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THE ROLE OF SENSORY NERVE ENDINGS IN NEUROGENIC INFLAMMATION INDUCED IN HUMAN SKIN AND IN THE EYE AND PAW OF THE RAT

BY

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We have previously shown that some substances which produce inflammation exert their effect by a mechanism which is dependent on the presence of intact sensory nerves. Examples of such substances are capsaicin and related acyl amides, mustard oil, xylene and ω-chloroacetophenone. In experiments with animals, an inflammatory response to the administration of these substances could be prevented by chronic denervation or by capsaicin desensitization (Jancsó, 1960; Jancsó, Jancsó-Gábor & Szolcsányi, 1967).

In this paper we report experiments in which the effect of local anaesthesia on the inflammation induced by these irritants was investigated in rats and on human skin. Furthermore, inflammatory effects were investigated on denervated and normal skin areas of two subjects with peripheral nerve injuries.

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METHODS

Induction of inflammation in normal and anaesthetized eyes and paws of the rat

In the eyes and the skin of the paws of rats, inflammation was induced with irritants which, according to our previous experiments, exert their effect by the neurogenic route (Jancsó, 1960; Jancsó *et al.*, 1967). Solutions of capsaicin (50 μg/ml.), ω-chloroacetophenone (1 mg/ml.) or a saturated aqueous solution of mustard oil were instilled into the eye. The skin of the paw was painted with xylene or 5% mustard oil in liquid paraffin. The inflammatory reaction was made visible by injecting, before the irritants, Evans blue dye 50 mg/kg into the tail vein. Ten minutes after the application of the irritant the animals were killed by bleeding and the amount of dye in the conjunctivae or skin of the paws was determined quantitatively by the suramin extraction method (Jancsó-Gábor, Szolcsányi & Jancsó, 1967).

In experiments with local anaesthetics, Cornecain (3%) or Psicain-Neu (1%) were instilled two or three times into the conjunctival sac. Five minutes later the corneal reflex could no longer be elicited. Evans blue was then injected intravenously and the local anaesthetics applied once again. The local anaesthetic by itself did not cause any appreciable exudation of the dye. Five minutes later the irritant was instilled. After this instillation the animal was held firmly so that it could not scratch or wipe its eyes. To reduce the possibility that the irritant was rinsed out with the tears, the instillation of the irritant was repeated.

The dorsal skin of the paws was anaesthetized with 0.1 ml. of a 1% procaine solution, injected subcutaneously. Five minutes later the Evans blue dye was given intravenously. Procaine by itself caused some dye exudation which could be prevented by injecting the 5-hydroxytryptamine antagonist methysergide (0.75 mg/kg) subcutaneously 30 min before procaine.

Inflammatory reactions induced by irritants in the normal, anaesthetized or denervated human skin

Inflammation in the skin of the forearm was induced by capsaicin, mustard oil, histamine, compound 48/80 or thurfyl nicotinate (Trafuril).

In the experiments designed to study the effect of capsaicin a small piece of very pungent paprika was fixed with a bandage to the arm so that its inner surface was in contact with the skin. The bandage was removed 30 min later and the skin washed with soap and water. This procedure proved to be more effective than the application of an ointment prepared with capsaicin.

To test the effect of xylene, a strip of blotting paper was soaked in it and then placed on the skin for 10 min. A 10% solution of mustard oil in liquid paraffin was applied in the same manner. The effects of histamine and compound 48/80 were tested by pricking the skin two or three times through a drop of an 0.5% and 0.4% solution, respectively.

Thurfyl nicotinate (Trafuril) was applied in form of a 5% ointment, which was smeared on an area of skin about 3 cm in diameter.

The effect of local anaesthesia on the inflammatory reaction in the skin was examined in several healthy individuals. On the volar surface of the forearm 1.5 ml. of a 1% lignocaine solution was injected superficially into the skin. The flare caused by the local anaesthetic disappeared after 10 min and the skin became insensitive over a circular area with a diameter of about 2.5 cm, as assessed with needle pricks. The irritant was then applied to the anaesthetized area and simultaneuosly to a corresponding unanaesthetized area of skin on the other arm. The inflammatory reaction was not of the same intensity in all individuals and therefore for serial experiments individuals were chosen whose skin responded with a suitable intensity to the different chemical stimuli.

