Motor and sensory nerve conduction velocities in Yucatan minipigs

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Summary

Motor and/or sensory conduction velocities are used to assess peripheral nervous system disorders. Although the miniature pig represents a model of choice for long-term pharmacological experimentation, no study has so far been reported on this model in relation to the measurement of nerve conduction velocities. We developed the present technique and applied it to 34 3–18-month-old Yucatan minipigs. Motor and sensory conduction velocities were measured using the anterior tibial nerve and the internal plantar nerve, a branch of the posterior tibial nerve, respectively. The nerve conduction velocity data of motor (MNCV) and sensory (SNCV) nerves, together with the amplitude of the sensory nerve signal, were logarithmically dependent on the age of the tested animals ($r^2 = 0.92, 0.81$ and 0.76, respectively). The mean values of MNCV and SNCV were $70.9 \pm 1.1$ and $67.9 \pm 0.2$ m/s, respectively, at the age of 16 months for these miniature pigs. In order to validate this model, we compared it with other known models when the velocities reached a plateau at the end of the study. These values were found to be higher than those in humans or rats, but are comparable to those of the baboon, one of the best large animal models for human pathologies. Because the physiology and metabolism of the minipig resemble those of humans, and due to its long lifetime, this animal represents a good model for studying the development of neuropathology.

Keywords

Minipig; longitudinal study; motor and sensory conduction velocities

Certain peripheral nerve system disorders are manifested by a decrease in motor and/or sensory conduction velocities, or a decrease in the amplitude of muscle or nerve potential. 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. For the rat, motor nerve conduction velocity (MNCV) is measured at the level of the sciatic nerve (Fullerton & Barnes 1966), or less frequently at the tail (Myoshi & Goto 1973, Knox et al. 1989). The sensory conduction velocity of the large proprioceptive fibres is measured indirectly through the H reflex (Stanley 1981). More recently, the sensory nerve conduction velocity (SNCV) has been measured on the external saphenous nerve (Hort-Legrand et al. 2001). Although rats have been extensively used to study SNCV and MNCV, their anatomy and physiology are too far from those of humans for them to be used as good animal models for such studies.

The same technique that has been used in humans has also been applied to monkeys (Birrell et al. 2000), which are considered the best experimental models. There are, however, several drawbacks to these models, including rearing, cost, and manipulation.

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No study on the miniature pig has been described so far. This large animal model, which has already been used for studies of cardiac autonomic neuropathy (Mésangeau et al. 2000), is particularly easy to work with for long-term pharmacological experiments. However, no method for the measurement of nerve conduction has been described using this model. The aim of the present article is to introduce a method for the measurement of motor and sensory conduction velocities in the miniature pig and to compare them with the measured values reported in humans and other reference species.

**Materials and methods**

A group of 34 healthy Yucatan minipigs (Charles River, St Aubin, France) was kept in the laboratory for 12 months. Animals were between three and 18 months old. Their weight increase followed the age increase according to the equation: \( \text{weight} = 19.6 \ln(\text{age}) - 11.5 \) \((r^2 = 0.85)\). The mean weight of the animals was 14.9 ± 3.1 kg between the third and the fifth months, and 43.2 ± 2.0 kg between the 13th and the 15th months. Six animals were examined three times during the course of the study; one of them was examined six times, another eight times, and the remaining 26 only one time.

The whole study was conducted on anaesthetized animals. Anaesthesia was induced and maintained with a mixture of isoflurane (2–2.5%) and medical carbogene (95% oxygen–5% carbon dioxide). Each animal was placed on a heating table in order to sustain a constant body temperature. Temperature in the limb was not measured. No animal was ever anaesthetized more than twice per month.

