DRUG ACTION IN GALVANOTROPIC RESPONSES.

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The responses of nemertean and gephyrean worms to galvanic currents, before and after treatment with drugs, have been taken (Crozier, 1926–27) to indicate that the primary action of strychnine in reversal effects has a locus which is not perikaryal, but is presumably synaptic. It was desirable to determine if unrelated forms would yield similar evidence.

The trough used for most of the observations on the galvanotropic responses of the animals which I have tested was made of "parawax," but one of glass served for the final experiments on *Planaria maculata*. Both were rectangular, 5 cm. wide, and 20 cm. long. Constant electric current derived from a 110 volt circuit passed through 1 cm. depth of tap water in the trough between "nonpolarizable" electrodes (*i.e.*, pads of absorbent cotton). The total effective current could be easily and gradually varied, by means of a rheostat, from 0 to 3 ma. or more, although 0.3 – 0.8 ma. (giving a current density of 0.06 – 0.16 ma. per sq. cm.) generally proved optimal; the potential was less than 5 volts with all the currents tried.

Isopods.—The effects of strychnine and of other neurophil drugs in relation to galvanotropism seemed of especial interest in arthropods, since their nervous system has revealed properties different from those characterizing other invertebrates (Crozier, 1922; Crozier and Pilz, 1924). Moreover, the anodic behavior of normal individuals of the fresh water isopod *Asellus communis* (Say) exposed to galvanic currents seemed favorable for these experiments.

If a current of moderate intensity (about 0.5 ma., or 0.1 ma. per sq. cm.) is switched on, a resting *Asellus* reacts sharply, usually starting forward if facing the anode, backward if facing the cathode, and at the same time usually turning the head end away from the cathode; if transverse to the current, the animal moves sidewise toward the anode and some evidence of U formation may appear, both anterior and posterior ends bending toward the anode, despite the creature's relative shortness and rigidity. Most often the animal will be more or less oblique

to the lines of current flow and the movements then elicited are corresponding resultants of the foregoing. Clearer orientation away from the cathode, as usual not completely toward the anode, resulted also and in the first instance from the oblique position. A swimming animal is halted on the make of an ascending current (*i.e.*, flowing postero-anteriorly) but not of a descending current. While weaker currents are simply less efficacious in prompting these responses (especially the sidling to the anode), stronger currents obscure them by causing ventral flexion of the head away from the anode and backward creeping toward the cathode. If the current is gradually increased from 0 to 0.8 ma., the anodal character of the resulting behavior remains as when the current is suddenly established, sidling (of transverse animals) towards the anode appearing with about 0.4 ma.; but sharp movements of any sort rarely occur in the absence of the make shock, which sometimes, however, acts just as touch to stimulate "death feigning" immobilization.[1]

When a few of these isopods, selected as active and showing typical anodal movements, were left immersed in strychine sulfate solutions (in tap water, 1:20,000 and 1:10,000), there was apparent in 18 hours no effect further than a possible tendency to ventral flexion of the head and to a drawing together of the legs. Also, after 3 days in the stronger solution, followed by removal to tap water, one individual was abnormally sluggish, tending to lie on its back, and showed poor coordination of the legs when prodded into creeping. Solutions of the strychnine base (1:10,000) had little effect other than to induce, after at least an hour's exposure, convulsive ventral flexures and entangling adductions of the legs from time to time; the animals acted normally between the short convulsions, running when prodded, and 2 days later, on a second trial, were even less affected. In the case of saturated solution, increased sensitivity to touch suggested itself after a few minutes only; after 1 to 18 hours, the only discernible effect was some disorganization of locomotion, with ventral flexure. In short, strychnine did not cause reversal of the reciprocal inhibition involved in normal movements, or other pronounced effects, in *Asellus*. The strychninized animals were, besides, anodic as normally, aside from the threshold being raised after several hours in the solution.

Atropine sulfate (in solution of 1:1000) failed to induce any discernible departure from the normal behavior or from the anodal galvanotropism of animals which

[1] A single individual, among the fairly large number examined, on first being taken from the aquarium in which all the stock was kept for a number of weeks, and again after 2 hours, exhibited at the make of 0.6 ma. responses of the sort described, except toward the cathode instead of the anode. When next tested, on the same day, this individual made the normal *anodal* movements in the same current, and no subsequent reversal was seen in the 2 days that it survived (while others lived weeks longer under similar conditions). This is of interest as it shows that galvanotropic reversal is possible in *Asellus*. The "death feigning" reactions, obtainable in all, seemed more frequent and of longer duration in animals which died within a few days.

were immersed in it for 48 hours, either during that period or within 3 subsequent days. As many as 42 hours in caffeine (1:10,000) yielded similar, negative results.

