Original article

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Influence of General Anesthesia and Surgical Procedure on Functional Recovery Following Rat Sciatic Nerve Transection and Repair

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Objective. To evaluate the effects of the type of general anesthesia and surgical procedure on sensory and motor functional recovery following sciatic nerve transection with immediate neurorrhaphy in rats.

Materials and methods. The study was conducted in male Wistar rats using an experimental model of complete sciatic nerve transection followed by immediate end-to-end suture repair employing either two or four interrupted epineurial sutures. General anesthesia was induced using either inhaled isoflurane or an intraperitoneal ketamine/xylazine mixture. During the postoperative period (up to 24 weeks), thermal and mechanical sensitivity were assessed, along with motor function recovery evaluated by the Sciatic Functional Index (SFI).

Results. The type of general anesthesia significantly affected the pattern and dynamics of sensory and motor functional recovery after neurorrhaphy. Animals operated under ketamine/xylazine anesthesia demonstrated more stable and complete recovery of both sensory and motor functions, as evidenced by the absence of persistent mechanical and thermal hypoalgesia and by significant improvement in SFI values, even when the minimum number of epineurial sutures was used. In contrast, the use of inhalational isoflurane anesthesia was associated with more prolonged postoperative sensory deficits, which were largely dependent on the surgical procedure. Under isoflurane anesthesia, the application of four epineurial sutures provided more favorable conditions for sensory recovery, particularly mechanical sensitivity, compared with neurorrhaphy performed using two sutures.

Conclusions. The type of general anesthesia and the characteristics of the suture repair technique are important determinants of sciatic nerve regeneration and functional recovery following transection. Ketamine/xylazine anesthesia is associated with more favorable conditions for sensorimotor recovery. The use of inhalational isoflurane anesthesia during neurorrhaphy, particularly when a minimal number of sutures is employed, should be approached with caution, as it may adversely affect the stability and functional outcomes of sciatic nerve recovery.

Keywords: *peripheral neuropathy; neurorrhaphy; peripheral nerve regeneration; isoflurane; ketamine; sciatic nerve; Hargreaves test; von Frey test; Sciatic Functional Index.*

Introduction

Peripheral nerve injury (PNI) is a relatively uncommon pathology during peacetime [1–8]; however, its incidence increases during military conflicts [9] due to the predominance of combat-related limb injuries [4, 6]. Although PNI is generally considered the least severe form of injury affecting the nervous system, it is characterized by persistent sensorimotor, trophic, and pain-related disorders [10–12], resulting in substantial direct [3, 13] and indirect economic losses. PNI predominantly affects working-age adults and occurs more frequently in men [1–3, 5]. Treatment is predominantly surgical [2, 8] and primarily involves restoration of the anatomical continuity of the injured nerve through direct suturing of its severed ends, i.e., neurorrhaphy [2, 14].

Despite the relatively high regenerative capacity of the peripheral nervous system and significant advances in surgical techniques for PNI management, treatment outcomes remain suboptimal [15]. The development of novel therapeutic approaches for PNI is therefore largely conducted in experimental settings [7, 16]. For obvious reasons, such research critically depends on the quality and reproducibility of experimental injury models. Despite its relative simplicity, the widely used rat sciatic nerve transection model in experimental neurosurgery is characterized by limited reproducibility of outcomes [17]. This limitation may be related to shortcomings of the functional–anatomical assessment method based on the sciatic functional index (SFI), differences in the duration of postoperative observation of experimental

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animals [17], variations in neurorrhaphy techniques, and differences in anesthetic management.

General anesthesia is an essential component of surgical procedures in experimental medicine and biology, ensuring the reduction of pain and distress in laboratory animals. This requirement has stimulated the development of noninvasive anesthetic techniques, particularly inhalational anesthesia [18]. Additional advantages of these methods include rapid induction and recovery, as well as the ability to control the depth of anesthesia during surgery [18]. However, following inhalational anesthesia, the rapid restoration of motor activity in animals subjected to PNI modeling and immediate nerve repair may compromise the integrity of the neurorrhaphy. Furthermore, early mobilization has been shown to impair nerve regeneration even when suture integrity is maintained [19]. Consequently, postoperative immobilization of the affected limb is routinely employed in clinical practice following peripheral nerve repair [20]. Another important strategy for enhancing the reliability of nerve coaptation is the application of an adequate number of epineurial or perineural interrupted sutures. Nevertheless, increasing the number of sutures has limitations, as the persistence of xenogeneic suture material may trigger local inflammatory responses that restrict and slow the regenerative growth of nerve fibers [21].

