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Electrophysiological Studies of the Sciatic Nerves in *Mycobacterium leprae* Foot Pad-injected Rats[1]

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Although involvement of nerve trunks is the most striking feature in leprosy, little is known about this neuropathy because of the technical limitations of biopsy and electrophysiology in man. Relatively few attempts have been made to provide an experimental model ([1-3, 19, 21, 25, 26]) which could be studied extensively as in diabetes ([22]) or dying back polyneuropathies ([14, 24]). The slow development of nerve lesions which generally are not clinically apparent in inoculated laboratory animals ([17]) can explain the poor interest in the development of such an experimental model.

Our study is concerned with the development of such a model in non-immunosuppressed Wistar rats. This animal was chosen to ensure precise conduction velocity measurements because of the sciatic nerve length. Segmental motor and sensory conduction velocities were calculated at several dates after hind foot pad inoculation with *Mycobacterium leprae* in young animals. Unfortunately, nerve impairment was detectable only at 21 months after inoculation. Nevertheless, our experiments point out the importance of electrophysiological recordings, easy to obtain and to repeat in laboratory animals, to detect experimental leprosy neuropathy. The results show that it is possible to develop such a neuropathy in rats, providing a model for paucibacillary leprosy. On the other hand, the use of this model for extensive therapeutic research would seem to extremely time consuming.

MATERIALS AND METHODS

Animals. Female non-immunosuppressed Wistar rats, six weeks old and weighing 120–140 g were inoculated. The animals were housed in plastic cages with floors covered with a deep layer of sawdust to avoid pressure neuropathy of the hind foot ([5]). All of the rats were fed a standard cube diet and water *ad libitum*. The animal room was kept at a constant 25°C. For recordings and sacrifice, animals were anesthetized with sodium pentobarbital (40 mg/kg).

***M. leprae* inoculation.** Sixty animals were divided into three matched groups of 20 each: a) Group I was inoculated in the left foot pad with 7×10^3 acid-fast bacilli (AFB); b) Group II was inoculated with 7×10^1 AFB; and c) Group III (controls) received only the vehicle solution. The *M. leprae* strain was provided by the laboratory of Professor Stefaan Pattyn, Antwerp, Belgium. This strain (No. 17547) was originally isolated from a biopsy of a lepromatous dapsone (DDS)-resistant patient ([10]) and had been maintained by serial passages every seven months in mice since 1969. Counting of the AFB was performed every two months, and negative growth in Löwenstein medium was obtained at each passage.

The preparation of the *M. leprae* solution was done according to the method previously described by Shepard ([16]). After counting and correct diluting in Hanks' solution ([18]), 0.1 ml of inoculum was injected into the left hind foot pad of the rats into the soft tissue using a 1 ml tuberculin syringe and a short (½ inch) 26-gauge needle. Simultaneously, Swiss mice were inoculated in both hind foot pads in order to assess the viability of the *M. leprae*. The rats and mice were sacrificed at different dates. The left hind foot pads were surgically excised, as well as the rat tibial nerve from the ankle to the knee. These tissues were treated as described by Shepard ([16]) in order to count microscopically the number of *M. leprae* in each tissue ([18]).

