

SCIATIC NERVE CONDUCTION VELOCITY AND LOCOMOTORY PATTERNS IN FROG, UROMASTIX AND RABBIT

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ABSTRACT

The complex interactions among the structural features of axon, its myelin sheath and its length, determine the nature of nerve impulse conduction. In addition, fibers of different diameters and degree of myelination determine the nerve conduction velocity (NCV). It is therefore, hypothesized that for the same nerve, the NCV may differ in animals, due to the differences in the leg architecture and its movement during locomotion. The purpose of this study was to compare right and left limb's sciatic NCV in three different classes of animals to identify differences, if any, and to relate these differences with different types of gait locomotory pattern observed in them. The Compound Action Potential (CAP) was recorded from both the right and left isolated sciatic nerves from adult Frog, Uromastix and Rabbit of both sexes, through Power Lab and its accessories. These CAP records were then used for the calculation of NCV. Significant difference was observed in sciatic NCV being greater in order of Frog>Rabbit>Uromastix. These differences were found well correlated with differences in their gait and locomotion. However, there was no significant difference among right and left sciatic nerve's NCV for both Uromastix and Rabbit, while it was significantly different for Frog. The differences in NCV among Frog, Uromastix and Rabbit's sciatic nerves reflect differences in these animals for their sciatic nerve diameter, leg architecture, gait and locomotion, as per their habitat.

Key Words: Compound Action Potential (CAP), Nerve Conduction Velocity (NCV), Sciatic Nerve, locomotion.

INTRODUCTION

The conduction velocity of a myelinated fiber depends on the complex interactions among the structural features of the axon and its myelin sheath, and on the nature and distribution of the conductance mechanisms of the axon (Waxman, 1980). The speed of propagation for mammalian motor neurons is from 10 to 120 meters/sec, while for non-myelinated sensory neurons it is from 5 to 25 meters/sec (Kenneth, 1996). Non-myelinated neurons fire in a continuous fashion, without the jumps; ion leakage allows effective closure of circuits, but it slows the rate of propagation (Kenneth, 1996). The evolution of the nervous system has been an important factor in the adaptation of animal to their environment (Abdelmelek *et al.*, 2003). In the elongated nerves, both the amplitude and conduction velocity of compound nerve action potential have been reported to decrease following leg lengthening (Harumitsu *et al.*, 2005). Body mass is an important factor regarding locomotion, heavier animals though using more total energy, require less energy per unit mass to move (Campbel and Reece, 2005). The sciatic nerve, which is the broadest nerve in the body lies deep to the gluteus *maximus*, superiorly. crossing posteriorly to the obturator *internus*, *gemelli* and the quadratus *femoris* muscles (Williams *et al.*, 1995). This nerve divides into its tibial and common peroneal

branches approximately halfway or more down the thigh. In, approximately 12% of the cases, this nerve also branch earlier in this course, as soon as it leaves the pelvis (Moore and Dalley, 1999). The fibers constituting the sciatic nerve in left leg have greater mean diameter but lower mean density than those constituting the right nerve. The diameter of its myelinated fibers and the density of both the myelinated and un-myelinated fibers do not vary from male to female. On the other hand, diameter of the un-myelinated axons do, since the nerves on the right side (in both sexes) have higher morphometric values, on average, than the contra-lateral ones (Muglia *et al.*; 1995). In addition, the structural/morphological characteristics of axon and nerve itself also determine the impulse conduction and propagation characteristics. Further, gait and locomotion has also been mentioned to depend on the characteristics of innervations to the muscles involved. Since, information is not available regarding comparison of NCV in different animal classes and species, and its relation with gait and speed of locomotion, therefore, in this study, CAP has been recorded for the calculation of NCV from the isolated sciatic nerve of three animals Frog, Uromastix and Rabbit. The calculated values of NCV have been compared among these animals to explain the differences in their gait and speed of locomotion.

MATERIALS AND METHODS

Animals: 8 adult Frogs (*Rana esculanta*) of fresh-water or near water in damp places on land were used. 9 Uromastix that lives in dry, soft, sandy tracts with scant vegetation in burrows and 8 adult rabbits (*Oryctolagus cuniculus*) lives in fields, grass-lands and open woodlands and in burrows underground were selected. All three animals of both sexes were selected for both right and left nerves for dissection. Nerves from both right and left sides used for recording of NCV.