Objective: To evaluate the effects of the type of general anesthesia and surgical procedure on the recovery of sensory and motor functions following rat sciatic nerve transection with immediate neurorrhaphy.

Materials and methods

Experimental animals and experimental groups

The study was conducted in accordance with current bioethical standards and research ethics guidelines and was approved by the Biomedical Ethics Committee of the O.O. Bogomolets Institute of Physiology, NAS of Ukraine (Protocol No. 3/22, November 16, 2022). All experimental procedures complied with the provisions of the Convention on Human Rights and Biomedicine (Council of Europe, 1997), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), the General Ethical Principles for Scientific Research adopted by the First National Congress on Bioethics of Ukraine (September 2001), and the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (2006).

Male Wistar rats aged 1.5–2.0 months and weighing 190–230 g were obtained from the animal facility of the O.O. Bogomolets Institute of Physiology, National Academy of Sciences of Ukraine. Animals were housed under standard laboratory conditions with a 12-h light/dark cycle, an ambient temperature of (22 ± 2) °C, and ad libitum access to food and water.

Animals were allocated into three experimental groups according to the type of general anesthesia (inhalational isoflurane or intraperitoneal ketamine/xylazine) and the number of sutures used during surgical modeling of peripheral nerve injury (PNI) with neurorrhaphy:

Group 1 — sciatic nerve transection under isoflurane anesthesia followed by immediate nerve reconstruction using two epineurial sutures (n = 6);

Group 2 — sciatic nerve transection under ketamine/xylazine anesthesia followed by immediate nerve reconstruction using two epineurial sutures (n = 5);

Group 3 — sciatic nerve transection under isoflurane anesthesia followed by immediate nerve reconstruction using four epineurial sutures (n = 6).

Sciatic nerve injury modeling and repair. In animals of Groups 1 and 3, general anesthesia was induced and maintained with inhalational isoflurane using an anesthesia machine manufactured by "RWD Life Science", China. Anesthesia induction was performed in an induction chamber with 3% isoflurane. After achieving a surgical plane of anesthesia, the animal was transferred to a heated surgical table and fitted with a specialized face mask through which isoflurane was delivered at a concentration of 1.5–2.5%, with continuous monitoring of respiration.

In animals of Group 2, general anesthesia was induced by intraperitoneal administration of a mixture of xylazine hydrochloride (15 mg/kg; "Biowet", Poland) and ketamine hydrochloride (75 mg/kg; "Farmak", Ukraine). Drug doses were individually calculated for each animal.

In both anesthetic protocols, anesthetic depth was assessed by response to hind paw pinch, whereas the degree of muscle relaxation was evaluated manually and by visual inspection. In cases of insufficient anesthesia or muscle relaxation, the duration of induction with 3% isoflurane was extended, or an additional dose of the ketamine–xylazine mixture (one-third of the calculated dose) was administered.

The technique for sciatic nerve injury modeling and neurorrhaphy in rats has been described in detail in a previous publication [17]. Following adequate analgesia and muscle relaxation, animals were positioned prone, with the limbs placed at an angle of approximately 45° relative to the longitudinal axis of the body. A support pad was placed beneath the left iliac region. The skin of the left posterior thigh was shaved, disinfected with a 10% Betadine® solution ("Egis", Hungary), and incised longitudinally, parallel to the femur. The sciatic nerve was exposed by blunt muscle separation, transected at the mid-thigh level, and immediately repaired by end-to-end approximation of the nerve stumps using either two interrupted epineurial sutures (Groups 1 and 2) or four interrupted epineurial sutures (Group 3) with a 9-0 atraumatic needle ("Olymp", Ukraine) under a stereomicroscope ("Olympus SZX7", Japan). The lateral margins of the thigh muscles mobilized during the surgical approach, as well as the skin edges, were closed in two layers using interrupted 4-0 sutures ("Olymp", Ukraine). The wound was subsequently treated with a 10% Betadine® solution ("Egis", Hungary). Postoperatively, Bicillin-5 solution (600,000 IU/kg; "Kyivmedpreparat", Ukraine) was administered subcutaneously into the posterior cervical region, while dexamethasone solution (5 mg/kg; KRKA, Slovenia) was injected into the right posterior thigh.