In subjects who had a sensory nerve injury, experiments were carried out on the area of skin which was completely insensitive to needle pricks. In the case of subject A.M., a 45-yr-old woman who 7 yr before had suffered a brachial nerve injury in an accident, the skin of the volar surface of the left forearm was completely insensitive. Subject S.V., a 20 yr old man, had an insensitive area of skin on the right upper arm and the forearm as a result of nerve injury caused by an accident 1 yr before. The innervated skin of these subjects was used as controls.

Levels of significance were calculated using Student's *t* test.

Drugs and irritants used were: *d*-benzoyl-pseudo-tropine-carbonic acid isopropylate (Psicain-Neu, Merck); *p-n*-propylamino-benzoic acid-γ-dimethylamino-β-hydroxypropylate (Cornecain, Hoechst); procaine hydrochloride; lignocaine hydrochloride (Lidocaine); methysergide bimaleate (Deseril, Sandoz;); Evans blue (Merck); suramin (Bayer 205); capsaicin, crystallized in our laboratory, or pieces of pungent paprika; mustard oil; xylene; ω-chloroacetophenone; histamine hydrochloride; compound 48/80; thurfyl nicotinate (Trafuril ointment 5%, Ciba).

RESULTS

Effect of local anaesthesia on neurogenic inflammation in the rat

When a solution of capsaicin (50 μg/ml.) was instilled into the eye of a rat after intravenous injection of Evans blue (50 mg/kg), the conjunctivae and eyelids turned blue. The instillation of the irritant evoked violent pain and blepharospasm. If the eye was completely anaesthetized, the irritant did not cause a pain reaction, but the blueing response was unchanged. Table 1 shows the amount of dye leakage from blood vessels after instillation of capsaicin in anaesthetized and non-anaesthetized eyes. The amount

of dye extracted from the eyelids was practically the same in the eyes pretreated with local anaesthetics and in the non-anaesthetized control eyes ($0.2<P<0.5$). The local anaesthetic by itself did not produce any appreciable exudation of dye. The results were the same when, instead of capsaicin, a solution of ω-chloroacetophenone or a saturated aqueous solution of mustard oil was applied as an irritant.

Local anaesthesia also failed to inhibit neurogenic inflammation in the dorsal skin of the rat paw because the inflammatory response was not altered by a previous administration of 1% procaine. The experiments on the skin were complicated by the fact that the

TABLE 1

AMOUNT OF EVANS BLUE EXTRACTED FROM THE EYELIDS AFTER CAPSAICIN INSTILLATION INTO NORMAL AND ANAESTHETIZED EYES OF RATS

Dose of Evans blue, 50 mg/kg.

Number of eyes	Local anaesthetic	Capsaicin 50 μg/ml.	Evans blue in the eye lids (μg/100 mg ± S.E.)	Ratio of dye content : capsaicin-treated/ non-treated
9	—	+	11·4±4·4	3·6
4	—	−	3·2±1·1	
7	Psicain-Neu 1%	+	9·7±2·9	2·6
4	Psicain-Neu 1%	−	3·7±1·1	
7	Cornecain 3%	+	11·0±3·1	3·1
3	Cornecain 3%	−	3·6±1·0	

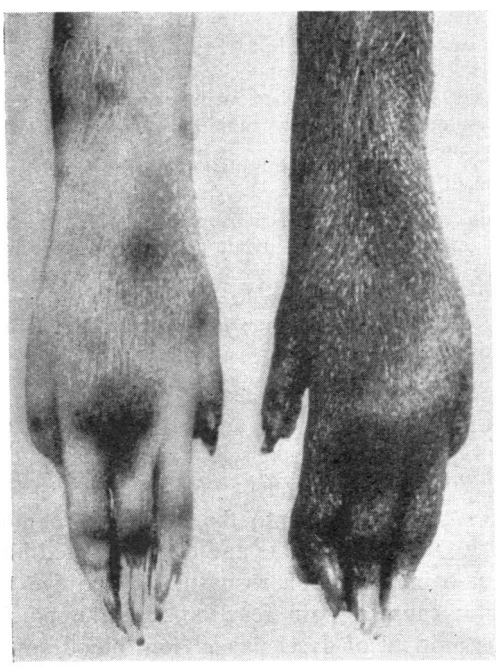

Fig. 1. Failure of local anaesthesia to decrease inflammatory response evoked by mustard oil in rat paw. 0.1 ml. of a 1% procaine solution was injected under the dorsal skin of both paws. The left paw (b) was then painted with 5% mustard oil. Evans blue dose, 50 mg/kg intravenously.

