Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
x (x): xx, 2025
DOI: 10.9775/kvfd.2025.35321

Research Article

The Effects of Urethane and Ketamine-Xylazine Anesthesia on Electromyographic Measurements in a Streptozotocin-Induced Diabetic Rat Model

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How to cite this article?

Menteşe B, Kale E: The effects of urethane and ketamine-xylazine anesthesia on electromyographic measurements in a streptozotocin-ınduced diabetic rat model. *Kafkas Univ Vet Fak Derg,* x (x): x-x, 2025.
DOI: 10.9775/kvfd.2025.35321

Article ID: KVFD-2025-35321
Received: 19.09.2025
Accepted: 05.12.2025
Published Online: 09.12.2025

Abstract

Diabetes mellitus is a rapidly increasing global health concern, and neuropathy constitutes one of its significant complications. In animal models of diabetic neuropathy, invasive electromyography (EMG) is a widely applied approach. However, the choice of anesthetic agent represents a critical methodological factor, as it can directly modulate nerve conduction and muscle responses, thereby influencing the reliability of electrophysiological outcomes. This study investigated the comparative effects of ketamine-xylazine and urethane anesthesia on EMG parameters in streptozotocin-induced diabetic rats. Electrophysiological assessments of the gastrocnemius muscle demonstrated that urethane anesthesia produced markedly higher amplitudes and prolonged compound muscle action potential (CMAP) durations, potentially masking neuropathic deficits. In contrast, ketamine-xylazine anesthesia preserved the expected electrophysiological hallmarks of diabetic neuropathy, including reduced amplitudes and shortened CMAP durations. These findings indicate that urethane is not a pharmacologically inert anesthetic but one that may artificially alter neuromuscular transmission, leading to misleading interpretations in neuropathy models. Conversely, ketamine-xylazine provides more consistent results aligned with the established pathophysiology of diabetic neuropathy. In conclusion, the selection of anesthetic agent has profound implications for both the validity and translational relevance of electrophysiological research. Therefore, in preclinical neuropathy studies, ketamine-xylazine should be preferred over urethane as a more reliable and methodologically appropriate anesthetic protocol.

Keywords: Diabetes mellitus, Diabetic neuropathies, Streptozotocin, Wistar rat, Electromyography, Anesthesia, Ketamine, Xylazine, Urethane, Neuromuscular transmission

Introduction

Diabetic neuropathy is one of the most common and serious complications of diabetes mellitus, characterized by impaired nerve conduction, muscle weakness, and sensory deficits. To elucidate the underlying mechanisms, experimental animal models are employed, with the streptozotocin (STZ)-induced diabetic rat model being widely preferred due to its reproducibility and similarity to human pathophysiology. Electrophysiological methods, particularly electromyography (EMG) and compound muscle action potential (CMAP) recordings, are considered the gold standard for evaluating peripheral nerve conduction and neuromuscular function in these models.

In animal experiments, anesthesia is mandatory for performing invasive electrophysiological recordings. However, since the anesthetic agent used may directly or indirectly influence nerve conduction, synaptic transmission, and muscle responses, it is regarded as a methodologically critical variable [1]. The ketamine–xylazine combination is one of the most commonly used protocols in rodent studies, owing to its rapid onset of action and ability to provide adequate surgical depth. It has been reported that ketamine and xylazine affect glucose metabolism and cardiovascular stability [2-4] On the other hand, urethane is known for providing long-lasting and stable anesthesia, with relatively limited effects on cardiovascular and respiratory functions, and is therefore preferred in electrophysiological studies [5,6].

Nevertheless, urethane has been shown to exert direct effects on neurotransmitter systems, with evidence indicating its ability to modulate nicotinic acetylcholine receptor activity and acetylcholine release [7-9] This situation raises methodological concerns, particularly in

experimental designs where neuromuscular transmission is a primary parameter. Considering the increased sensitivity of diabetic animals to anesthetic agents, the selection of an appropriate anesthesia protocol is critically important not only for animal welfare but also for the reliability and reproducibility of the data obtained.

This study aims to provide a methodological contribution to the electrophysiological approaches used in diabetic neuropathy models by comparing the effects of ketamine-xylazine and urethane anesthesia on EMG recordings in STZ-induced diabetic rats.

