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Minimally invasive techniques for sensory and motor nerve conduction in Wistar albino rats

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Sir,

Decrease in motor and/or sensory conduction velocities, or a decrease in the amplitude of muscle or nerve potential indicates certain peripheral nerve system disorders. Techniques for the measurement of motor and sensory conduction velocities are well established in humans as well as in numerous animal models such as the rat and the monkey. Scientists widely use rats as experimental animals for scientific research for their viability, fast reproduction and simplicity, which makes them indispensable in experimental researches. Particularly, rats are used for investigation of models of peripheral axonal and demyelinating neuropathies, neural tissue injury and regeneration.[1-3] In rats, motor nerve conduction velocity (NCV) is measured at the level of the sciatic nerve[4] or less frequently at the tail.[5-6] The sensory nerve conduction velocity (SNCV) has been measured on the external saphenous nerve and sural nerve.[7] Recording of compound muscle action potentials and selective stimulation of the nerve in rats are done by surgical exploration of the sciatic nerve or other nerves and muscles which results in sacrificing the animal, as the trauma leads to severe disability or death. This is the major drawback in studies where the effect of drugs cannot be studied, as in the same group of rats pre and post recordings cannot be done due to the death of animals after surgical exploration of nerves. For these reasons we have used non-invasive methods of motor and sensory nerve conduction study of the sciatic and sural nerves in rats without exploration of nerves and animal scarification or disability. Although the normal values of sciatic motor nerve conduction velocity were studied by using minimally invasive methods in Wistar rats,[8] to our knowledge no studies have described the normal values of sensory nerve conduction velocity of sural nerve in these rats by using non-invasive methods. Therefore, we studied to find out the normal values of motor and sensory conduction velocities of sciatic and sural nerves in wistar rats by using non-invasive methods. For this study eight normal growing male adult Wistar Albino rats of 10-12 wks age, weighting 180-220 g were obtained from JIPMER central animal house. This study was a part of PhD work. Approval of Scientific Advisory Committee and Institute Animal Ethics Committee, JIPMER, Puducherry were obtained. All the animals were housed in individual cages with 12/12 h light/dark cycle and food, water *ad libitum,* in 25 ± 2°C temperature and humidity controlled room for habituation at the Animal Research Laboratory in the Department of Physiology for a period of one week followed by nerve conduction study. No animal were killed or disabled during the present study. The animals were anesthetized with 70 mg/kg ketamine Hydrogenchloride to prevent discomfort. Skin temperature was maintained at 34° C during NCV. Body temperature was maintained at 37°C after NCV using a warming pad to ease animal stress from anesthetic. The animals were positioned prone with maximally straightened hind limbs. Electrophysiological measurements were taken using LabScribe (Iworx) device. The platinum needle electrodes were cleaned with 70% alcohol to maintain pathogen-free status. Measures of NCV were performed per Animal Models of Diabetic Complications Consortium (AMDCC) protocols;[9] similar techniques were followed by Sang *et al.* for the measures of NCV in mice.[10]

Sciatic-tibial motor NCV (SMNCV) was determined by recording at the dorsum of the foot and orthodromically stimulating with supramaximal stimulation (current intensity 30% above the value to evoke the maximal compound muscle action potential) first at the ankle, then at the sciatic notch. Latencies were measured in each case from the initial onset of the compound muscle action potential. The sciatic-tibial motor NCV was calculated by subtracting the measured ankle distance from the measured notch distance. The resultant distance was then divided by the difference in the ankle and notch latencies for a final nerve conduction velocity.

Sural Sensory NCV (SNCV) was determined by recording at the dorsum of the foot and antidromically stimulating with supramaximal stimulation at the ankle. NCV was calculated by dividing the distance by the take-off latency of the sensory nerve action potential. All the data were expressed as mean ± SD.

The mean latency of sciatic nerves was 0.55±0.129 ms and the sciatic-tibial motor NCV was 46.38±12.77 m/s. The mean latency of sural nerve was 0.75±0.24 ms and the sural sensory NCV was 39.24±8.32 m/s. We used subcutaneous stimulating and recording needle

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electrodes to measure the SNCV as well as the SMNCV. The stimulating needles in the iliac notch were close to the sciatic nerve, whereas surface electrodes were separated from it by a thick fat layer, forming an effective electrical insulator. We used an antidromic technique for bipolar recording of sensory potential. At the level of the last phalanx of the digit, the nerve endings contained only sensory axons. The nerve potential was triphasic, the latency was measured at the onset of the potential, which was always clearly distinct here. The sensory velocity was 39.24±8.32 m/s at the age of 12 weeks, which was similar to the CD-strain rats of the study conducted by C. P. V. deJESUS, MD *et al*.[11] This study is first one of its kind study to use subcutaneous needle electrodes in Wistar albino rats for sural sensory nerve conduction whereas the sciatic motor nerve conduction studies by needle electrodes were performed in rats in earlier studies.[12-14] The SMNCV in 12 weeks old Wistar rats in our study was less than the SMNCV of Wistar rats studied by Nergiz Huseyinoglu *et al*.[8] (46.38±12.77 m/s Vs 58.90±5.07) which might be due to the difference in age of the rats. Our results are similar to a study conducted by Kurokawa K *et al*. in which SMNCV was 46.3±12.4 m/s.[15] Additionally, some studies, which were performed on isolated sciatic nerve segments and on the exposed nerve, support our results, Head RJ *et al*. reported that the sciatic nerve MNCV as 46.9±2.2, 53.0±2.1 and 48.9±3.8 m/s depending on dietary supplementation.[16] Similar results *in vitro* and *in vivo* studies prove the objectivity and reliability of our nerve conduction technique by bipolar needle electrodes on the non-exposed nerves. Study conducted by Nergiz Huseyinoglu *et al*. explained a minimally invasive procedure for Sciatic MNCV in wistar rats in which cecording bipolar needle electrodes were placed in medial gastrocnemius muscle belly.[18] However, authors considered that recording CMAPs from the gastrocnemius muscle in rats should be treated with caution, especially, if monopolar needle electrodes are used for recording.[17] In conclusion, the results of present study shows that minimally invasive sciatic and sural nerves conduction studies with needle electrodes may be the suitable methods in rat models of peripheral nervous system diseases. These methods of nerve conduction may be preferable in cases where researchers need further observation of surviving animals after examination. On the other hand, from an ethical point of view, these methods are minimally invasive and not the cause of death or disability of animals, such as in the present study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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