Animals in Groups 1 and 3 were maintained under elevated ambient temperature conditions until full recovery of spontaneous motor activity (approximately 30 min), whereas animals in Group 2 were kept at room temperature until reaching a comparable recovery state (approximately 2 h).

Motor functional assessment. The SFI was determined in all experimental groups over a 24-week period following surgery. Paw prints required for SFI calculation were obtained using the Gait2SFI system (***Fig. 1***), which enables high-resolution video recording of animal locomotion with both high spatial resolution (5.3 K; 5312 × 2988 pixels) and temporal resolution (60 fps), as well as a substantial depth of field. The system also allows identification of paw–surface contact areas using the frustrated total internal reflection (FTIR) method [22]. Optimization of illumination parameters combined with the large depth of field enabled simultaneous high-quality visualization of both the contact regions and the entire paw. Subsequent processing of the recordings using dedicated software permits frame-by-frame analysis of anatomical paw landmarks and semi-automated calculation of SFI values [23].

SFI values were calculated according to the Bain–Mackinnon–Hunter formula [24], using morphometric measurements obtained from the highest-quality paw-print images:

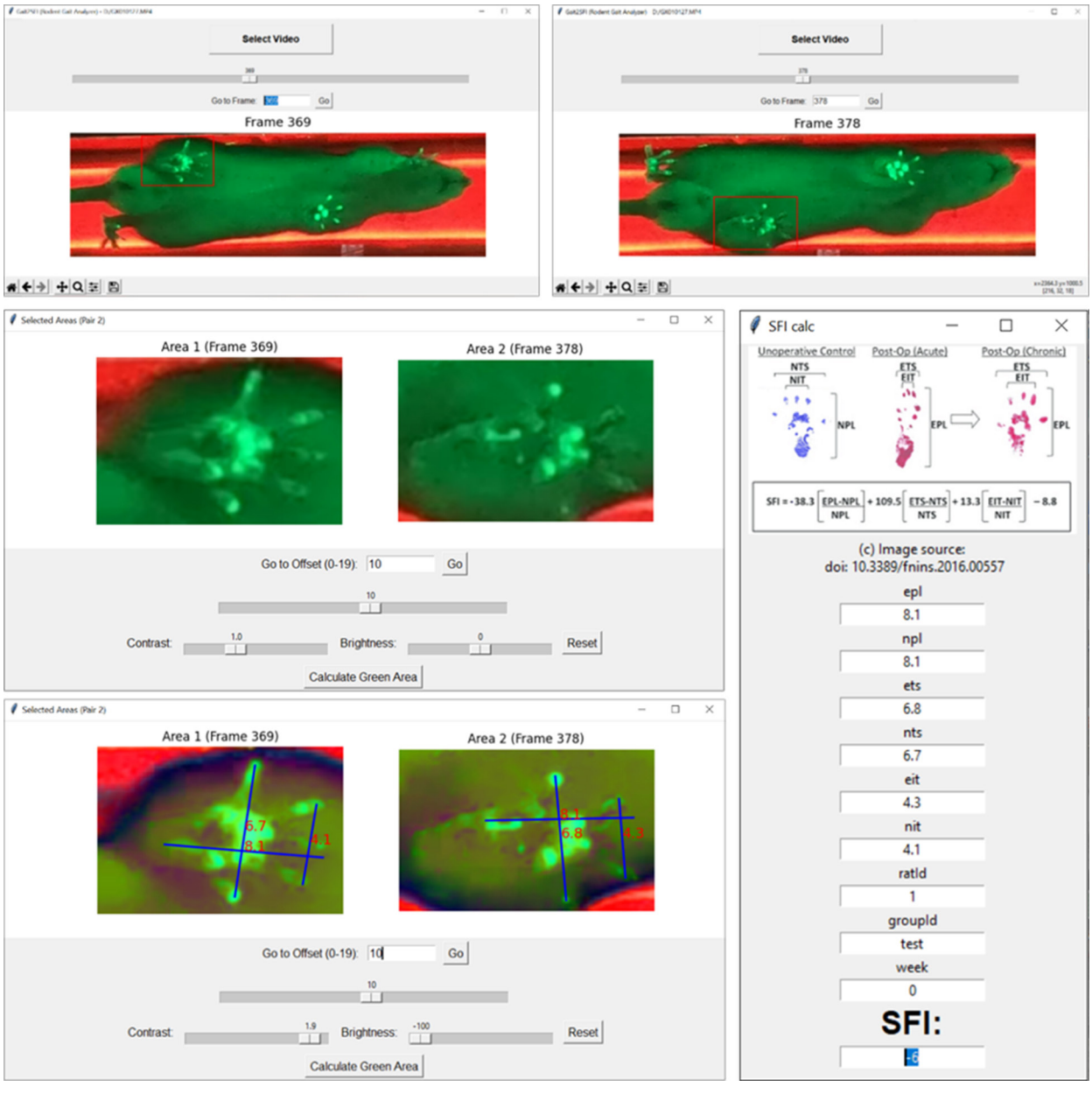

Fig. 1. The Gait2SFI hardware–software platform enables acquisition of video recordings during animal gait testing with high-contrast paw prints, in which surface contact areas are highlighted in green, and provides semi-automated calculation of the SFI. The upper row presents sequential frames from a single step cycle of a rat, displayed in the software interface and used for SFI determination. The middle row shows cropped regions of the same frames illustrating the hind paw prints. Bright green areas indicate locations in direct contact with the walking surface, whereas the remaining portions of the paw are visible due to illumination by diffuse green light. In the lower row, adjustment of image contrast and brightness within the software enhances visualization of contact areas and enables measurement of parameters required for SFI calculation. The measured parameters are entered into a calculation table (right panel). The software performs SFI calculation automatically, and the resulting values are stored together with the corresponding morphometric parameters in a unified database

$$SFI = -38.3 \times \frac{PL_E - PL_N}{PL_N} + 109.5 \times \frac{TS_E - TS_N}{TS_N} + 13.3 \times \frac{IT_E - IT_N}{IT_N} - 8.8 ,$$

*where **E** denotes the injured limb and **N** the intact limb; **PL** is the print length, measured as the distance from the heel to the tip of the longest toe; **TS** is the toe spread, defined as the distance between the first and fifth toes; and **IT** is the intermediary toe spread, defined as the distance between the second and fourth toes.*

