Short Communication

In vivo recording of nerve conduction velocity of spinal CNS fibers in the mouse

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Abbreviations:

CAP, compound action potential; CNS: central nervous system; NCV, nerve conduction velocity.

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Abstract

Anesthetic and surgical procedures and an electrophysiological method were developed for recording nerve conduction velocity (NCV) of CNS fibers in the murine spinal cord. Under intravenous anesthesia and artificial ventilation the lumbar spinal cord segments L1 to L4 and dorsal roots L3 to L5 on the left side were exposed by laminectomy. After stimulation of the dorsal root L4, a compound action potential (CAP) was recorded at the ipsilateral left fasciculus gracilis at the spinal cord level L1. The latency from stimulation to the CAP together with the measured distance between the electrodes was used for the determination of the NCV. NCV of the fastest fibers in the fasciculus gracilis was observed to be approximately 28 m/s. Reversible decrease of the NCV was measured, *in vivo,* under general hypothermia. The technique described serves for *in vivo* electrophysiological investigations of spinal central fibers in wildtype and mutant mice.

Keywords:

In vivo electrophysiology in mice, spinal cord, CNS fibers, nerve conduction velocity.

Text

In vivo electrophysiological investigations in mice for the purpose of recording nerve conduction velocity (NCV) have been performed predominantly in the peripheral nervous system. Measurements of the NCVs of afferent and efferent fibers in different peripheral nerves of the mouse have been carried out (Steffens et al. 2012). Further, *in vivo* measurements have been described on different peripheral nerves in mice (Moldovan and Krarup 2006; Schulz et al. 2014, Hoffmann et al. 2015). Within the CNS, *in vivo* motor and somatosensory evoked potential (MEP and SSEP) recordings have been used to determine differences in NCV of central fibers between wildtype mice and knock-out mice for an oligodendrocyte protein (Lee et al. 2011). However, the accuracy of NCV of central fibers measured by MEP and SSEP is compromised due to the contribution of the synaptic delay to the measured latencies. NCV measurements within the CNS have been mainly realized *ex vivo* using dissected optic nerves in incubation chambers (Etxeberria et al. 2016).

In this report, we describe a technique for *in vivo* electrophysiology of sensory fibers of the dorsal spinal cord in the mouse. The experiments were performed according to the ethical guidelines of the national animal protection law and were authorized by the ethical committee of the state of lower Saxony. Adult wildtype C57BL/6 mice of 3 to 12 month of age were used.

For initial anesthesia, 80 mg/kg pentobarbital was injected intraperitoneally. After placing the mouse on a heated support and cannulation of the jugular vein with a 0.61 mm catheter, anesthesia was continued intravenously with 40 to 60 mg methohexital-sodium per kg and hour. Tracheotomy was performed and a tube inserted for artificial ventilation with a gas mixture of CO_2 (2.5%), O_2 (47.5%) and N_2 (50%) at 120 strokes/minute (120-160 µl/stroke depending of the weight of the

mouse). To avoid recording artefacts by active respiratory and reflexogenic movements the mice were paralyzed (800 µg/kg pancuronium, supplemented i. p. every hour). Rectal body temperature (kept at 37-38°C by manually regulating the power of the heated support), heart rate (ECG was recorded via platinum wires into both forelegs) and O_2 blood saturation (sensor in the inguinal region; MouseOx®, Starr Life Sciences, Oakmont, USA) were continuously monitored (Figure 1A). Appropriate dose of anesthesia was initially proofed by the absence of corneal and pinna reflexes. Later after injection of pancuronium, it was controlled by falling body temperature and heart rate.

After incision of the skin and removal of tendons and muscle tissue from the lumbar dorsal spinal processes and spinal arcs, a laminectomy was performed from vertebrae L1 to L5 to expose the spinal cord segments L1 to L4 and the left dorsal roots L3 to L5 (Figure 1A). After paralyzation, the dura mater was removed to mobilize the dorsal roots. To avoid motion artefacts the vertebral column was rigidly fixed with two custom-made clamps (Figure 1A; for more details regarding anesthetic and surgical procedures refer our previous reports: Dibaj et al. 2010; Dibaj et al. 2011; Dibaj et al. 2012; Steffens et al. 2012; Schomburg et al. 2013).

