NERVE CONDUCTION STUDY TECHNIQUE IN THE ARMADILLO

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SUMMARY: The authors present a technique to perform nerve conduction study in armadillos *(D. novemcinctus)* and suggest that 66.17 m/s could be the mean normal nerve conduction velocity for the sciatic nerve, a parameter that could be used for assessment of the peripheral nervous system in this animal, considered the choice for experimental development of leprosy neuropathy and even in other peripheral neuropathies.

Key-words: *Armadillo; Nerve Conduction Study; Leprosy; Peripheral Neuropathies .*

1. INTRODUCTION

For a long time M. leprae, the causative agent of leprosy, could not be cultivated experimentally. More recently, some laboratory animals has been successfully used to produce *M. leprae* growth to some extent, such as the timectomized mouse, the nude mouse, among others. The growth of *M. leprae* in these animals showed to be limited although enough to allow the performance of many studies, including the mouse foot-pad model of SHEPARD (1960) for drug resistance determination.

STORRS (1.971) has established the armadillo as an experimental model for leprosy. Further research in this field has shown that, indeed, armadillos, namely *Dasypus novemcinctus,* seem to be the best available animal to reproduce leprosy and provide research laboratories with massive quantities of live bacilli for many purposes. Since then, D*. novemcinctus* has been considered the choice animal for studies in experimental leprosy. For this reason, an increasing interest has rised in regards to different ecological, anatomical and biological aspects of this animal (OPROMOLLA, 1980) in order to help researchers to understand its biological behavior. Nerve damage is one of the most challenging aspects of leprosy and the possibility of development of such damage in armadillos could be of a great value to better understand this issue. Many articles have demonstrated that armadillos inoculated withM. *lepraecan* develop nerve damage (MEHTTA, 1984). The production of a localized granuloma in a specific peripheral nerve of the lower limb of the armadillo, although not yet obtained and difficult due to the characteristic immunology of this animal, could be also another important contribution to stablish a model of entrapment neuropathy caused by leprosy and other diseases (VIRMOND, 1994). Nerve conduction velocity is an important parameter for the study of peripheral nerve damage and the determination of the normal range of this parameter in the armadillo, as well as the technique to achieve this, is necessary for this kind of research. This is the purpose of this work.

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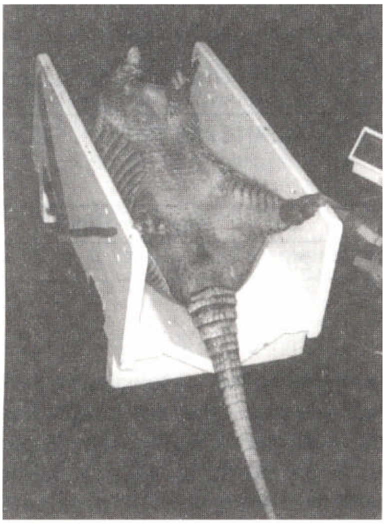
2. MATERIAL AND METHOD

Ten healthy adult armadillos (a novemcinctus) from the armadillo farm of the Instituto Lauro de Souza Lima, Bauru-Brazil were used in this study, being 6 male and 4 female with an average weight of 3,913.0 g.

Animals were sedated with Droperidol (0.11 ml/Kg) and kept secure and safe in a special wood device in order to expose the ventral aspect of the animal as well as the four limbs(fig.1). The room temperature was taken with a standard wall termomether and the animal temperature was also taken with a four channel digital surface termomether.

Fig. 1 - Special wood device to keep secure the animal.

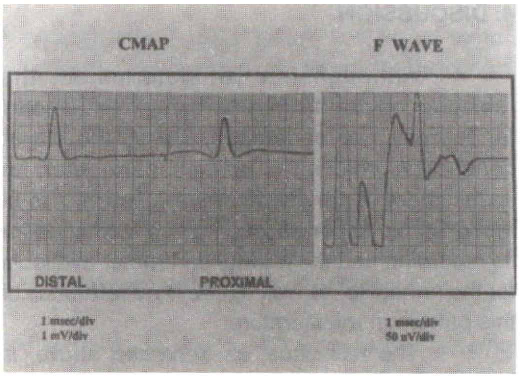


Nerve conduction studies were performed with an electromyograph, POLIMED-1002, and the results were printed in a graphic recorder. For this equipment the maximum amplification is 5 V/division, the maximum sweep is 0.2 ms/division, being the electrical stimulus of rectangular impulses of 0.5 to 1.0 s. The surface stimulation electrode used was a plastic plug of two metal pins with 2 mm of diameter with a space of 11 mm between cathode and anode. The surface recorder electrode was a plastic bar with 2 metal discs of 9 mm diameter and 14 mm of distance between the active and the reference electrodes.

Motor nerve conduction studies were performed in both hind limbs of each animal, being the recorder electrode placed in the foot pad and fixed with adhesive tape. The plastic bar was oriented in such a way that the active electrode was the more proximal. The stimulation electrode was placed distally above the ankle of the animal, and proximal over the 3rd skin band in the thigh. The filter of low frequency was 20 Hz and high frequency filter 2 KHz,. the latencies were obtained manually, and the gain used was 200 V, as suggested by the literature (STALBERG, FALK, 1993). The measurement of the distance between the two stimulation sites was done with a tape line, keeping the ankle at 1202, for this was used a gonniometer.