Electrophysiological recordings. Serial measurements of both motor and sensory

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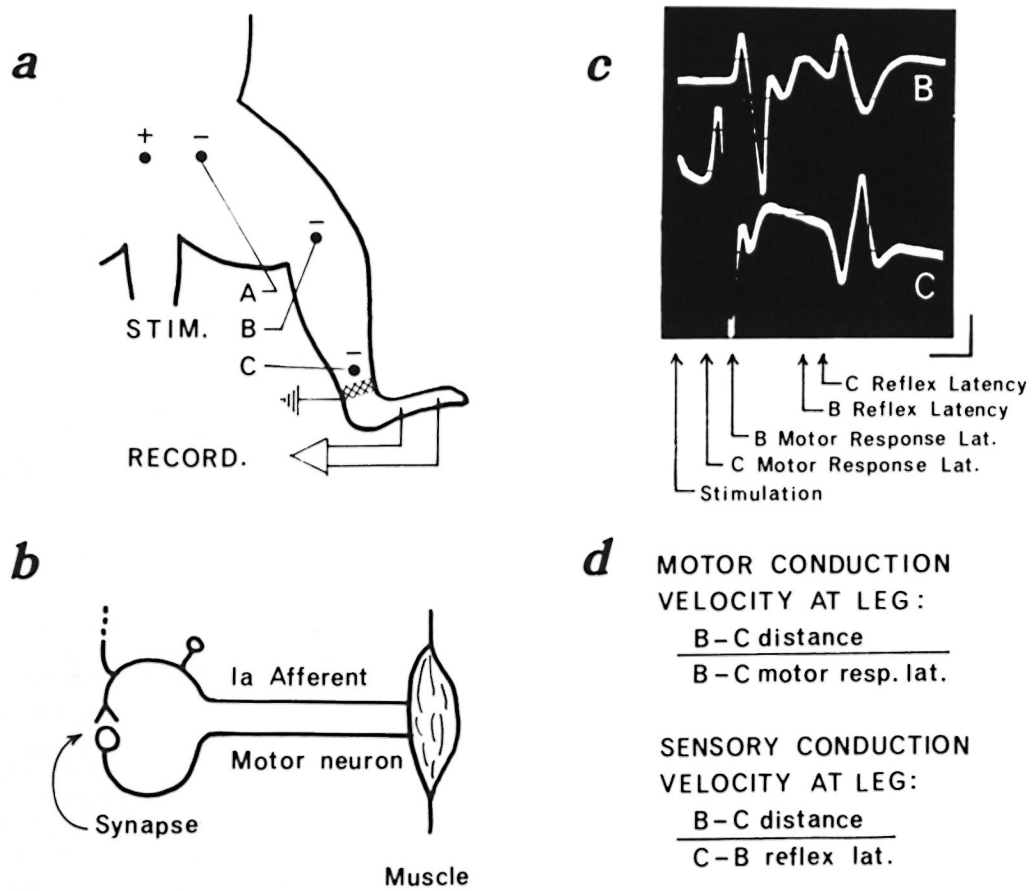

THE FIGURE. Method used to calculate motor and sensory conduction velocities of the rat sciatic nerve.

a) Sciatic nerve was stimulated by means of needles at three levels of the hind limb (A, B, C) with the cathode (anode on the back), and the evoked muscular potentials of the plantar muscles were recorded by means of sharp needles.

b) Stimulation of the sciatic nerve elicited a depolarization both of motor neurons and of proprioceptive Ia afferent neurons supporting the monosynaptic reflex.

c) Therefore, two evoked muscle potentials were recorded, with different latencies. The short latency one resulted from the motor neuron stimulation, and the long latency one resulted from the monosynaptic pathway. The respective latencies of these responses differed with the level of the stimulation (here B and C).

d) After measuring of the distance between stimulation points and the differences in latency, the conduction velocities were calculated as shown.

conduction velocities in the tibial nerve fibers supplying the muscles of the plantar hind foot were made according to the method described by Fullerton [4] in the guinea pig. This method of determining motor [4] and sensory [23] conduction velocities in the rat is simple and useful in the study of experimental neuropathies [5, 23]. To prevent anesthesia heat loss during the experiment, the animals were placed on cotton wool and heat was provided from a lamp. With this procedure, rectal temperature was kept between 35–38°C as in the Fullerton study [4].

Electromyograph (Emg) records were taken with two, sharp, monopolar needle electrodes from the foot muscles. Stimulating cathodes were needles inserted near the sciatic and the tibial nerves through the skin a) at the sciatic notch, b) at the knee, and c) at the ankle. The stimulating anode was a clip attached to the skin over the back (The Figure a). Rectangular pulses (0.1 ms;

TABLE 1. *Number of acid-fast bacilli (AFB) in foot pads of mice and rats and nerves of rats after inoculation with* M. leprae.