Materials and Methods

Ethical Statement

This study was approved by the Manisa Celal Bayar University Local Ethics Committee for Animal Experiments (Approval date and number: 29/07/2025; 77.637.435/331).

Experimental Animals

A total of 18 male Wistar albino rats (22-24 weeks old, 350-400 g) were obtained from the institutional Experimental Animal Application and Research Center. The animals were housed under standard laboratory conditions (22±2°C, 50±10% humidity, 12/12 h light-dark cycle) with free access to water and standard pellet food. Rats were kept in polycarbonate cages containing corn-cob bedding, which was replaced once per week. Trained personnel monitored the animals daily for general health, activity, grooming, and hydration. All housing and husbandry procedures complied with institutional animal care guidelines and the ARRIVE recommendations.

Experimental Groups

The animals were randomly divided into four groups:

KetamineC (n=5): Control rats under ketamine-xylazine anesthesia

KetamineD (n=4): Diabetic rats under ketamine-xylazine anesthesia

UrethaneC (n=4): Control rats under urethane anesthesia

UrethaneD (n=5): Diabetic rats under urethane anesthesia

Diabetes Induction

In the KetamineD and UrethaneD groups, diabetes was induced after 6-8 h of fasting by a single intraperitoneal (i.p.) injection of streptozotocin (STZ; 60 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) freshly prepared in 50 mM sodium citrate buffer (pH 4.5). To prevent acute hypoglycemia, the animals were provided with 10% sucrose solution for 48 h following the injection. Seventy-two h after the injection, blood glucose levels were measured from the tail vein (Accu-Chek Active, Roche, Germany), and animals with blood glucose levels > 300 mg/dL were considered diabetic [10]. Throughout the 4-week experimental period, the body weights and fasting glucose levels of the animals were recorded on a weekly basis.

Anesthesia Protocols

At the end of the fourth week, prior to electrophysiological recordings, the following anesthetic protocols were administered to the animals:

Ketamine groups (KetamineC, KetamineD): Ketamine 75 mg/kg + Xylazine 10 mg/kg, i.p.

Urethane groups (UrethaneC, UrethaneD): Urethane 1.5 g/kg, i.p.

The depth of anesthesia was assessed by pedal reflex, and rectal temperature was continuously monitored and maintained at approximately 36.5°C.

Electrophysiological Recordings

Under deep anesthesia, an incision of approximately 2.5 cm was made on the posterior surface of the right hind limb to expose the sciatic nerve. Bipolar hook electrodes (10 mm length, 0.35 mm diameter, 3 mm interelectrode distance) were placed on the nerve. The recording electrode (needle type) was inserted into the gastrocnemius muscle between the tendons, approximately 15 mm distal to the sciatic trifurcation. Electrical stimuli were delivered at supramaximal intensity with a duration of 0.2 msec, a frequency of 1 Hz, and an initial current of approximately 0.1 mA [11]. Electromyographic recordings were obtained using the LabChart 7 software (ADInstruments, Australia), and the following parameters were evaluated: latency (msec), amplitude (mV), and CMAP duration (msec).

At the end of the electrophysiological recordings, all animals anesthetized with urethane were humanely euthanized before regaining consciousness, in accordance with the institutional animal ethics approval and the AVMA Guidelines for the Euthanasia of Animals (2020). Urethane was used solely for acute terminal EMG procedures, and no survival or long-term follow-up was performed in urethane-treated rats.

Statistical Analysis

Data were presented as median (minimum-maximum). Normality was assessed using the Shapiro-Wilk test. The Kruskal-Wallis test was employed for comparisons among groups, and the Mann-Whitney U test was used for pairwise comparisons. Values of $P<0.05$ were considered statistically significant. Statistical analyses were performed using SPSS version 30.0 software (IBM Corp., Armonk, NY, USA). A post-hoc power analysis was performed in G*Power 3.1 using the CMAP duration data from diabetic

rats anesthetized with Ketamine (n=4) and urethane (n=5). The standardized effect size for the difference between these groups was calculated as Cohen's d=2.5567. Using a two-tailed α=0.05, the achieved power of the study was 0.90.