The records were visualized in the monitor screen and later on registered in paper (fig. 2).

Fig. 2 - CMAP distal and proximal of the ciatic nerve responses, and F wave response.



3. RESULTS

Results from the study are presented in table 1 and 2.

TABLE 1 - Nerve conduction velocity, limb temperature, room temperature, sex and weight

	NCV	NCV	Temperature		Room temp	Sex	Weight
	Right	Left	Right	Left			
1	56.25	55.00	29.20	28.60	24	female	4,000 g
2	81.60	84.00	30.40	30.70	25	male	4,100 g
3	45.45	42.50	31.00	30.40	26	female	2,700 g
4	44.16	47.27	30.70	31.00	26	female	4,000 g
5	72.85	82.50	29.50	29.50	23	male	4,500 g
6	82.85	88.33	29.40	29.40	21	female	4,700 g
7	58.75	60.00	28.50	28.70	18	male	4,100 g
a	65.55	63.63	27.90	27.60	17	male	4,000 g
9	75.00	71.42	30.80	30.50	27	female	3,060 g
10	71.42	75.00	31.70	31.60	28	female	3,970 g

NCV: nerve conduction velocity

TABLE 2 - Mean conduction velocity and SD, Mean Fwave and SD, Mean limb temperature and SD

MEAN CV m/s		SD		MEAN F w ms	SD	MEAN LIMBT °C		SD	
Right	Left	Right	Left	12.1	2.26	Left	Right	Right	Left
65.38	66.96	13.85	15.85			29.91	29.80	1.2	1.25

CV: conduction velocity; SD: standard deviation; Fw: F wave;

4. DISCUSSION

Although not yet determined, the armadillo could be an optimum experimental model for leprosy nerve damage. Being the nerve conduction velocity a precise parameter for assessment of peripheral nerve function, it is of recognizable importance to have a technique for its recording and the normal parameter available for the armadillo, as well. There is no reference of this pattern in the literature.

The technique, as described above, is feasible. The sedation of the animal does not interfere with results and allows easy and friendly manipulation of the animal. Fastening the animal in an appropriate wood lay permits good exposure of the limbs for the surface electrodes and electrodes placement.

During the NCS procedure the sensitivity of the amplifier must be keptthe same, duetothefactthat the longer latencies value are recorded with low amplification rather than with high amplification. The stimulus intensity must be always supramaximal, and the intensity must be progressively increased until the maximum amplitude is achieved, than the stimulus intensity is again increased 10 to 25% (STALBERG, FALK 1993).

The Compound Motor Action Potential (CMAP) waveform must be carefully analyzed, a nerve stimulus can spread to nerves other than the one being tested, and it can change the CMAP waveform and consequently the latencies (JOHNSON, 1980, KIMURA, 1984). In this technique it happens more frequently when the sciatic nerve is stimulated in the proximal site, where the peroneus nerve and the tibial nerve are adjacents. The volume conducted from the muscles of the leg (extensor of the toes and the foot) may "contaminate" the CMAP recorded in the foot pad muscles. The CMAP was measured from the negative peak to positive peak, the mean amplitude obtained was, distally 4,735 V and proximal 4,295 V, the variation was also large, distally 1,600 to 9,600 V and proximal a 1,400 to 9,400 V, but no significant proximal amplitude decay, neither temporal dispersion was observed in each nerve itself.

The mean conduction velocity obtained was 66.17 m/s, with a large variability, with a 1 standard deviation of 15.85 m/s. The most important physical factor affecting the parameters is the temperature, the decrease in temperature of a nerve segment decreases the conduction velocity by 1.2 m/s/°C to 2.4 m/s/°C (STALBERG, FALK, 1993). The mean limb temperature obtained was 29.91°C, with a SD of 1.2 in the right side, and 29.8°C, with SD of 1.25, in the left, however it cannot be the only responsible of these variations of the nerve velocity. This high variability is most concerned to the short distance segment studied, specially distances less than 100 mm, and specially distancies around 50 mm, coherent with data in the literature (MAYNARD, STOLOV, 1972), in wich the latencies measurements were also performed manually. In this study we didn't have any discrepancies in NCS between each side, which exclude the hypothesis of gross errors.

F wave was also tested in 16 nerves and was present in 8, including 2 animal where it was obtained bilateraly. The mean amplitude was 0.49 mV with a variation of 0.10 to 1.5 mV and mean latency of 12.1 ms, and SD: 2.26 ms. For the F wave we need to use repetitive stimules of 1 Hz what causes a reaction of the animal and makes difficult the recording in 100% of the tested nerves.

5. CONCLUSION

The mean motor nerve conduction velocity for the sciatic nerve in D. novemcinctus, of both sexes and of 3,913 g mean weight, was 66.17 m/s, with a standard deviation of 15.85 m/s. No C MAP amplitude decay was observed proximal in the examined animals.

The NCS in armadillos is easy and feasible to reproduce and can be used to assess peripheral neuropathy experiments.

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