For electrophysiology studies, the spinal cord was covered with mineral oil and the mice were grounded via the clamps fixating the vertebral column. Stimulation and recording were performed using thin bipolar platinum wire electrodes (Figure 1A). Rectangular constant voltage pulses with a duration of 0.1 ms were used to stimulate the left dorsal root L4. Different stimulation strengths were used: 1.5T, 2T, 5T, 10T, 20T and 50T (the strength being indicated by multiples of the threshold strength for the activation of the lowest threshold fibers T; see also Saab et al. 2016). The relatively high stimulation strength of 5T was used for the determination of the NCV

(Patzig et al. 2016). CAP recording was performed with a sampling rate of 50 kHz with a surface electrode at the ipsilateral left fasciculus gracilis at spinal cord level L1. The signal was amplified (around 10k) and appropriately filtered (low pass 0.1 Hz; high pass 30 kHz). At the end of the experiment, the distance was measured in situ between the stimulation electrode (cathode) and the recording electrode. The latency from stimulation to the first peak of the CAP together with the distance between the electrodes was used for the determination of the NCV (Figure 1B). NCV of the fastest fibers in the fasciculus gracilis was approximately 28 m/s. Since the temperature of the oil over the spinal cord was around 34-35°C (core temperature 37-38°C), the NCV is lightly (approximately 10%) underestimated (cf. Figure 1C). The same NCV was determined by recording from fasciculus gracilis at two different distances from the root entry zone L4. Therefore, NCV of ascending fibers in dorsal root as well as in fasciculus gracilis can be assumed to be similar. Under hypothermic condition, induced by continuous reduction of the support power, a reversible decrease of the NCV was observed *in vivo* (Figure 1C). We measured a NCV reduction of approximately 40% (28 m/s to 16.5 m/s, decreasing temperature from 37-38°C to 23°C).

In summary, a method was developed for stable *in vivo* electrophysiology of sensory CNS fibers within the dorsal spinal column. The advantage of our presented method with i. v. anesthesia and artificial respiration is the possibility of the paralyzation of the mouse which distinctly reduces movement artefacts. The method enables the determination of conduction properties of nerve fibers in the CNS of wildtype mice and in mice with transgenic modifications of CNS properties.

Figure legends

Figure 1 *In vivo* recording of dorsal spinal fibers in the mouse

A shows the exposed lumbar spinal cord after laminectomy. Stimulation at the left dorsal root L4 and recording further cranial (left in the picture) at the ipsilateral left fasciculus gracilis at spinal cord level L1 were performed by respective bipolar platinum wire electrodes. Vertebral column was rigidly fixed with two custom-made clamps. ECG was recorded via platinum wires into both forelegs. O_2 blood saturation was recorded via a sensor in the right inguinal region. Body temperature was monitored by a rectal sensor. B shows exemplarily a compound action potential (CAP); red dotted line marks the rising slope (upstroke) of the CAP. The latency between the stimulation time and the begin of the rising slope together with the distance between the electrodes was used for the measurement of the NCV. C shows a reversible reduction of the NCV under hypothermic condition. Body temperature was reversibly reduced from 38 °C to 23 °C. n = 5 adult C57BL/6 mice; SEM.