Foot pad inoculum size	Tissue	Days after inoculation									
		60	90	120	150	180	210	240	270	420	630
7×10^3	mouse foot pad	$<10^4$	$<10^4$	$<10^4$	9×10^4	9×10^4	2×10^4	8×10^5			
		$<10^4$	$<10^4$	$<10^4$	1×10^5	2×10^5	5×10^4	9×10^5			
		$<10^4$	2×10^4	$<10^4$	2×10^5	2×10^5	1×10^5	2×10^6			
		$<10^4$	2×10^4	2×10^4	2×10^5	3×10^5	4×10^5	2×10^6			
		$<10^4$	4×10^4	2×10^4	2×10^5	3×10^5	6×10^5				
		5×10^4	7×10^4	2×10^4	2×10^5	4×10^5	7×10^5				
	rat foot pad								$<10^4$	2×10^5	3×10^5
									9×10^4	1×10^6	4×10^5
	rat nerves									$<10^4$	$<10^4$
										$<10^4$	$<10^4$
7×10^1	mouse foot pad						4×10^4	1×10^5	1×10^5		
							1×10^5	1×10^5	1×10^5		
							1×10^5	2×10^5	3×10^5		
	rat foot pad										$<10^4$
											7×10^4
	rat nerves										$<10^4$
											$<10^4$

0.3 Hz) were delivered by a constant current stimulator. Intensity was adjusted to observe both the motor response and the H reflex according to Meinck ([9]) and Stanley ([23]). Muscle potentials were amplified (×1000, 7 Hz–10 KHz) and displayed on a cathode ray oscilloscope. The latencies of the potentials were measured from the onset of the stimulus to the onset of the first deflection of each evoked potential from the baseline (The Figure c). The distance between stimulating cathodes was measured over the skin after full extension of the limb. Conduction velocities were calculated as indicated in The Figure d. Using this procedure, our results in the control rats were comparable to those of others ([4, 5, 9, 23]).

Statistical analysis. Statistical analysis was carried out on the data obtained from each group. Assuming near normal distribution of the results, the Student *t* test was used for intergroup comparisons. Values are expressed as mean ± one standard deviation.

RESULTS

Table 1 gives the multiplication of *M. leprae* in the foot pads of the rats and the mice at several time intervals and the absence of detectable multiplication in the rat nerves. Electrophysiological recordings were performed 7, 14, and 21 months after inoculation. Motor and sensory conduction velocities were not modified 7 and 14 months after inoculation. Table 2 gives the results of the recordings performed 21 months after inoculation. The sensory as well as the motor conduction velocities were significantly decreased ($p < 0.01$) in the left leg (inoculation side) for Group I mice which were inoculated with 7×10^3 bacilli. The same group also showed a significant decrease ($p < 0.01$) of the motor conduction velocity of the right leg. Group II mice (inoculated with 7×10^1 bacilli) showed a significant decrease ($p < 0.05$) of the motor conduction of both legs. No differences were observed in the sensory or motor conduction velocities of the hip part of the sciatic nerve in either of the groups.

DISCUSSION

This study was carried out to explore the possibility of electrophysiologically detecting a latent experimental neuropathy during the multiplication of *M. leprae* in the foot pads of Wistar rats.