Results

Demographic Characteristics of the Experimental Diabetic Groups

The demographic evaluation of the experimental diabetic groups revealed that both KetamineD and UrethaneD animals exhibited persistent hyperglycemia throughout the study period. On day 3 following STZ induction, mean blood glucose levels were markedly elevated in both groups (KetamineD: 600.00±0.00 mg/dL; UrethaneD: 484.20±76.06 mg/dL). During the first week, glucose values remained high, with the UrethaneD group showing slightly higher mean levels (558.00±93.91 mg/dL) compared to the KetamineD group (449.50±42.68 mg/dL). By weeks 2–4, both groups sustained glucose concentrations above the diabetic threshold, with mean values exceeding 500 mg/dL across all time points (Week 2: 582.00±20.78 vs. 575.20±55.45 mg/dL; Week 3: 514.00±53.63 vs. 550.20±45.73 mg/dL; Week 4: 523.25±91.84 vs. 591.40±19.23 mg/dL, for KetamineD and UrethaneD respectively).

In terms of body weight, both groups demonstrated a gradual reduction following STZ administration, consistent with the diabetic phenotype. The mean body weight of KetamineD rats decreased from approximately 370 g at baseline to ~314 g at week 4, whereas UrethaneD rats declined from ~345 g to ~324 g in the same period. These findings confirm the successful establishment of experimental diabetes in both groups and indicate comparable metabolic alterations across anesthetic regimens.

As shown in *Table 1*, significant overall differences were observed among the groups in terms of latency, amplitude, and CMAP duration. The urethane-anesthetized groups exhibited shorter latencies and higher amplitude values compared with the ketamine groups. In addition, CMAP durations tended to be longer in the urethane groups, particularly in diabetic animals, whereas the shortest durations were observed in the ketamine diabetic group.

Table 1. *Electrophysiological parameters of experimental groups*

EMG Parameter	KetamineC Median (Min-Max)	KetamineD Median (Min-Max)	UrethaneD Median (Min-Max)	UrethaneC Median (Min-Max)	P Value
Latency (msec.)	1.25 (1.25-1.75)	2.00 (1.00-2.25)	1.75 (1.50-2.25)	0.88 (0.75-1.00)	0.015
Amplitude (mV)	14.05 (11.09-21.09)	15.25 (9.60-19.62)	22.46 (18.39-41.22)	24.44 (19.02-33.53)	0.016
CMAP duration (msec.)	4.50 (4.00-4.75)	2.13 (1.75-2.50)	3.00 (2.75-4.00)	3.13 (2.75-4.00)	0.003

Values are presented as median (minimum-maximum). KetamineC: Ketamine control (n=5), KetamineD: Ketamine diabetic (n=4), UrethaneC: Urethane control (n=4), UrethaneD: Urethane diabetic (n=5). Statistical analysis was performed using the Kruskal–Wallis test. P<0.05 was considered statistically significant.

As shown in *Table 2*, the KetamineC group exhibited a significantly longer CMAP duration compared with the KetamineD group, accompanied by an extremely large effect size. In addition, latency values in the UrethaneD group were significantly higher than those in both the KetamineC and UrethaneC groups. A significant difference was also observed between UrethaneD and UrethaneC, where UrethaneD demonstrated markedly higher latency with a very large effect size. Furthermore, the latency comparison between KetamineD and UrethaneC was significant and associated with a very large effect size. Although the remaining comparisons were not statistically significant, several displayed large effect sizes, suggesting substantial anesthetic-related differences.

As shown in *Fig. 1*, individual CMAP latency values demonstrated a clear separation between groups. Ketamine-treated control rats (KetamineC) exhibited

Table 2. *Bonferroni-adjusted pairwise comparisons and effect sizes (Cohen's d) for electrophysiological parameters among experimental groups*

Comparison	Parameter	Adj p	Cohen's d
KetamineC vs KetamineD	Latency	1.000	−1.03
	Amplitude	1.000	0.07
	CMAP duration	0.001	7.86
KetamineC vs UrethaneD	Latency	0.023	−1.43
	Amplitude	0.091	−1.40
	CMAP duration	0.255	3.19
KetamineC vs UrethaneC	Latency	0.560	2.71
	Amplitude	0.142	−1.89
	CMAP duration	0.393	2.93
KetamineD vs UrethaneD	Latency	1.000	0.03
	Amplitude	0.204	−1.36
	CMAP duration	0.405	−2.56
KetamineD vs UrethaneC	Latency	0.041	2.32
	Amplitude	0.204	−1.83
	CMAP duration	0.255	−2.53
UrethaneD vs UrethaneC	Latency	0.023	3.51
	Amplitude	1.000	−0.02
	CMAP duration	1.000	−0.10