Treatment Pattern: In all of the experimental animals, the sciatic nerve was exposed from its origin (lumber vertebrae) up to knee and calf. Throughout the dissection, care was taken to prevent drying of exposed sciatic nerve during the removal of surrounding connective tissues and muscles. For this purpose, respective buffer solutions all the chemicals used in the preparation of solutions were obtained from e. merck, germany or anala-r. For the experiments on uromastix *hardwickii*, similar composition of reptilian buffer was used as described by Khalil and Masseih (1954), for frog, ringer’s solution (Franhenhauser and Widen 1965) for rabbit, Kreb’s solution (Winegrad and Shames, 1962) was used to be dribbled over the exposed surface of nerve, intermittently. During this dissection, secondary and tertiary fine branches that were observed to arise from sciatic nerve were not traced and cut off with scissor, to follow its gross course through pelvic girdle, thigh, knee and termination in calf for exposure and isolation.

Recording of cap: For this purpose, isolated sciatic nerve was kept in the nerve bath having respective buffer solution. Care was taken to fill the bath to an extent with buffer, to avoid short circuiting of stimulation current. The PowerLab was kept ready. The settings file

associated with Power Lab experiment for NCV contains Chart macros that automate recording and analysis functions. The voltage that was nearest to the one that first elicited a maximal CAP from the respective nerves was selected. The Settings used in order to have access to a pre-configured Chart Settings file for experiments includes sampling rate 40k/sec. For Channel 1 and 2, Input Amplifier ranged at 50 mV. Positive checkbox and Negative checkbox kept checked. Low pass filter kept off and main filters checkboxes were also kept unchecked. For Stimulator settings, mode was kept at pulse. Its output was chosen at, ‘once only’. Marker channel kept at, ‘OFF’.

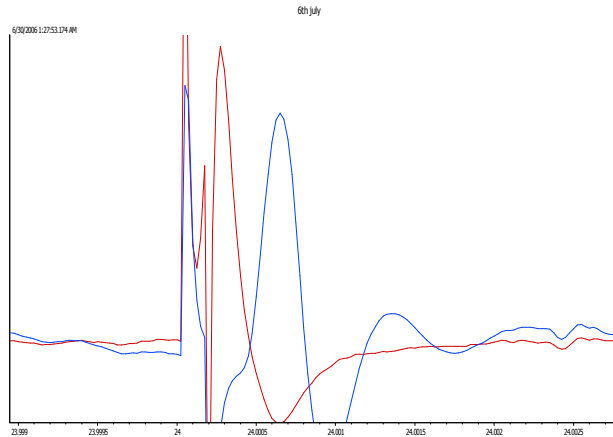
Calculation of Nerve Conduction Velocity: The time difference between the two simultaneous CAPs measured from the first and second sets (pair) of recording electrodes was measured according to the procedure (PowerLab manual–Model No.ML825, Serial No. 225-0815). In addition, for the distance between the two sets (pair) of recording electrodes was also measured for the calculation of NCV by using the following formula stated in PowerLab manual–Model No.ML825, Serial No. 225-0815:

$$\text{Conduction velocity (meters/sec)} = \frac{\text{distance between electrodes (Cm)}}{\text{time interval between two CAPs (Sec)}} \times 100$$

RESULTS

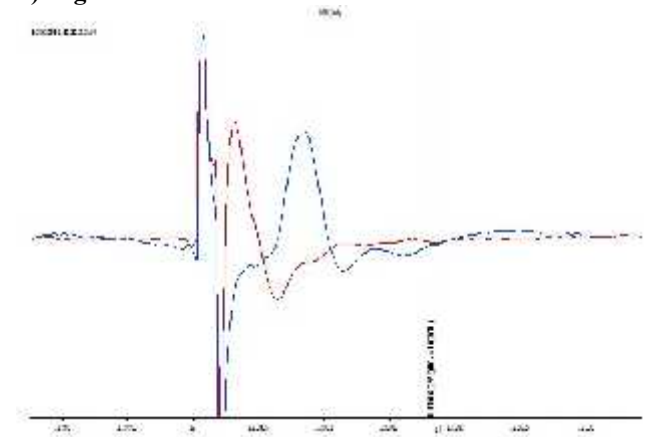
Fig. 1a, b and c, exhibits representative records of CAP recorded at two different sites of stimulation at the sciatic nerve for Frog, Uromastix and Rabbit, respectively. The distance between the latency (time of stimulation to the onset of CAP) has been found different for Uromastix when compared with Frog and Rabbit.