***M. leprae* multiplication in the rat foot pad.** The strain of *M. leprae* used in this study multiplied in mouse foot pads as quickly as reported by Levy ([7]). This multiplication was slower in the Wistar rat foot pad. It did not exceed 4×10^5 AFB 21 months after inoculation, while no AFB were detectable in the sciatic nerves homogenates. This slow multiplication of AFB in the rat foot pad could possibly explain the late detection of electrophysiological abnormalities in the sciatic nerve. But abnormalities in the conduction velocity of mye-

TABLE 2. *Sciatic nerve motor and sensory conduction velocities (meters per second) 21 months after inoculation.*

Sciatic nerve	Group I Inoculation = 7×10^3 AFB		Group II Inoculation = 7×10^1 AFB		Group III Controls	
	No. animals	Mean ± one S.D.	No. animals	Mean ± one S.D.	No. animals	Mean ± one S.D.
Motor conduction velocities						
Right leg	10	43.1 ± 11.1[a]	10	46.9 ± 16.9[b]	10	61.4 ± 16.6
Right thigh	8	74.9 ± 23.1	10	88.9 ± 27.0	10	73.7 ± 20.0
Left leg	9	40.9 ± 6.7[a]	10	37.8 ± 15.5[a]	11	57.4 ± 13.9
Left thigh	9	69.8 ± 9.9	10	72.3 ± 37.6	9	64.8 ± 17.1
Sensory conduction velocities						
Right leg	9	55.6 ± 13.7	10	52.7 ± 31.6	10	64.8 ± 19.1
Right thigh	9	55.4 ± 10.5	10	77.9 ± 40.0	11	62.8 ± 15.1
Left leg	9	46.0 ± 11.9[a]	10	57.1 ± 24.4	11	65.4 ± 19.5
Left thigh	8	63.6 ± 23.4	10	64.7 ± 32.7	11	63.6 ± 17.3

[a] Significantly less than controls, Student's t test; $p < 0.01$.
[b] Significantly less than controls, Student's t test; $p < 0.05$.

linated fibers in the sciatic nerves of inoculated mice were previously detected by Shetty, *et al.* ([21]) only 18 months after inoculation, despite the faster AFB multiplication. On the other hand, the absence of AFB in the rat sciatic nerve is not surprising when we consider their absence in the dermal nerves of mice until 14 months after inoculation ([11]).

Validity of electrophysiological techniques. The nerve fibers innervating the plantar muscles were stimulated at the sciatic notch, the knee, and the ankle in order to calculate the segmental motor and sensory conduction velocities of the sciatic nerve. The stimulus intensity was adjusted to record two muscular responses (The Figure c). The short latency one (named M wave) is due to the stimulation of the motor fibers. These latencies were used to calculate the motor conduction velocities (The Figure d). The long latency response (named H reflex[6]) results from the stimulation of the Ia proprioceptive fibers innervating the muscle and supporting the monosynaptic reflex (muscle spindle—Ia afferent sensory fibers—spinal cord synapse—motor efferent fibers—muscle fibers). In man, it is known that direct stimulation of the Ia fibers elicits an H reflex ([8]). In the rat, the long latency response of the plantar muscles is considered as an H reflex on the basis of the following observations ([9, 23]): a) latency decreases with more proximal stimulation; b) the recruitment of the response is identical to that of the H soleus reflex in man when the stimulation intensity is modified; and c) the response is abolished by transection of the dorsal roots. Therefore, the latencies of the late muscular response was used to calculate the sensory conduction of the Ia afferent fibers (The Figure d) which are the largest myelinated fibers of the peripheral nerves.

Slowing of the sciatic nerve conduction velocities. This study gave the following three results: a) Conduction velocities were never modified at the level of the thigh. b) Leg motor conduction velocities were bilaterally decreased in all animals. c) Sensory conduction velocities were decreased in the inoculated side only in animals inoculated with 7×10^3 *M. leprae.*

The constancy of the normal values of the conduction velocities in the proximal part of the hind limbs strongly suggests that the nerve impairment in this model affects the colder part of the limb as in human leprosy ([13]). The impairment of the motor conduction velocity in both legs could be explained either by a dissemination of the AFB ([11]) resulting in an early, distal, electrophysiologically detectable neuropathy as in humans ([20]), or by an autoimmune phenomena as suggested by Crawford, *et al.* ([3]) which leads to the possibility of the nerve invasion by inflammatory cells, soluble antigens, and edema ([12]). In the latter case, an entrapment

neuropathy could develop above the tarsal tunnel, resulting in a motor conduction velocity decrease ([5]) in both legs. This concept of entrapment neuropathy is consistent with the early impairment of unmyelinated fibers ([19]) (four months after inoculation) and later impairment of the larger myelinated fibers observed by Vidyasagar, *et al.* ([25]) in mouse sciatic nerves.