Sensory function assessment. Quantitative evaluation of changes in nociceptive thresholds following surgery was performed using two standardized behavioral assays: the Hargreaves test (assessment of thermal nociception) and the von Frey test (assessment of mechanical nociception), as previously described [25,26]. Testing was conducted on the lateral plantar surface of the hind paw within the cutaneous innervation territory of the tibial and sural branches of the sciatic nerve on both the operated and intact limbs. Baseline measurements were obtained before surgery, whereas subsequent assessments were performed between Weeks 2 and 24 postoperatively.

Mechanical sensitivity, encompassing both tactile and nociceptive components, was evaluated using the von Frey test. Animals were individually placed in cages with a wire-mesh floor and allowed to acclimatize for at least 30 min. Mechanical thresholds were determined using a calibrated set of nylon monofilaments (Touch-Test, Stoelting, Cat. No. 58011), which were applied perpendicularly to the plantar surface of the hind paw until the filament bent, thereby delivering a constant force (expressed in grams) for 2–3 s. A positive response was defined as paw withdrawal, licking of the tested limb, or vocalization during or immediately after filament application. Testing was performed only when animals were awake, calm, immobile, and not engaged in grooming behavior.

In Group 2 (ketamine/xylazine anesthesia, two epineurial sutures) and Group 3 (isoflurane anesthesia, four epineurial sutures), paw withdrawal thresholds (PWT) were determined using the "up–down" method. Testing began with a 26-g filament. If a positive response was observed, a filament with a lower force was subsequently applied; if no response occurred, a filament with a higher force was used. Following the first reversal in response direction, at least five additional stimulations were performed according to the up–down algorithm. The PWT was defined as the force corresponding to a 50% probability of response and was calculated using the standard Dixon–Chaplan formula [27] based on the sequence of positive and negative responses:

$$50\%\ \text{threshold (g)} = \frac{10^{x+kd}}{10\,000} ,$$

*where **X** is the logarithmic value of the force of the final filament used; **k** is the tabulated value corresponding to the observed response pattern; and **d** is the mean logarithmic increment between adjacent filament forces. Threshold values were calculated using the Von Frey 1.0 online calculator (DTU Health Tech) [28]. The resulting values were expressed in grams and used for statistical analysis.*

In Group 1 (isoflurane anesthesia, two epineurial sutures), a "bottom-up" von Frey paradigm was employed. Testing began with the filament exerting the lowest force, followed by sequential application of filaments with progressively greater forces. Each filament was applied five times with intervals between stimulations. A response was considered positive when a clear protective reaction was observed, including paw withdrawal, shaking, or licking. The PWT was defined as the force of the filament that elicited a protective response in at least three of five applications. This value was used as the individual measure of mechanical sensitivity for the corresponding animal and time point.