**Measurement of motor velocity**

The sciatic nerve was stimulated on its proximal part, in the sciatic notch (Figure 1), with rectangular current pulses delivered through a pair of subcutaneous steel electrodes (0.5 mm diameter, 20 mm length) with an impedance of less than 1 kΩ (MEI, Montreuil, France). The cathode was the closest electrode to the recording electrodes. The anode was placed 3 cm above. Distal stimulation, consisting of exciting the anterior tibial nerve, a branch of the external sciatic nerve going downward, inward, and forward, was applied on the external side of the tibia (Montané et al. 1964). The subcutaneous electrodes were placed just outside the median line above the anterior annular ligament. The cathode was the closest to the recording electrodes, with the anode located 3 cm above. The power intensity was 30 mA.

The muscle potential was recorded by using two subcutaneous electrodes. The cathode was placed on the median line, 2 cm above the metatarsal–phalangeal joint, under the anterior annular ligament. It was kept in contact with the extensor-digitorum-brevis muscle innervated by the external terminal branch of the anterior tibial nerve. The anode was positioned at a distance, in the notch between digits III and IV.

The ground electrode was placed between the stimulating and the recording electrodes. The electric signals were recorded with a Myto device (EBNeuro, Florence, Italy). The analysis period was 10 ms, the sensitivity 5 mV per division, and the bandpass was 2–2000 Hz.

The distance between the two cathodes was measured. Latency difference of the onset of the motor potentials was used for the MNCV calculation.
Measurement of sensory velocity

The SNCV was measured on the internal plantar nerve, a branch of the posterior tibial nerve that emerges to a superficial location at the external malleolus. The nerve divides at the scaphoid into two terminal branches: an internal one and an external one. At the tip of the space between digits II and III, the digital nerve of the second space (terminal ramification of the external branch of the internal plantar nerve) divides itself into the external collateral nerve of digit II and the internal collateral nerve of digit III.

The stimulating electrodes were placed in contact with the nerve on the inside of the tendon, 2 cm above the cuboid bone (Figure 2). The anode was 3 cm above. The recording electrodes were placed at the tip of the nerve: the cathode was placed at the level of the division of the digital nerve of the second space, and the anode at the tip of the phalanx of digit III.

The ground electrode was placed between the stimulating and the recording electrodes. The stimulus intensities, between 3 and 8 mA, were applied maximally for the sensory nerve potentials. Amplitude of the sensory nerve action potential did not vary for higher stimulus intensities. The frequency of stimulation was 1 Hz. The averages of 100 nerve potentials were determined. A rejection of the artefacts was always present. The analysis period was 10 ms, the sensitivity 2 μV per division, and the bandpass 2-2000 Hz.

The distance between the stimulating cathode and the recording electrode was measured.

The latency of the first negative peak (peak 1) and the latency of the first positive peak (peak 2) of the mean response (Figure 3) were used for the calculation of the SNCV (Metral & Ropert 1984). The amplitude of the nerve potential was measured from peak to peak, between the first positive and the second negative peaks of the response.

Results

Body temperature and nerve velocities

The mean central body temperature was 38 ± 0.1°C. Within the range of temperature (36.4–39.4°C), nerve conduction velocities can be considered independently of the central body temperature.

Conduction velocities according to age

Motor nerve

The animals were grouped into different ages of 3–5, 5–7, 7–9, 9–11, 11–13, 13–15, 15–18 months, and the mean velocities were determined. The relationship between age and MNCV measured at the onset of the muscle potential followed a logarithmic regression ($r^2 = 0.92$). It was seen to be equal on average to 60.5 ± 0.9 m/s at the fourth month and to 70.9 ± 1.1 m/s at the age of 16 months (Figure 4).

![Figure 2](image2.png)

**Figure 2** Recording of sensory nerve conduction velocity on the left posterior limb. A = anode; C = cathode; G = ground; II = second digit; III = third digit; R = recording electrode; S = stimulating electrode

![Figure 3](image3.png)

**Figure 3** Sensory nerve (internal plantar) potential in a 16-month-old minipig
Sensory nerve  The SNCV, using peak 1, and MNCV varied in parallel as the animal aged, with SNCV being $7.3 \pm 1.1$ m/s more than the MNCV. The conduction velocity measured at peak 1 of the nerve potential followed a logarithmic regression ($r^2 = 0.81$). It was equal to $68.0 \pm 2.0$ m/s at the fourth month and $81.5 \pm 1.8$ m/s at the age of 16 months (Figure 5). The conduction velocity measured at peak 2 of the nerve potential was parallel to, but lower than, the one measured at peak 1. The velocities were $57.9 \pm 2.1$ and $67.9 \pm 0.2$ m/s at four and 16 months, respectively (Figure 5). The dispersion of data within the age categories was limited, as compared with the data obtained from peak 1.