i. Left Sciatic nerve



i. Left Sciatic nerve

ii) Right Sciatic nerve



ii) Right Sciatic nerve

Fig.1a Recorded trace of CAP from Sciatic nerve of Frog

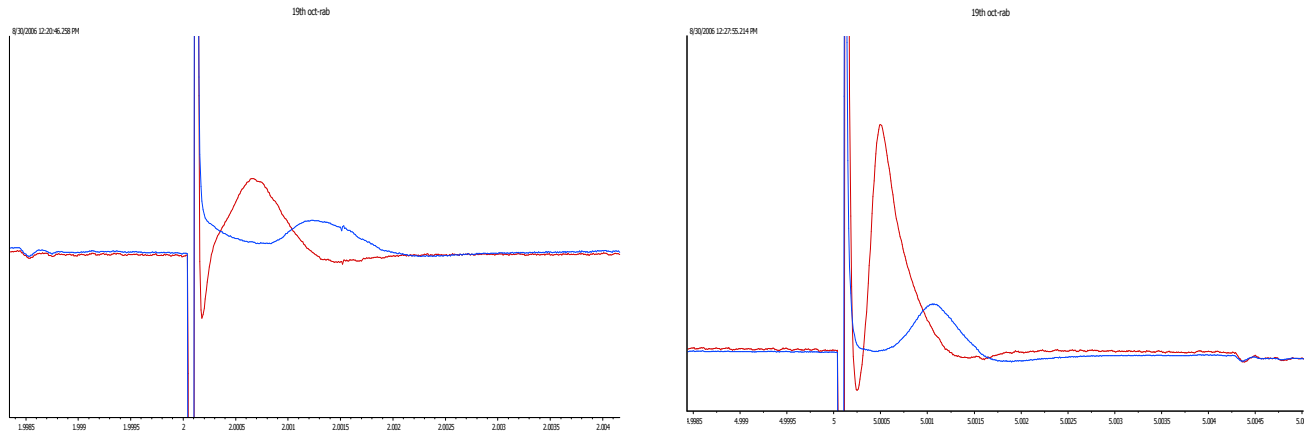


Fig.1b Recorded trace of CAP from Sciatic nerve of Uromastix.

i. Left Sciatic nerve

ii) Right Sciatic nerve

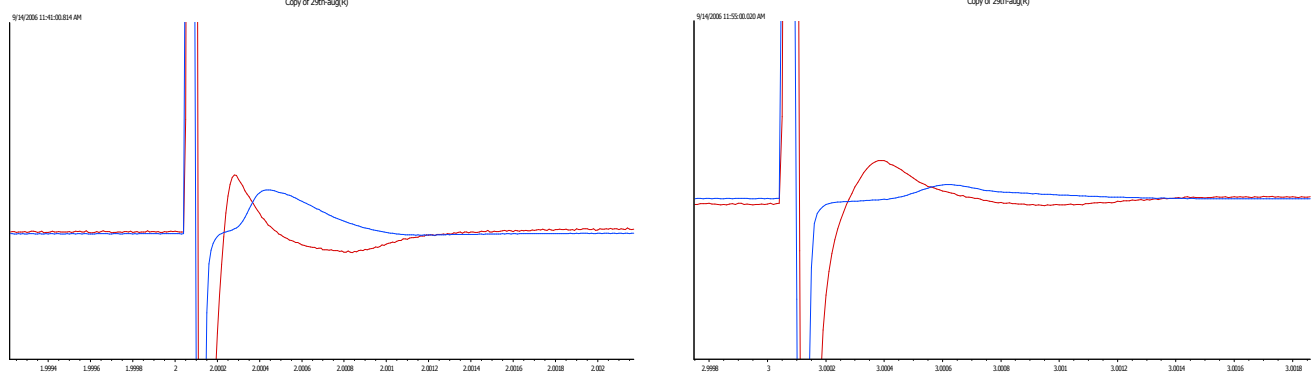


Fig.1c Recorded trace of CAP from Sciatic nerve of Rabbit.

The comparison of conduction velocity of left and right leg's sciatic nerve of Frog, Uromastix and Rabbit has been presented in Table 1. Accordingly, significant difference ($P < 0.05$) was found between right

and left sciatic nerves of frog. However, this difference was statistically non- significant ($P > 0.05$) between right and left sciatic nerves of Uromastix and Rabbit.