Thus, mechanical sensitivity was assessed using two distinct von Frey paradigms. The "bottom-up" method provides a discrete measure of suprathreshold responsiveness, whereas the "up–down" method estimates the force corresponding to a 50% probability of paw withdrawal. Because these measures are not numerically equivalent, direct between-group comparisons of absolute values were not performed. Instead, statistical analyses of time-dependent changes were conducted separately within each methodological series.

Thermal nociception was evaluated using the Hargreaves test with a plantar analgesia meter ("Ugo Basile", Cat. No. 37370UB, Italy). Animals were placed in transparent chambers with a Plexiglas floor and allowed to acclimatize before testing (30–40 min before the first session and 20–30 min before subsequent sessions). Acclimatization was considered complete once grooming behavior had ceased. A focused radiant heat source was directed at the plantar surface of the hind paw in the heel region, and the paw withdrawal latency (PWL) was recorded as the time elapsed from stimulus onset to the first pain-related behavioral response (paw withdrawal, licking, or vocalization). Baseline withdrawal latencies in preoperative animals and on intact paws ranged from 10 to 12 s. A cut-off latency of 33 s was imposed to prevent tissue injury. Each limb was tested five times per session, with an interstimulus interval of 3–5 min.

Statistical analysis

The statistical analysis was designed not to assess differences between individual measurements, but rather to identify patterns at the level of animal groups over time. Accordingly, a two-way repeated-measures analysis of variance (two-way repeated-measures ANOVA, RM-ANOVA) was employed, with "Group" as the between-subjects factor and "Time" as the within-subjects factor. Because the tests were conducted repeatedly in the same animals, the analysis accounted for intra-individual changes and the correlation among repeated measurements. The primary questions addressed by this statistical model were:

1. Do sensory sensitivity and motor function change over time after surgery within each group?
2. Do experimental groups subjected to different types of anesthesia or different surgical procedures differ overall?
3. Does the pattern of change over time depend on the type of anesthesia or surgical procedure?

A statistically justified answer to the third question (the "Group" × "Time interaction") was of central importance to this study.

For the "Time" factor, the assumption of sphericity was evaluated. When this assumption was violated, the Greenhouse–Geisser correction was applied, which

is the standard procedure for longitudinal data with multiple time points. The sphericity assumption is a key requirement for the valid application of RM-ANOVA. Within this framework, the assumption of normality pertains to the distribution of residuals rather than to the distribution of the measured variable itself; moreover, with small sample sizes, formal testing of normality is of limited informativeness [29].

Post hoc comparisons were performed only when a statistically significant effect of "Time", "Group", or the "Group" × "Time" interaction was detected. Within-group comparisons between baseline values and each postoperative time point were conducted using the Wilcoxon signed-rank test. To evaluate postoperative recovery of motor function, additional within-group comparisons were performed between the first postoperative measurement and each subsequent postoperative time point. Between-group post hoc comparisons at individual time points were conducted using the Mann–Whitney U test. If testing was not performed in one of the comparison groups during a particular week, data obtained from the other group during that week were excluded from the analysis.

The validity of pooling data from multiple time points was assessed using either the Friedman test or the Kruskal–Wallis test.

For the von Frey tests, only within-group statistical analyses were performed using one-way repeated-measures analysis of variance (one-way repeated-measures ANOVA, one-way RM-ANOVA).

All statistical analyses were conducted in Python 3 using the pandas, numpy, scipy.stats, and matplotlib libraries. Statistical significance was set at $p < 0.05$.

Results

Effect of anesthesia type on sensory recovery following neurorrhaphy

To evaluate the effect of anesthesia type on sensory sensitivity, the animals were allocated into two experimental groups:

Group 1 – animals subjected to sciatic nerve transection followed by immediate end-to-end repair using two interrupted epineurial sutures under inhalational isoflurane anesthesia (n = 6);

Group 2 – animals subjected to the same surgical procedure under intraperitoneal ketamine/xylazine anesthesia (n = 5).