With nicotine (1:50,000) marked changes from normal posture and movements appeared in from 10 minutes to 3 hours. Animals immersed longer (as much as 7 hours) showed increased immobility and cramped attitudes involving now dorsal flexure of a whole animal, with or without raising and spreading of the legs, and now the reverse; at the same time, the antennæ were folded together anteriorly or spread stiffly back along the body, and the leg movements were disorganized, or if creeping could be induced by prodding from behind it was generally reversed, namely backwards, progress forward seeming impossible. These effects appeared to recur periodically, for at intervals the behavior was practically normal, including even forward creeping on stimulation. Numerous trials, made in the course of the nicotinization, failed to reveal either reversal or exaggeration of the typical anodal galvanotropic responses. When the just described nicotine effects were more evident, significant movements could not be elicited with up to two or three times the usually effective current. On the other hand, when the usual or the stronger currents were effective, the responses were anodal as before and remained such for the several days during which the nicotine effects were still observable.

The practical ineffectiveness of strychnine as concerns *Asellus* agrees with previous findings for other arthropods (Crozier and Pilz, 1924), which may also be said for the absence of any reversal of inhibition (Crozier, 1922). Although impermeability of the exoskeleton may be a factor in the failure of the isopods to show any reaction to atropine and caffeine, clearly nicotine, at least, can penetrate. Observations by Crozier (1922) indicate that in caterpillars an effect of nicotine may be to induce reversal of certain motor responses to touch, as appeared in *Asellus*. The galvanic current effects, however, threw no further light on the nature of the action of nicotine on different or related invertebrates (*cf.* Crozier, 1926–27[2]).

Planarians.—*Planaria maculata* (Leidy) not only forms a U and orients toward the cathode (Hyman and Bellamy, 1922), but, consistently with Loeb's (1918) explanation of galvanotropism (*cf.* Moore, 1922–23), shows slightly strained, differential contraction of the longitudinal and the circular muscles, by lengthening (if not already elongated) and perhaps moving forward when facing the cathode, and by stopping and shortening when facing the anode. A total current of 0.3 to 0.5 ma., established either abruptly or gradually,

[2] Crozier (1926–27), p. 405.

generally sufficed to induce essentially these same responses in *P. agilis* (Stringer) and *P. velata* (Stringer), as likewise in *P. maculata*, and increased current density did not alter their cathodic character. Most of these experiments were on *P. agilis*, but *P. maculata* also figured, and decisively, in those with strychnine.

Whether the strychnine used be a 1:10,000 solution of the base or of the sulfate, the planarians begin in 3 minutes of immersion to elongate excessively and to twist about convulsively rather than creep. While remaining longer in the solution, or for some time after removal (from 10 or more minutes immersion) into tap water, the worms exhibit reversal of inhibition in that they elongate markedly or remain extended in response to touch, which normally, applied to the anterior half, elicits at least initial retraction of the head, if not complete shortening, of an active individual (*cf.* Moore, 1918–19).

Under these conditions, the absence of coordinated locomotor activity and the persistence of coiled and spiral elongation make it difficult to determine galvanotropic orientation. Strychninized planarians fail to show the usual cathodal responses to the ordinary or to increased current strengths; the latter, indeed, tend to set off more convulsions. The lengthening and shortening responses, however, are certainly reversed though trebled current may be required, while such bending as may become evident is (reversed) toward the anode. Although the whole animals do not uncoil so far that they can be placed entirely parallel to the current, they elongate still further when the head end is nearer the anode and *vice versa*. These responses are more readily distinguished if the curvature be lessened by cutting the animals in two. Both parts, but especially a head portion extending about to the mouth (and best in *P. maculata*), then elongate or shorten in currents which bring about the opposite effects in corresponding unstrychninized parts. The drugged head portions may even be manœuvred into nearly natural positions on the substrate and into locomotion. Putting such portions into the trough together with untreated but similarly amputated pieces provides an excellent demonstration of the opposite responses to the same currents. The strychninized "heads" progress, lengthened, toward the anode as the unstrychninized "heads" stop and shorten; or as the latter progress, lengthened, toward the cathode, the former stop and

shorten. Furthermore, recovery occurs within a very few hours after limited strychninization, so that all the reversal phenomena disappear; thereupon another strychnine treatment will reinduce them. One may add that when strong ascending currents cause some lengthwise contraction in the normal whole or part planarian, it appears in a retraction of the anodal end, which also is the end most affected by descending currents; upon strychninization, on the other hand, it is the cathodal end which first is drawn in.[3] Evidently the action of strychnine and of the galvanic current cannot both be to excite the same nervous elements. Also, in view of the responses exhibited by the caudal halves of the bisected planarians, the affected structures cannot be exclusively localized in their "heads."