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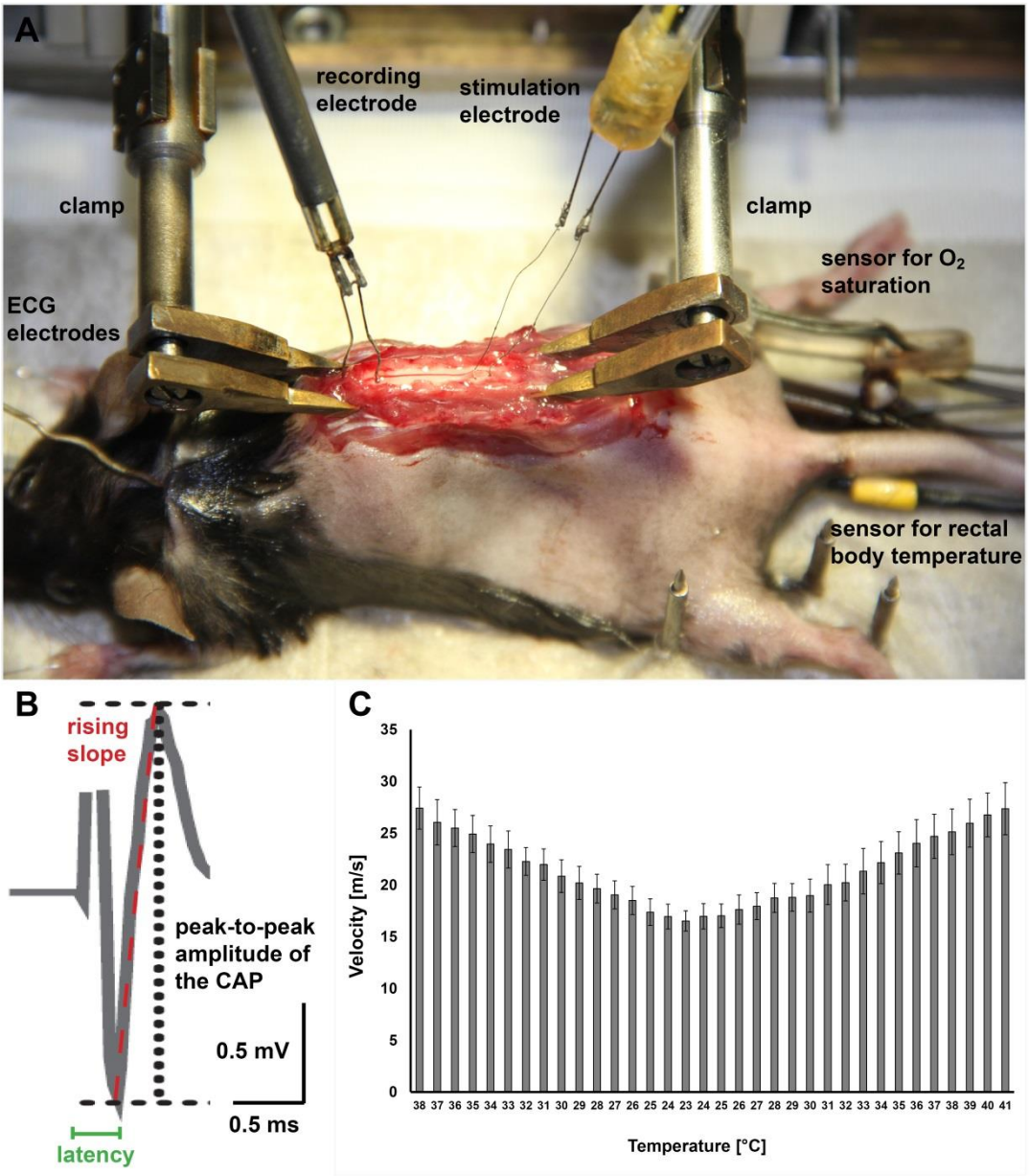
A
recording electrode
stimulation electrode
clamp
clamp
sensor for O_2 saturation
ECG electrodes
sensor for rectal body temperature
B
rising slope
peak-to-peak amplitude of the CAP
0.5 mV
0.5 ms
latency
C
Velocity [m/s]
0
5
10
15
20
25
30
35
38 37 36 35 34 33 32 31 30 29 28 27 26 25 24 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41
Temperature [°C]