Table 1. Comparison of conduction velocity of sciatic nerve between left & right limbs of frog, uromastix & rabbit

S.NO	ANIMAL	NCV (m/Sec)		p*
		RIGHT	LEFT	
1	FROG	51.371±2.011(8)	45.242±1.981(8)	P<0.05
2	UROMASTIX	23.805±1.561(12)	22.457±1.837(12)	P>0.05
3	RABBIT	46.798±2.737(8)	47.94±3.168(8)	P>0.05

DISCUSSION

According to our results, significant difference in NCV among the right and left sciatic nerves of Frog, indicate that the flexion and extension that occurs during Frog's jump, did not result probably in simultaneous landing of both the legs on ground, probably due to imbalance of its body during flight after jump (Rotem, 2008). It is quite possible that Frogs may be having preferred side or leg for landing on to the ground at the

end of each locomotory cycle. However, the non-significant differences observed in the NCV of right and left sciatic nerves of Uromastix and Rabbit (Table 1), indicate non-preference in the use of right or left hind limb in the two animals during crawling or hopping movements, respectively. It is further explained that during locomotion either in Uromastix or Rabbit, both the limbs moves simultaneously for extension and flexion for the completion of each of the locomotory cycle. In addition, Adam and Friede (1988), collected

morphological data on Frog's sciatic nerve which and demonstrates continuous addition of new myelinated and non-myelinated fibers with body growth. Adam and Friede (1988) also found that along with an increase in fiber number, there is always a marked change in axon calibers and in the relative thickness of the myelin sheaths as well. We assume that such changes during sciatic nerve growth may be different for both the right and left legs in Frogs. According to Adam and Friede (1988), these changes are not found in mammals. We suggest that such changes in nerves are also not associated with Uromastix (reptiles) during their growth probably. Under normal circumstances, the movement of the electrical impulse down the length of a nerve is very fast, being 35–60m/Sec (Rosenfalck and Rosenfalck, 1975). According to our results, NCV is highest in Frog, less in Rabbit and least in Uromastix in the order of Frog>Rabbit>Uromastix. In this connection, Waxman (1977 and 1980) stated that nerve fibers that exhibit geometrical similarity, their conduction velocity is nearly proportional to their diameter. In addition, the width of an axon varies greatly among species. The diameter of a squid axon is about 500 μ m, lobster nerve and frog muscle axons are about 75 μ m, and mammalian motor neurons average about 10 μ m (Kenneth, 1996). In our opinion, the differences in the NCV among three classes of animals, observed in the present study, also reflects the differences in their sciatic nerve diameters. Therefore, it is suggested that the diameter of sciatic nerve might be

least in Uromastix in comparison to the Frog and Rabbit and thus the obtained NCV is lowest in it. However, this suggestion needs confirmation through comparative morphometric studies on sciatic nerve of these animals. In addition, both the Frog and Rabbit have almost the same mode of style jumping and hopping (Jordan and Verma 1997). In our opinion, the hopping movement of Rabbit exhibits, forward extension, then flexion, followed by backward extension in its hind limbs, parallel to the ground (Fig. 2). While, jumping of Frog exhibits vertical and straight jumping movement against ground, but with backward extension followed by forward flexion of its hind limbs (Fig. 3). Hence, both the Frog and Uromastix demonstrate very fast movement during locomotion. In addition, both these animals have strong hind limbs (Jordan and Verma 1997), having more fast muscles innervated by sciatic nerve's fast fibers, having greater conduction velocity. It is the reason that the results of the present study showed non-significant difference in the NCV obtained from Frog and Rabbit. While, Uromastix, exhibits comparatively slow mode of locomotion (crawling), depending upon its gait showing lateral movements in hind limbs requiring lesser force. These movements are lateral against the ground (Fig. 4). In addition, the fast locomotory muscles of Uromastix are probably innervated by small distal nerves, having slow conduction velocity. Therefore, significantly ($P<0.0005$) lesser sciatic NCV (right and left) was obtained for Uromastix than Frog or Rabbit (Table 2).

Table 2. Comparison of conduction velocity of sciatic nerve between frog and uromastix, uromatix and rabbit, & frog and rabbit

LIMB SIDE	NCV (m/Sec)		p*
	FROG	UROMASTIX	
LEFT	45.242±1.981(8)	22.457±1.837(12)	P<0.0005
RIGHT	51.371±2.011(8)	23.805±1.561(12)	P<0.0005
	UROMASTIX		
	RABBIT		
LEFT	22.457±1.837(12)	47.94±3.168(8)	P<0.0005
RIGHT	23.805±1.561(12)	46.798±2.737(8)	P<0.0005
	FROG		
	RABBIT		
LEFT	45.242±1.981(8)	47.94±3.168(8)	p>0.05
RIGHT	51.371±2.011(8)	46.798±2.737(8)	p>0.05



Fig. 2: Drawings showing sitting position (a) and sequence of Frog's jumping movements having vertical but backward extension (b) & (c), followed by their flexion (d) in its hind limbs.