Median (min-max) values are reported for each pairwise group comparison. Adjusted P-values represent Bonferroni-corrected Mann-Whitney U post-hoc tests, and values of P<0.05 were considered statistically significant. Effect sizes (Cohen's d) were calculated using the SPSS "Independent Samples Effect Sizes" module based on pooled standard deviations. Positive d values indicate higher parameter values in the first group of each comparison, whereas negative d values indicate higher values in the second group

relatively lower and more clustered latency values, whereas diabetic rats under ketamine anesthesia (KetamineD) showed a wider distribution with generally higher latencies. In the urethane-anesthetized diabetic group (UrethaneD), latency values remained elevated and displayed greater variability compared with controls. In contrast, urethane-treated control rats (UrethaneC) presented the lowest and most narrowly distributed latencies among all groups.

As shown in *Fig. 2*, individual CMAP amplitude values exhibited a distinctly different distribution across the groups. The ketamine-treated control group (KetamineC) displayed lower amplitudes with a narrow distribution range. The diabetic ketamine group (KetamineD) showed similarly low amplitudes, although the variability was slightly greater. Diabetic rats evaluated under urethane anesthesia (UrethaneD) demonstrated higher and more widely distributed amplitude values, clearly separating them from the ketamine groups. The highest amplitude values were observed in the urethane-treated control group (UrethaneC), which appeared markedly different from all other groups in terms of both amplitude magnitude and distribution range.

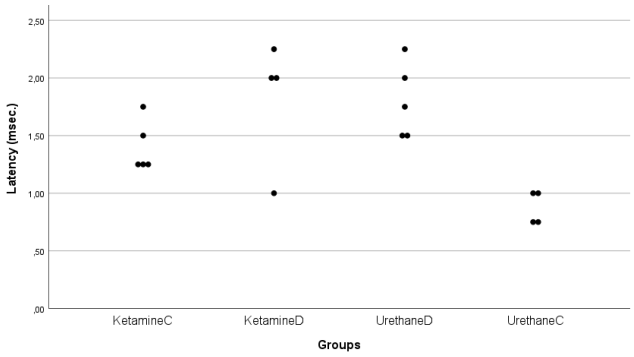

Fig 1. Individual CMAP latency values across experimental groups. KetamineC: Ketamine-Control; KetamineD: Ketamine-Diabetic; UrethaneD: Urethane-Diabetic; UrethaneC: Urethane-Control

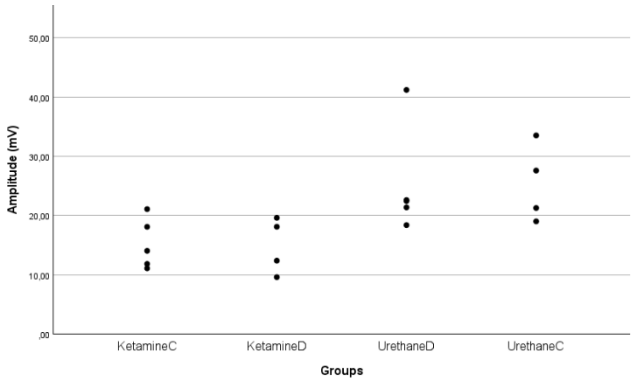

Fig 2. Individual CMAP amplitude values across experimental groups. KetamineC: Ketamine-Control; KetamineD: Ketamine-Diabetic; UrethaneD: Urethane-Diabetic; UrethaneC: Urethane-Control

As shown in *Fig. 3*, individual CMAP duration values exhibited a distinct distribution pattern across the groups. The ketamine-treated control group (KetamineC) displayed the highest CMAP durations, with values tightly clustered within a narrow range. In contrast, the diabetic ketamine group (KetamineD) showed markedly lower CMAP durations, with values clustered at the lower end of the distribution. Diabetic rats evaluated under urethane anesthesia (UrethaneD) presented moderate CMAP durations, with a broader distribution compared to the ketamine groups. The urethane-treated control group (UrethaneC) demonstrated a distribution pattern similar to that of the UrethaneD group, with CMAP durations remaining within a moderate range.