The specificity of the strychnine effect becomes clear by comparison with the action of nicotine and atropine, neither of which bring about similar reversal of inhibition or reversed galvanotropism in planarians. About 1 minute in a 1:50,000 solution of nicotine is enough to cause convulsive twisting, lengthening, and shortening. In a few more minutes, however, the planarians tend more and more to remain very shortened. The galvanotropic responses continue to be cathodal, although less obvious, even with increased current, on account of the animals' sluggishness and rounded-up posture. The latter effects of nicotine agree with Rico's (1926) observations on *Ascaris*.

Atropine sulfate (1:1000) acts in the first instance to excite the planarians to rapid creeping in a rather elongated state. This effect appears within 5 minutes. After about 20 minutes the animals become sluggish and, between periods of activity and occasional writhing, rest in a shortened condition which, since they are pointed at head and tail, differs from that induced by nicotine. In neither the excited nor the sluggish state does a change occur from the normal cathodal responses, except that in some cases the latter appear enhanced after the immersion in the drug solution has lasted at least 40 minutes.

Oligochætes.—Some years ago Knowlton and Moore (1917) reported reversal of inhibition by strychnine in the earthworm. When the

[3] Since it is hardly possible to judge if the direction of the (strong) currents is an influence in any dorsoventrally differential, longitudinal contraction, strychnine cannot be observed to enhance such effects, but does the contrary, as it seemed to me.

typical effects are in evidence (as after the worm has been immersed in a 1:8000 solution of strychnine sulfate for 2 hours), the normal cathodal responses to the galvanic current (Moore, 1922–23) are also reversed, though about eight times the usual current of 0.3 ma. may be required to evoke them.[4] *Allolobophora fœtida* (Savigny), especially its cephalic portion, turns and *elongates* toward the anode and *shortens* and bends from the cathode, instead of contrariwise. Meanwhile the caudal end contracts away from the anode, or, if in a transverse position, may bend in conjunction with the head toward the anode to form a U, in both respects likewise showing reversal. The anodal responses may be seen, too, in any group of segments cut from a strychninized worm.

Since a given movement of a planarian or an earthworm, whether induced by a drug or by a galvanic current, may be taken to depend upon the same nerve-muscle units, the strychnine reversal of galvanotropism in these forms bespeaks, in agreement with the conclusions based on nemerteans and gephyreans (Crozier, 1926–27), a localization of the strychnine effect primarily outside the nerve cell bodies. In fact, the results obtained with the planarians are evidence for the existence in their nervous system of structures homologous to synapses.

SUMMARY.

Under strychninization involving reversal of reciprocal inhibition of the circular and longitudinal muscles, planarians and earthworms show reversed, *i.e.*, anodal, galvanotropic responses, which neither nicotine nor atropine induce (in planarians). The results strengthen the conclusion (Crozier, 1926–27) that in causing reversal of inhibition strychnine acts primarily on central synapses or on homologous elements.

Like other arthropods, on the other hand, *Asellus* is little affected by strychnine; no reversal occurs. Caffeine and atropine are even less effective. Nicotine evokes abnormal posture and movements, perhaps reversed, but no alteration of anodal galvanotropism.

[4] Recognition for the initial demonstration of this result is due to Mr. V. C. Harnish, working in this Laboratory.

CITATIONS.

Crozier, W. J., 1922, *Biol. Bull.*, xliii, 239; 1926–27, *J. Gen. Physiol.*, x, 395.
Crozier, W. J., and Pilz, G. F., 1924, *Am. J. Physiol.*, lxix, 41.
Hyman, L. H., and Bellamy, A. W., 1922, *Biol. Bull.*, xliii, 313.
Knowlton, F. P., and Moore, A. R., 1917, *Am. J. Physiol.*, xliv, 490.
Loeb, J., 1918, Forced movements, tropisms, and animal conduct, Monographs on experimental biology, J. B. Lippincott Co., Philadelphia and London.
Moore, A. R., 1918–19, *J. Gen. Physiol.*, i, 97; 1922–23, v, 453.
Rico, J. T., 1926, *Compt. rend. Soc. biol.*, xciv, 918.