the only direct reference to the ambulatory activity of these two animals was that the animals moved occasionally 'for a few steps with the hind legs alternating as in walking'; otherwise the animals displayed 'a change from normal which was immensely greater than one would expect from simply adding up the effects of forelimb and hindlimb de-afferentation' (Weiss, 1936, p. 469) and this locomotory activity was 'extremely poor'. In our experience, this description applies quite satisfactorily to animals in which de-afferentation is not strictly complete in the sense that one at least of the spinal roots remains intact. Throughout his discussion Weiss (1941*b*) attributes considerable significance to the co-ordinated movements displayed by supernumerary limbs which have been grafted on to the body of the Axolotl. We hesitate to assess the precise significance of such movements, but would point out that it is difficult to interpret such movements in a way which differs essentially from that applicable to the movement of a de-afferentated limb; so long as one or more of the normal limbs possess an intact sensory and motor nerve supply, the supernumerary limb is free to receive reflex stimulation from peripheral sense organs as well as patterns of impulses from an inherently central source. Until the supernumerary limb can be shown to function normally in the absence of such peripheral sources of excitation, positive evidence for the existence of a central type of control must remain lacking. It must be remembered that, during ambulation, the limbs of an animal can only function satisfactorily if the muscles are free to alter in length. Isometric contraction and locomotion are mutually exclusive. If the central nervous system plays a predetermined 'score' (Weiss) the resultant muscular pattern can have no functional significance unless the environment is such as to allow the muscle to undergo an appropriate change in length.

As previously mentioned (Gray & Lissmann, 1940), the behaviour of a de-afferentated mammalian limb is somewhat different from that of an amphibian; recent work suggests, however, that there are, at least, certain features in common. Smith, Mettler & Culler (1940) have succeeded in eliciting a phasic response in the intact forelimb of the cat by bipolar stimulation of the cerebral cortex; after cutting the dorsal roots of the limb, however, the response became monophasic and the limb was sustained in full flexion. In a later paper, Mettler & Mettler (1940) reported that so long as three limbs of a cat were intact and available for locomotion, the fourth limb, if de-afferentated, exhibited phasic movements superimposed on a semiflexed posture—the movements corresponding in a general way with the locomotory pattern. They concluded that normal phasic movement is essentially due to an ability on the part of kinaesthetic impulses to alter the balance of the 'spinal discharge reserve' now in one direction and now in another. Our observations on the toad bring us into substantial agreement with this conclusion.

SUMMARY

1. An examination of a large number of toads has confirmed the conclusion that unilateral integrity of the sensory and motor nerve supply to one segment is necessary for the maintenance of the normal diagonal pattern of ambulation. The site of the intact segment is immaterial; segments of the body are equally effective as those of a limb provided the number of sensory nerve fibres is approximately the same.

2. If three limbs and the body are de-afferentated, a stimulus applied to the fourth intact limb can evoke well co-ordinated ambulation in all four limbs. If the motor roots of the fourth limb are cut, a stimulus applied to this limb invariably elicits only a monophasic response in each of the remaining three limbs. The nature of the monophasic response is always such as tends to move the body away from the source of stimulation.

3. A central nervous system totally isolated from stimulation of proprioceptor or labyrinthine origin cannot sustain co-ordinated movements of a toad either on land or in water.

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