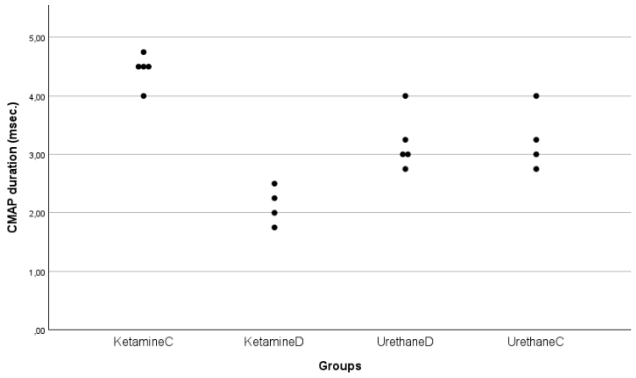

Fig 3. Individual CMAP duration values across experimental groups. KetamineC: Ketamine-Control; KetamineD: Ketamine-Diabetic; UrethaneD: Urethane-Diabetic; UrethaneC: Urethane-Control

Discussion

In this study, the effects of different anesthesia protocols on electrophysiological measurements were investigated in STZ-induced diabetic rats. Our findings demonstrate that ketamine-xylazine anesthesia revealed the expected neuropathic patterns, whereas urethane anesthesia significantly altered the compound muscle action potential (CMAP) parameters. In particular, the marked increase in amplitude and the prolongation of CMAP duration were found to have the potential to mask neuropathic impairments. This suggests that urethane may lead to misleading electrophysiological outcomes in disease models.

Urethane is frequently preferred in electrophysiological studies due to its ability to provide long-lasting and stable anesthesia, along with its relatively limited effects on cardiorespiratory functions [7,12]. However, it is not a pharmacologically inert agent. Studies in the literature have demonstrated that urethane enhances GABAergic transmission, suppresses glutamatergic signaling, and may increase nicotinic receptor activity within the cholinergic system through acetylcholinesterase inhibition [7-9,13-15]. These pharmacological properties may account for

the increase in CMAP amplitude and the prolongation of duration observed in our study. Furthermore, a recent investigation demonstrated that urethane and alternative agents produced distinct transmission profiles in visual system electrophysiology, showing that the choice of anesthetic exerts a significant impact on signal amplitude and temporal dynamics [16,17]. This finding supports the notion that urethane may artificially alter electrophysiological outcomes from a methodological perspective.

Urethane has also been reported to modify endocrine function, including an increase in plasma insulin levels that become evident approximately 20 min after administration in rats [18,19]. In the present study, however, urethane was administered as a single terminal bolus, and EMG recordings were completed within approximately 5-6 min of injection, with each individual recording lasting about 1 min. Thus, the time window during which electrophysiological data were acquired is likely to precede or only minimally overlap with the delayed endocrine effects described in that study. Moreover, diabetic neuropathy is a chronic complication, and it remains uncertain to what extent a short-lived, acute change in circulating insulin could rapidly modify nerve or muscle electrophysiology within a few minutes. Taken together, these considerations suggest that, while subtle metabolic influences cannot be fully excluded, they are unlikely to be the primary driver of the differences observed between anesthetic protocols. Instead, our findings are more consistent with the direct neurophysiological actions of urethane on synaptic transmission and membrane excitability.

The NMDA receptor antagonism of ketamine and the sedative and analgesic effects of xylazine through α2-adrenergic agonism have made this combination one of the most widely used protocols in experimental neurophysiology [20]. Several studies have reported that ketamine-xylazine anesthesia has minimal effects on peripheral nerve conduction and CMAP recordings [2,3,5]. In our study, the low amplitudes and short CMAP durations observed in the ketamine groups were found to be consistent with the expected electrophysiological pattern of diabetic neuropathy [21]. In addition, the literature has reported that in STZ-diabetic rats, sufficient anesthetic depth could not be achieved with ketamine + xylazine or medetomidine + ketamine, and that diabetes increases anesthetic sensitivity [22]. In one study, it was shown that the induction time with ketamine-xylazine was shortened in diabetic rats; however, this difference was not associated with glucose level or body weight [23]. These data suggest that ketamine-xylazine can preserve electrophysiological reliability even in diabetic models.

