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The effects of anesthesia on measures of nerve conduction velocity in male C57Bl6/J mice

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ABSTRACT

Animal models, particularly mice, are used extensively to investigate neurological diseases. Basic research regarding animal models of human neurological disease requires that the animals exhibit hall mark characteristics of the disease. These include disease specific anatomical, metabolic and behavioral changes. Nerve conduction velocity (NCV) is the predominant method used to assess peripheral nerve health. Normative data adjusted for age, gender and height is available for human patients; however, these data are not available for most rodents including mice. NCV may be affected by animal age and size, body temperature, stimulus strength and anesthesia. While the effects of temperature, age and size are documented, the direct and indirect effects of anesthesia on NCV are not well reported. Our laboratory is primarily concerned with animal models of diabetic neuropathy (DN) and uses NCV to confirm the presence of neuropathy. To ensure that subtle changes in NCV are reliably assayed and not directly or indirectly affected by anesthesia, we compared the effects of 4 commonly used anesthetics, isoflurane, ketamine/xylazine, sodium pentobarbital and 2-2-2 tribromoethanol on NCV in a commonly used rodent model, the C57Bl6/J mouse. Our results indicate that of the anesthetics tested, isoflurane has minimal impact on NCV and is the safest, most effective method of anesthesia. Our data strongly suggest that isoflurane should become the anesthetic of choice when performing NCV on murine models of neurological disease.

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Nerve conduction velocity (NCV) is a reproducible measure of peripheral nerve function used to assess and diagnose human neurological disease ranging from heavy metal toxicity [15], metabolic diseases [16], inflammation [24], chemotherapy [13] and genetic disorders [22]. This technique is also used to examine experimental neuropathy based on methods established in human patients and miniaturized for mice [31].

Our laboratory examines the mechanisms driving diabetic neuropathy (DN), a debilitating complication of type 1 and 2 diabetes [7]. Using multiple techniques including behavior, anatomical changes and NCV, we compared the development of DN in multiple models of diabetes in mice [30]. We surveyed over 20 years of literature regarding the methods and models used to examine DN [31]. Of the 65 papers surveyed in 2007, over one-third used NCV as a primary measure of DN.

Multiple factors affect NCV beyond the disease condition being studied, including ambient temperature, needle placement, the intensity of the electrical stimulus and the degree and type of anesthesia. NCV in humans is performed while the patient is awake and responsive. This is usually not the case for animal experiments. By definition, anesthesia slows or blocks nerve impulses and affects synaptic transmission and neuronal function [1,2,27]. We examined four commonly used anesthesia methods including isoflurane (ISO), pentobarbital (PB), ketamine/xylazine (KX) and 2,2,2 tribromoethanol (TBE) and documented their effects on NCV in the C57Bl6/J mouse. We report significant reductions in NCV following PB, KX and TBE induced anesthesia and significant animal mortality with TBE. Comparing across reagents, ISO had the least effect on NCV and surface temperature and was well tolerated by the mice.

Male C57Bl/6 mice ($n = 40$) were purchased from The Jackson Laboratory (Bar harbor, ME) at 12 weeks of age. The animals were provided standard mouse chow (Lab diet 5001, Purina Mills Inc.; Gray Summit, MO), had free access to food and water and were maintained on a 12:12 h light–dark cycle. Mice were randomly assigned to one of four groups receiving the following anesthesia, isoflurane (ISO), ketamine/xylazine (KX), pentobarbital sodium (PB) and 2-2-2 tribromoethanol (TBE), $n = 10$ per group. All animal experiments were performed in compliance with the University

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Table 1
Experimental Protocol and Doses of Anesthesia.

Group ($n = 10$)	Drug dosage (12 weeks, 24 weeks)	Source
Isoflurane (ISO)	4–5% for induction 1–2% for maintenance	Hospira, Inc., Lake Forest, IL
Ketamine/xylazine (KX)	Ketamine 100 mg/kg Xylazine 5 mg/kg	Fort Dodge Animal Health, Fort Dodge, IA/Ben Venue Laboratories; Bedford, OH
Pentobarbital (PB)	50 mg/kg	Ovation Pharmaceutical Inc., Deerfield, IL
2-2-2 Tribromoethanol (TBE)	200 mg/kg	Sigma–Aldrich, St. Louis, MO

All drugs were administrated by intraperitoneal injection with the exception of isoflurane.

Committee on Use and Care of Animals at the University of Michigan.

The experimental protocol and dosages are presented in Table 1. Onset of anesthesia was judged by diminish righting reflex and decreased pedal withdrawal [3–5,9,28,35,36]. Physiological parameters were recorded during the first 5 min of anesthesia. The ambient room temperature was maintained at 22 °C. Monitoring included skin temperature (ear, proximolateral hind limb, tail and dorsal foot) by infrared thermometer (Fluke 63, Everett, WA), core body temperature (Cole-Parmer Instrument Company, Vernon Hills, IL), and cardiopulmonary variables (oxygen saturation, heart rate, and respiratory rate) using a pulse oximeter (Mouse Ox; Starr Life Sciences Corp, Oakmont, PA).