local anaesthetic itself caused a certain amount of dye exudation. This factor was completely eliminated by treating the rats 30 min before the experiment with methysergide (0.75 mg/kg subcutaneously). In the experiments illustrated in Fig. 1, 0.1 ml. of a 1% procaine solution was injected under the dorsal skin of both paws of a rat and 5 min later Evans blue 50 mg/kg was administered intravenously. Five minutes after giving the dye the left paw was painted with 5% mustard oil and 10 min later the animal was killed by bleeding. Figure 1 shows that the local anaesthetic did not reduce the reaction induced by the mustard oil: a massive oedema and intensive blue colorization developed in the entire left paw (b), whereas the right paw (a), treated with procaine only, remained uncoloured with the exception of a small patch at the site of the needle prick.

When the above experiment was performed on rats in which the saphenous nerve had been severed some days before, the innervated skin turned blue despite the local anaesthesia but the denervated skin area remained uncoloured. This result clearly demonstrates that the inflammatory response cannot be inhibited by local anaesthesia although it is neurogenic in nature.

Effect of denervation on inflammatory reactions induced in the human skin

These observations were made on completely insensitive areas of skin of the arms of two subjects with nerve injuries. A round piece of paprika was tied on to the completely insensitive volar skin area of the forearm of the subject A.M. and a similar piece of paprika was applied to the corresponding area of the innervated skin of the other arm. Capsaicin caused a burning sensation on the healthy arm, but not on the injured one. After 30 min the pieces of paprika were removed. The result of the experiment is illustrated in Fig. 2. In the normal skin the response consisted of an intensive flush and slight oedema, and a surrounding flare with irregular outlines. On the denervated skin no sign of an inflammatory reaction could be detected. A similar result was obtained after application of pieces of paprika on the arms of the subject S.V. The inflammatory reaction on the healthy (innervated) arm disappeared in the course of 2–3 hr, the flare always sooner than the contact reaction, so that finally only an erythema corresponding to the shape of the piece of paprika could be seen.

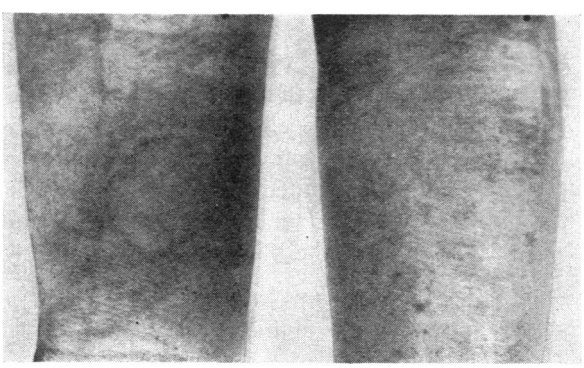

Fig. 2. Effect of capsaicin on the normal (a) and denervated (b) human skin (Subject A.M.). A piece of very pungent paprika was tied on to the skin of both forearms for 30 min.

These observations show that in human skin also capsaicin induces inflammation by a neurogenic route.

Substantially similar results were obtained with mustard oil and xylene, which are chemically quite different from capsaicin but also stimulate sensory nerves. In these experiments a strip of blotting paper, soaked in a 10% solution of mustard oil or xylene, was placed on the skin for 10 min. Figure 3 shows that in the innervated skin xylene evoked a slight central oedema surrounded by an intensive hyperaemic flare. On the denervated skin there was only minimal hyperaemia in the middle of the contact area and no flare.

Fig. 3. Effect of xylene on the normal (a) and denervated (b) human skin (Subject S.V.). A strip of blotting paper soaked in xylene was placed for 10 min on the skin.

These experiments show that the inflammatory action of xylene and mustard oil on the human skin depends chiefly on the presence of intact nerves, although a slight direct action on non-neuronal tissues might be involved, as in the rat (Jancsó, 1960; Jancsó *et al.*, 1967).