Thermal sensitivity was assessed using the Hargreaves test on the day before surgery, every 2 weeks after surgery for 12 weeks, and additionally at weeks 18, 20, and 22. The results are presented in ***Fig. 2*** and were analyzed using repeated-measures analysis of variance (RM-ANOVA).

The analysis revealed no significant main effect of group ($F(1,9) = 2.26$, $p = 0.167$), indicating the absence of an overall difference between the groups when averaged across all time points. In contrast, a large and statistically significant main effect of time was observed ($F(9,81) = 3.17$, $p = 0.003$), demonstrating that thermal sensitivity changed over the course of the observation period in both groups. A significant difference between the groups was detected prior to week 8 ($p = 0.025$), whereas no further significant between-group differences were observed from week 8 onward (***Fig. 2***).

Importantly, the "Group" × "Time" interaction across the entire postoperative period demonstrated a clear trend toward differential temporal dynamics between the

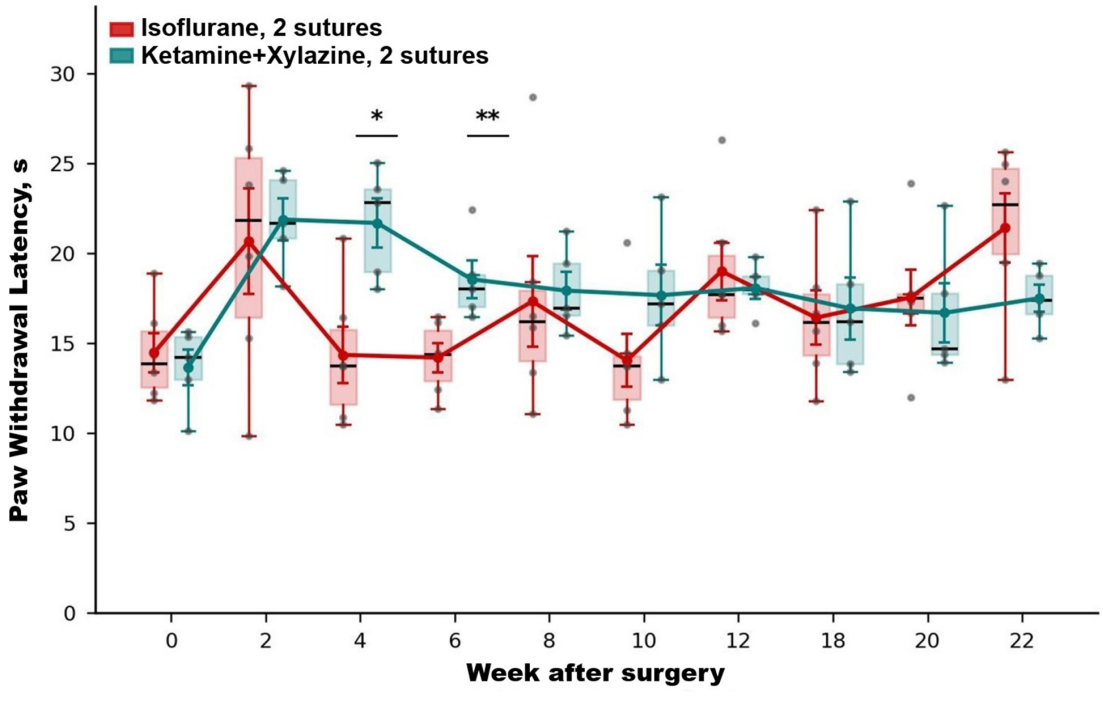

Fig. 2. Dynamics of PWL to a thermal stimulus throughout the experiment in the isoflurane and ketamine/xylazine anesthesia groups (2 sutures). Data are presented as the median with the interquartile range to illustrate data distribution. Statistical analysis was performed using RM-ANOVA followed by planned post hoc comparisons. Horizontal bars indicate significant differences between groups at the corresponding time points (post hoc Mann–Whitney U test, *$p < 0.05$; **$p < 0.01$)

groups, reaching the threshold of statistical significance ($F(9,81) = 1.98$, $p = 0.05$). Specifically, thermal sensitivity progressively deteriorated throughout the experiment in the isoflurane anesthesia group, whereas gradual improvement was observed in the ketamine/xylazine anesthesia group (***Fig. 2***).