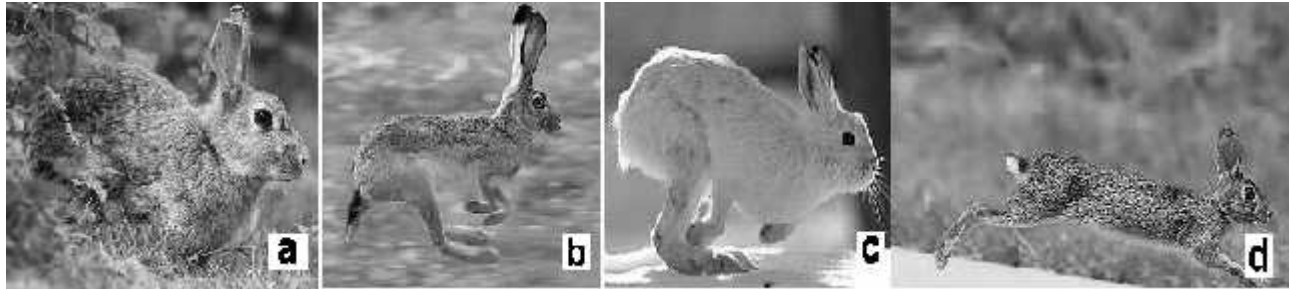


Fig. 3: Showing photographs of Rabbit; a) sitting and b, c & d hopping movements, b) forward leg extension, c) leg flexion for landing and d) backward extension for running

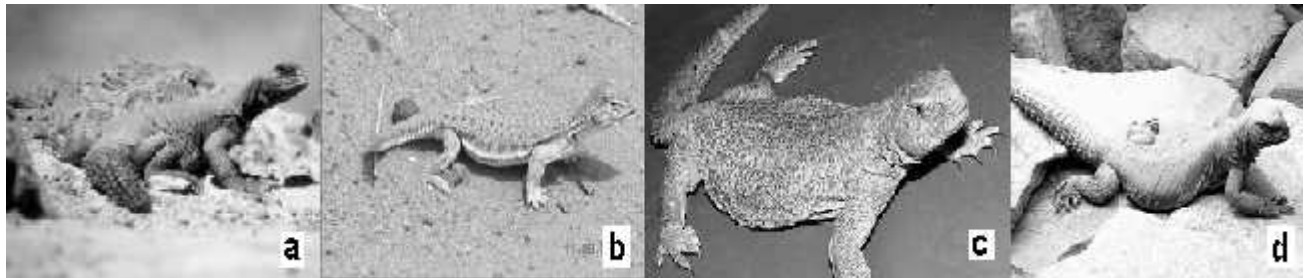


Fig. 4: Showing photographs of Uromastix; a) sitting. (b, c & d) Lateral movements in hind limbs. b) Slight forward extension. c) Full forward extension. d) Backward flexion for forward movement against ground.

A comparison of NCV between Frog and Rabbit showing non-significant difference ($P > 0.05$) in both the left and right sciatic nerves (Table 2) is due to the fact that both of these two animals having strong and long hind limbs, and having more or less similar mode of locomotion, i.e., hop or jump. Further, as suggested by Necker *et al.* (2000), there is specialization in the lumbo-sacral vertebrae and spinal cord in birds, which can be assumed for animals moving on ground. We suggest that such specialization of the lumbo-sacral vertebrae is definitely and significantly different for Uromastix than Frog and Rabbit. Thus our results indicate significant differences in NCV obtained from sciatic nerve of Uromastix to those of Frog and Rabbit (Table 2), as sciatic nerve character must be influencing the locomotory pattern of the innervated locomotory muscles.

Conclusion: The significant difference of sciatic NCV of Uromastix in comparison with Rabbit and Frog reflects differences in these animals for their sciatic nerve course, diameter, leg architecture, gait and locomotion, as per their habitat. However, unlike Rabbit and Uromastix, the significant difference in NCV observed among right and left sciatic nerves of frog, cannot be explained unless detailed morpho-metric and neurophysiological character of sciatic nerve is mapped on both the right and left sides.

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