The inflammatory action of histamine, compound 48/80 and thurfyl nicotinate (Trafuril), on the other hand, is to a large extent not mediated by the neurogenic route. In the innervated skin a histamine puncture elicited the typical triple response in both subjects, whereas in the denervated skin it produced an inflammatory reaction without flare. The oedematous swelling in the denervated skin was flatter and of a pale red colour; that is, the reaction was inhibited to a certain extent, and the pressure exerted by the oedema fluid was insufficient to compress the blood vessels completely.

A similar result was obtained if the skin was pricked through a drop of a 0.4% solution of compound 48/80 instead of histamine.

Thurfyl nicotinate elicited on the healthy arm of subject A.M. an oedematous swelling which was surrounded by a hyperaemic flare. On the denervated skin only an intense hyperaemia appeared at the site of treatment, without oedema or flare (Fig. 4). The

reaction in the innervated skin of the subject S.V. was the same as in subject A.M., however, on the denervated skin there was also an oedematous swelling but without any flare.

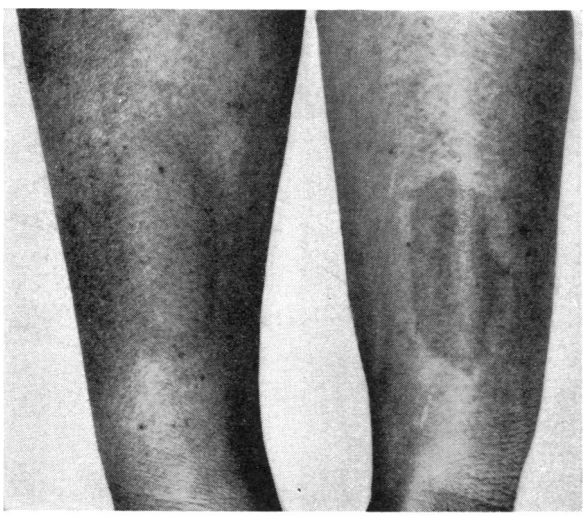

Fig. 4. Effect of thurfyl nicotinate (Trafuril) on the normal (a) and denervated (b) skin (Subject A.M.).

Effect of local anaesthesia on inflammatory reactions induced in the human skin

The effect of local anaesthesia on neurogenic inflammatory reactions was examined on the volar surfaces of the forearms of several normal subjects.

Local anaesthesia abolished the development of the flare, but in the area of direct contact of the skin with the irritant there was the usual vascular reaction, uniform redness and oedema. The result of a typical experiment is illustrated in Fig. 5, which shows that the intense redness and slight oedema induced at the site of contact by a piece of paprika was not prevented by lignocaine anaesthesia. The only effect of local anaesthesia was to prevent the development of the flare. A similar result was obtained if, instead of paprika, a strip of blotting paper soaked in mustard oil or xylene was applied to the skin.

DISCUSSION

The experiments described in this paper show that local anaesthesia does not diminish inflammatory reactions induced in rats by capsaicin, mustard oil, ω-chloroacetophenone or xylene.

The effects of local anaesthetics on the development of inflammation have been studied for a long time, but the experimental findings have usually been contradictory. As early as 1910 Bruce and later Ricker & Regendanz (1921) reported that mustard oil chemosis

in the conjunctiva of the rabbit was inhibited by cocaine or alypin anaesthesia. Shimura (1924) could not confirm this, but more recently Fearn, Karády & West (1965) reported that cocaine inhibited the increased vascular permeability produced by xylene.

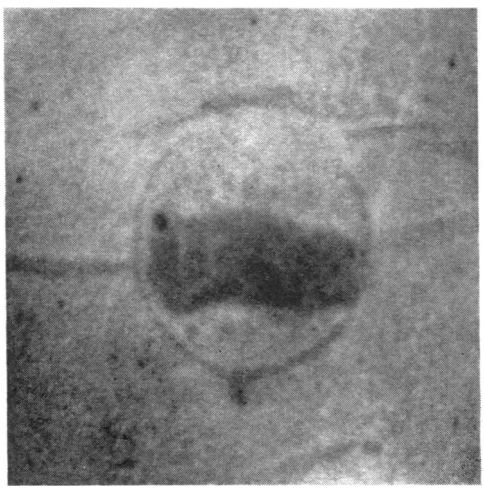

Fig. 5. Effect of capsaicin on the anaesthetized innervated human skin. A long piece of paprika was tied on the forearm for 30 min. The faint marks are caused by the bandage.