Measures of NCV were performed per our published protocols [30]. Mice were anesthetized and core temperature maintained at 34 °C with a heating lamp. The platinum needle electrodes (ViaSys, Madison, WI) were cleaned with 70% alcohol between animals. Tail sensory NCV (TSNCV) was determined by orthodromically stimulating the proximal 30 mm segment of tail. NCV was calculated by dividing the distance in mm by the take-off latency (measured in ms) of the sensory nerve action potential (Fig. 1). Tail motor distal latency (TDML) was determined by orthodromically stimulating the proximal 30 mm segment of the tail. Latency was measured from initial onset of the compound muscle action potential. Sural sensory NCV (SNCV) was determined by recording at the dorsum of the foot and antidromically stimulating with supramaximal stimulation at the ankle (Fig. 1). NCV was calculated by dividing the distance by the take-off latency of the sensory nerve action potential. Sciatic-tibial motor NCV (SMNCV) was determined by recording at the dorsum of the foot and orthodromically stimulating with supramaximal stimulation first at the ankle, then at the sciatic notch (Fig. 1). 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.

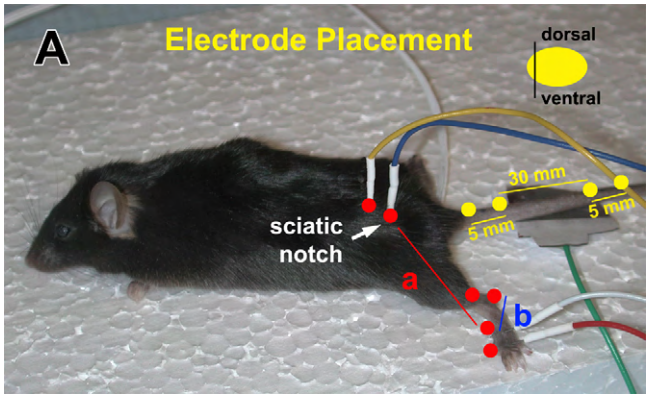

Fig. 1. The recording/stimulating electrodes in the tail (yellow dots) are placed 30 mm apart. In the sciatic nerve, the recording electrode is placed in the dorsum of the foot (b, red dots) and the stimulating electrode in the ankle and sciatic notch (a, red dots). A reference electrode is placed 5 mm distal from the recording/stimulating electrodes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Following NCV measurements at 24 weeks, mice were euthanized by intraperitoneal injection of a sodium pentobarbital overdose (Fatal-plus; Dearborn, MI). Blood samples were collected by vena puncture, placed in 1.5 ml eppendorf tubes and maintained at 22 °C for 20 minutes followed by centrifugation at 10,000 rpm (9.3 rcf) for 15 min. The serum was collected and frozen in liquid nitrogen and stored at −80 °C until analyzed for aspartate transaminase (AST) and alanine transaminase (ALT) activity.

ANOVA test was performed on the data using a null hypothesis set at 05. A Bonferoni post hoc test was used to compare all columns. We assumed a Gaussian distribution of the data.

Motor and sensory NCV were measured in the tail and hind limb. The expected increase in both motor and sensory NCV with age was noted in the hind limb and tail and was not affected by anesthesia. Sensory NCV measured in the hind limb and tail was not differentially affected by any of the anesthetics (Table 2). A significant decrease in SMNCV was detected in the KX and TBE groups at 12 weeks and the PB, KX and TBE groups at 24 weeks of age compared to ISO (Table 2). Additionally, there was a significant increase in latency in the TDML at 12 weeks in the TBE group compare to the ISO group (Table 2).

Peripheral nerves lie close to the surface; therefore, we assessed the effects of anesthesia on surface temperature. When compared to ISO, mice anesthetized with KX exhibited a significantly lower surface temperature measured at the ear, dorsal foot and hind limb at 12 weeks of age (Table 3). This effect was also observed in the hind limb at 24 weeks of age (Table 3). At 24 weeks of age, PB, KX and TBE treated animals exhibited significantly decreased surface temperature measured at the hind limb (Table 3). Core temperature measured in mice at 24 weeks of age demonstrated similar effects of anesthesia as surface measures with significant decreases in animals treated with PB, KX and TBE (Table 3).

Anesthesia depresses heart function and blood flow [26] which may affect both surface temperature and potentially blood flow to the peripheral nervous system. We assessed the effects of ISO, PB, KX and TBE on heart rate, arterial oxygen saturation and respiratory rate. Compared to ISO anesthesia (451.1 ± 7.109), heart rate (HR) was significantly reduced by PB (321.7 ± 16.28), KX (197.2 ± 13.46) and TBE (381.7 ± 8.125) (Fig. 2A). Oxygen saturation was also significantly decreased by PB (90.85 ± 1.8) and TBE (88.97 ± 3.5) compared to ISO ($99.36 \pm .04$) (Fig. 2B). The method of anesthesia also had an impact on respiratory rate. Compared to ISO, KX anesthesia significantly decreased respiratory rate (Fig. 2C).

When performed carefully, NCV may be measured at multiple time points throughout the developmental time course of a disease. Therefore, any long-term toxicity may affect both the disease under study and independently affect NCV. We assessed liver toxicity following the two doses of anesthesia. Serum activities of aspartate transaminase (AST) and alanine transaminase (ALT) were measured (data not shown) to determine the effect of anesthetic agents on

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