Diabetic neuropathy is characterized by reduced conduction velocity, prolonged latency, and decreased amplitude [24]. While these alterations were observed in the ketamine group in accordance with the literature, the increase in amplitude and prolongation of duration seen in the urethane group may present neuropathic impairment as less severe than it actually is. This methodological issue complicates the interpretation of findings in diabetic neuropathy studies and may lead to conflicting results across laboratories.

CMAP measurements are widely used objective parameters not only in experimental neuropathy models but also in clinical studies [25]. Therefore, the significant alteration of electrophysiological outcomes by urethane represents a methodological risk in the translation of preclinical data to human disease. Our findings indicate that the ketamine–xylazine protocol is more appropriate in terms of translational reliability.

In conclusion, this study demonstrates that urethane anesthesia can influence electrophysiological outcomes in diabetic rats and may lead to changes in CMAP parameters that partially obscure neuropathic alterations. Our findings indicate that, particularly in disease models where subtle neuropathic changes must be assessed with precision, electrophysiological data obtained under urethane anesthesia should be interpreted with caution. In contrast, the ketamine–xylazine combination provides more consistent and interpretable CMAP results in this experimental model. Nevertheless, further studies with larger sample sizes and additional physiological parameters are needed to more clearly determine the comparative suitability of these anesthetic agents in diabetic neuropathy research.

This study has several limitations. First, only a diabetic neuropathy model was used, and it remains unknown whether urethane exerts similar electrophysiological effects in neuropathies induced by chemotherapy, trauma, or other systemic diseases. Second, receptor-level or molecular analyses that could directly clarify the pharmacological actions of urethane were not performed. Third, while electrophysiological alterations in CMAP parameters support the presence of neuropathic involvement, EMG findings alone cannot definitively confirm diabetic neuropathy. Comprehensive confirmation would require additional assessments such as sensory nerve conduction studies, motor unit number estimation (MUNE), intraepidermal nerve fiber density measurements, or behavioral pain evaluations. Fourth, the total number of animals used in this study was relatively small, and the group sizes were slightly unbalanced, which may limit the generalizability of the findings. Although a post-hoc power analysis (Cohen's $d=2.5567$; $1-\beta=0.90$) demonstrated adequate statistical strength for the primary electrophysiological comparison, the exploratory pilot nature of the study requires cautious

interpretation. Larger future studies with prospectively calculated sample sizes are necessary to validate and expand these observations. Fifth, advanced physiological monitoring (e.g., HR, MAP, SpO_2, $EtCO_2$, EEG/BIS) was not performed. Reflex-based assessments were used, but the lack of continuous cardiorespiratory data limits the precision with which anesthetic depth can be interpreted. Future studies incorporating invasive or non-invasive physiological monitoring systems may better characterize the relationship between anesthetic depth and EMG outcomes.

In future research, combining pharmacological antagonists with neurotransmitter analyses may provide deeper insight into the neural mechanisms underlying urethane's electrophysiological effects. Furthermore, the inclusion of additional electrophysiological assessments such as sensory nerve potentials or MUNE could strengthen methodological robustness. Considering the markedly elevated amplitude values observed in the urethane group, future studies may also explore whether urethane has potential applications in investigating -or possibly mitigating-muscle damage associated with neuropathy.

Declarations

Availability of Data and Materials: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments: The authors would like to thank the staff of the Experimental Animal Application and Research Center for their valuable technical assistance during the animal experiments.

Funding Support: This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing Interest: The authors declare that they have no conflict of interest.

Declaration of Generative Artificial Intelligence (AI): No generative AI tools were used to create or write the scientific content, tables, figures, or analyses presented in this manuscript. AI-based tools (OpenAI) were used solely to improve the clarity and readability of the language. Reference organization was performed using Mendeley software.

Author Contributions: B.M. contributed to the study conception and design, development of the experimental protocol, execution of EMG recordings, data analysis, and drafting of the manuscript. E.K. contributed to animal care and daily monitoring, diabetes induction, administration of anesthesia, and technical assistance during electrophysiological procedures, as well as the critical revision of the manuscript for important intellectual content. Both authors contributed to the interpretation of the results, reviewed the manuscript critically, and approved the final version of the article. The authors affirm that all listed contributors meet the journal's Authorship Rights and Ethical Principles, and no individuals other than the authors contributed to the scientific content of this study.

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