Heite & Höland (1956) found that procaine inhibited to a certain extent the oedema induced by formalin in the rat paw. They did not attribute this effect to the local anaesthetic action but to the " membrane clogging " effect of the local anaesthetics. According to Fleckenstein (1956) local anaesthetics inhibit the effect of depolarizing agents not only on nerve and muscle membranes, but probably also on the membranes of other cells. The latter, he thinks, is the reason why local anaesthetics inhibit chemosis by mustard oil.

With our method for measuring changes in vascular permeability, we could not confirm previous reports that local anaesthesia prevents the inflammatory reactions induced by mustard oil or xylene. On the contrary, no significant difference could be detected between the actions of the irritants on anaesthetized and normal conjunctivae or on anaesthetized and non-anaesthetized areas of the skin of the paws of rats.

The experiments on human skin confirmed in every respect the results obtained in rats. Local anaesthetics did not inhibit inflammatory reactions induced by capsaicin, xylene or mustard oil. They did, however, prevent the flare elicited by the axon reflex. The inflammatory effects of histamine, compound 48/80 and thurfyl nicotinate (Trafuril) are largely independent of the presence of nerves, but neural factors do play some part in them. After the application of these agents to a denervated skin the flare fails to develop and the oedematous reaction at the site of contact is also reduced. We suggest that this is because of the absence of the neurogenic component. In the case of thurfyl nicotinate (Trafuril) we failed to confirm the finding of Crockford, Hellon & Heyman (1962) that chronic denervation of the skin completely inhibits the effect of this substance,

because in our experiments the inflammatory reaction was also seen in the denervated skin although it was not quite as pronounced as in the innervated skin.

The finding that neurogenic inflammation induced by capsaicin and other substances cannot be inhibited by local anaesthetics seems at first to be paradoxical. Local anaesthetics abolish conduction in nerve fibres and thus the results cannot be explained on the basis of the axon reflex theory as proposed by Bruce (1910), Bayliss (1923), Lewis (1927) and Chapman & Goodell (1964). On the other hand, the electrophysiological investigations of Gray (1959) and others have shown that local anaesthetics, in concentrations at which they paralyse the conduction of impulses completely, do not affect sensory receptors. Thus, in our opinion, it seems reasonable to assume that the neurogenic inflammatory agents release the substance responsible for the enhancement of the permeability of blood vessels from sensory receptors, and the axon reflex is not necessary in the induction of neurogenic inflammation (Fig. 6B). The axon reflex, however, is needed for the flare component of the response in the human skin (Fig. 6A). The permeability-enhancing neurohumour seems to be released from the sensory nerve endings at both terminals of the axon reflex arc and to gain access to the vessels by diffusion. This is supported by the fact that the vasodilatation elicited by antidromic stimulation of a sensory nerve—in contrast to the vasodilatation evoked by stimulation of autonomic nerves—ensues only after a prolonged latency, develops slowly and may persist for 10 min after the stimulation (Uvnäs, 1954).

According to previous experiments with animals (Jancsó, 1960; Jancsó *et al.*, 1967) local or parenteral pretreatment with capsaicin prevents the pain producing and inflammation inducing effect of neurogenically acting phlogogenic agents (for example, capsaicin, mustard oil, xylene) just as denervation does. Similar results were also obtained by local desensitization of the human skin (Jancsó, 1960). In such cases the synthesis or release of the mediator is inhibited and thus capsaicin-desensitization blocks the inflammation induced by capsaicin and other irritants at the site of contact (Fig. 6C).

The inflammation elicited by antidromic electrical stimulation of the sensory nerve can also be almost completely inhibited by capsaicin desensitization (Jancsó *et al.*, 1967).

It may therefore be assumed that the permeability enhancing neurohumour is released from nerve endings which can be stimulated and subsequently desensitized with capsaicin. After capsaicin desensitization, the action potentials can be elicited in sensory nerves by tactile and mechanical stimuli and, according to Green & Tregear (1964) by cold stimuli as well. Thus it seems reasonable to assume that the neurohumour is released from the chemosensitive pain nerve endings and perhaps from the heat-sensitive nerve endings.