The evolution of the amplitude SNCV signals followed the logarithmic regression ($r^2 = 0.76$) (Figure 6). The amplitudes calculated at the age of four months were $4.9 \pm 0.3$ and $14.5 \pm 2.2 \mu V$ at the age of 16 months.

Discussion
We used subcutaneous stimulating and recording needle electrodes to measure the SNCV as well as the MNCV. The stimulating needles in the iliac notch were close to the sciatic nerve, whereas surface electrodes would have been separated from it by a thick fat layer, forming an effective electrical insulator. In humans, recording of the muscle potential is usually made with surface electrodes. Exceptionally, subcutaneous needle electrodes can be used to measure MNCV when muscles are very much atrophied by peripheral neurological pathologies. Subcutaneous needle electrodes were used in the pig because of the small size of the extensor digitorum brevis muscles and their location under the anterior annular ligament.

The bipolar recording of the sensory potential here used an antidromic technique. At the level of the last phalanx of the digit, the nerve endings contained only sensory axons. The nerve potential is triphasic: the latency was measured at the onset of the potential (peak 1), which was always clearly distinct here. The sensory velocity was $81.5 \pm 1.8$ m/s at the age of 16 months, greater than the motor velocity, which was $70.9 \pm 1.1$ m/s at the same age. However, the determination of the latency of the first positive peak (peak 2) clear in every species, allowed comparison of SNCVs from one species to the other.

SNCVs depend on age and follow a logarithmic regression. A one-year study period (from the fourth to the 16th month)
represents on average only one-fifteenth of the lifetime of the miniature pig. Although SNCV had not reached an asymptotic value at the end of the study period, it seemed that the value tended to stabilize between the 14th and the 16th months. This testified to the near completion of the phase of maturation of the peripheral nervous system, the growth continuing independently.

Assuming that the velocities nearly reach a stable value at the age of 16 months for the miniature pig, we can compare them with the velocities of other species. The SNCV measured at peak 2 is 67.9 ± 0.2 m/s at the age of 16 months in the minipig on the internal plantar nerve, 60.6 ± 0.25 m/s in the rat between six and 13 months on the external saphenous nerve (Hort-Legrand et al. 2001), 67.5 ± 7 m/s in the baboon between five and nine years on the internal saphenous nerve (Birrell et al. 2000), and 46.1 ± 3.5 m/s in the human between 15 and 35 years on the external plantar nerve (Behse & Buchthal 1971).

The MNCV in the rat, measured on the posterior tibial nerve, is 52.6 ± 0.33 m/s at the age of 13 months; equal to 72.5 ± 7 m/s in the baboon between five and nine years on the sciatic nerve; 46.1 ± 4.1 m/s in humans between 15 and 35 years on the anterior tibial nerve; compared with 70.9 ± 1.1 m/s in the minipig at the age of 16 months.

The MNCVs and SNCVs of the adult pig are comparable to those of the baboon, but are still much higher than in humans and in the rat.

Although electrophysiology showed significant differences between humans and the minipig, Swindle (1992) has reviewed the physiological and metabolic similarities between the two species. Indeed, the minipig is really close to humans from a physiological and metabolic point of view, as has been reviewed elsewhere (Swindle 1992). Moreover, its ease of use, compared with monkeys, and its long lifetime allow the study of pathologies with slow developments, in particular peripheral (e.g. diabetic) neuropathies (Mésangeau et al. 2000).

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References

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