The variation between the sensory findings in the two limbs might be due to a direct involvement of the muscle spindle and the proprioceptive fibers at the inoculated site at an earlier stage from that in the other limbs. Impairment of the H reflex in leprosy patients, resulting from a reduction of proprioceptive fibers, has been previously observed ([15]).

In summary, AFB inoculated Wistar rats develop a paucibacillary sensori-motor neuropathy similar to borderline tuberculoid (BT) or tuberculoid (TT) leprosy patients. These abnormalities could be detected by means of electrophysiological technics which are easy to apply and to repeat, but they were observed only as late as 21 months after inoculation. Further histological and fiber teasing studies are needed to correlate with these electrophysiological abnormalities. The interest of this experimental model for therapeutic research is open to question.

SUMMARY

This study tested the possibility of developing an experimental model of neuropathy in female Wistar rats inoculated with *Mycobacterium leprae* in the foot pad and assessed by repeated electrophysiological methods. *M. leprae* multiplied in the rats but considerably less than in simultaneously inoculated mice. No acid-fast bacilli were found in nerves. Motor and sensory conduction velocities remained normal at the thigh level of the sciatic nerve. At the leg, they decreased significantly bilaterally for motor conduction and in the inoculated side for sensory conduction at 21 months after inoculation. These results suggest the possibility of developing an experimental model of leprosy neuropathy which might be useful for therapeutic research. Further histopathological studies are needed to assess this paucibacillary model.

RESUMEN

Se estudió la posibilidad de desarrollar un modelo experimental de neuropatía en ratas Wistar hembras inoculadas con *Mycobacterium leprae* en el cojinete plantar. Las lesiones se evaluaron por métodos electrofisiológicos. El *M. leprae* se multiplicó considerablemente menos en las ratas que en los ratones inoculados en forma simultánea. No se observaron bacilos ácido resistentes en los nervios. Veintiún meses postinoculación, las velocidades de conducción motora y sensitiva del nervio sciático permanecieron normales a nivel femoral. En la pata, disminuyó significativamente su conducción motora bilateral y en el lado inoculado disminuyó su conducción sensorial. Estos resultados sugieren la posibilidad de desarrollar un modelo experimental de neuropatía leprosa que podría ser de utilidad en investigaciones terapéuticas. Se requieren más estudios histopatológicos para evaluar mejor este modelo paucibacilar.

RÉSUMÉ

Cette étude a visé à étudier la possibilité de développer un modèle expérimental des neuropathies chez des rats Wistar femelles inoculés avec *Mycobacterium leprae* au niveau du coussinet plantaire; on a eu recours pour évaluer les troubles neuropathiques à des méthodes électrophysiologiques répétées. *M. leprae* s'est multiplié chez les rats, mais beaucoup moins que chez des souris qui avaient été inoculées au même moment. Aucun bacille acido-résistant n'a été trouvé au niveau des nerfs. Les vitesses de conduction motrice et sensorielle sont restées normales dans le nerf sciatique au niveau de la cuisse. Aux pattes, 21 mois après l'inoculation, ces vitesses ont décru significativement des deux côtés en ce qui concerne la conduction motrice, et du côté inoculé pour ce qui est de la conduction sensorielle. Ces résultats suggèrent qu'il est possible de développer un modèle expérimental des neuropathies lépreuses, qui pourrait être utile pour la recherche thérapeutique. Des études histopathologiques supplémentaires sont requises pour évaluer ce modèle pauci-bacillaire.

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