Our hypothesis that the chemosensitive nerve endings release a neurohumour when they are activated would afford a functional explanation of more recent results on the ultrastructure of the receptor organs. The corial and intraepithelial free nerve endings, frequently contain small vesicles and granules as well as accumulated mitochondria (Cauna, 1966). They therefore closely resemble terminals of efferent nerves from which the release of transmitters is well established.

SUMMARY

1. The neurogenic inflammation induced by capsaicin, mustard oil, xylene or ω-chloroacetophenone in the eye or paw of the rat is not inhibited by local anaesthesia,

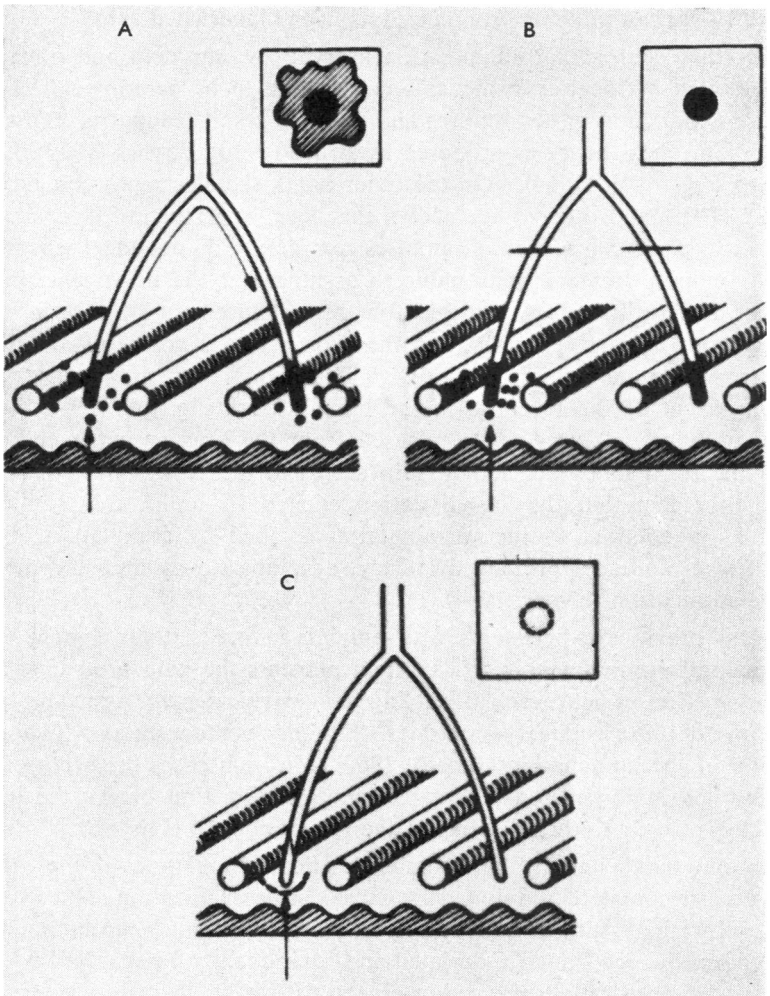

Fig. 6. Hypothesis of the neurogenic inflammatory mechanism. A, Intact skin ; B, anaesthetized skin ; C, capsaicin-desensitized skin. In the small inset squares the corresponding cutaneous reactions of the human skin to irritants are indicated. For full explanation see text.

but, as previously shown, can be prevented by sensory denervation or capsaicin desensitization.

2. In denervated human skin it could have been proved that capsaicin exerts its effect entirely, and mustard oil or xylene predominantly by a neurogenic route. The inflammatory effects of histamine, compound 48/80 and thurfyl nicotinate (Trafuril) are largely independent of a neural mechanism but neural factors contribute to it.

3. In human skin local anaesthesia prevents the flare component of the inflammatory reaction induced by neurogenically acting irritants, without affecting the direct effect on the site of contact.

4. The hypothesis is put forward that in neurogenic inflammation a neurohumour which increases permeability is released from sensory nerve terminals which cannot be blocked by local anaesthesia. It is suggested that in human skin release of the neurohumour occurs at both terminals of the axon reflex arc.

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