Post hoc analysis within each group did not reveal any significant deviation from baseline values, although a significant overall tendency toward changes in thermal sensitivity of the limb with the injured nerve was detected for both anesthesia regimens ($F(9,81) = 3.17$, $p = 0.003$). At week 2, non-significant hypoalgesia was observed, with no significant difference in PWL between the groups (***Fig. 2***). It is possible that this hypoalgesic effect would have reached statistical significance with larger group sizes. In both experimental groups, the observed hypoalgesia was attributable to substantial denervation of the plantar region following sciatic nerve transection.

Post hoc analysis (Mann–Whitney U test) also revealed significant differences between the groups at weeks 4 ($p = 0.017$) and 6 ($p = 0.009$). Thus, significant differences were detected only during the early stage of postoperative recovery. During this period, the isoflurane anesthesia group (2 sutures) exhibited a significantly shorter PWL than the ketamine/xylazine anesthesia group (2 sutures), indicating more rapid recovery of thermal nociception under isoflurane anesthesia. Therefore, the results of this experiment demonstrate a significant difference in postoperative changes in thermal sensitivity of the ipsilateral limb depending on the type of anesthesia used. Notably, this difference persisted for several weeks after surgery. These findings suggest that a relatively short anesthetic procedure (1–2 h) can influence postoperative alterations in thermal sensitivity during the early recovery period (up to 6 weeks), promoting functional recovery. At the same time, a tendency toward impaired thermal sensitivity during the late postoperative period was observed in animals anesthetized with isoflurane compared with those receiving ketamine/xylazine anesthesia.

Given that thermal sensitivity is largely mediated by nerve fibers expressing TRPV1 receptors [30], it may be assumed that the type of anesthesia used during sciatic nerve injury modeling substantially affects the recovery of these fibers.

To determine whether the recovery of another major class of sensory fibers—mechanoreceptive fibers—also depends on the type of anesthesia used during sciatic nerve injury modeling, changes in mechanical sensitivity were evaluated in animals from the two aforementioned groups.

For the von Frey test results, direct between-group comparisons of absolute threshold values were not performed because of differences in the algorithms used to determine the mechanical sensitivity threshold. Instead, within each group, changes relative to baseline (week 0) were assessed using one-way repeated-measures ANOVA (one-way RM-ANOVA) followed by the Wilcoxon signed-rank test for paired comparisons.

As shown in ***Fig. 3A***, animals anesthetized with isoflurane (2 sutures) demonstrated a pronounced and significant effect of time ($F(10,30) = 8.022$, $p < 0.001$), characterized by a gradual increase in PWT, reaching a maximum at week 18 (~80 g compared with a baseline value of ~21 g). Significant deviations from baseline values persisted at weeks 12, 14, 16, 18, and 22 ($p = 0.031$). These findings most likely indicate the progressive development of persistent mechanical hypoalgesia and the absence of a tendency toward recovery of mechanical sensitivity by the end of the experiment. In contrast, animals anesthetized with ketamine/xylazine exhibited a significant overall effect of time ($F(10,40) = 2.602$, $p = 0.016$), which did not result in significant deviations from baseline values at any subsequent time point (***Fig. 3B***). PWT fluctuated (~13–46 g) around the baseline level (~24 g). This pattern suggests recovery of mechanical sensitivity following an initial tendency toward mechanical hypoalgesia, which, as in the case of thermal sensitivity, most likely resulted from denervation of the paw following nerve injury.

Although direct comparison of the groups is inappropriate because different measurement algorithms were used, a normalized graph (***Fig. 3C***) is presented for illustrative purposes. It demonstrates that, following isoflurane anesthesia, the parameter progressively and significantly increased during the post-injury period (reaching approximately 200–400% of baseline values), whereas under ketamine/xylazine anesthesia the values remained close to baseline levels.

Thus, the von Frey test results are consistent with the findings of the Hargreaves test, supporting the conclusion that sensory recovery differs significantly depending on the type of anesthesia used. Furthermore, the results indicate more favorable conditions for restoration of sciatic nerve sensory function following transection and immediate reconstruction with two epineurial sutures when the procedure is performed under ketamine/xylazine anesthesia.

Effect of anesthesia type on motor function recovery following neurorrhaphy. Motor function recovery was assessed using the SFI [24] on the day before surgery and at 4, 8, 12, 16, 20, and 24 weeks postoperatively. The results are presented in ***Fig. 4*** and were analyzed using repeated-measures analysis of variance (RM-ANOVA). The analysis revealed no significant main effect of group ($F = 0.36$, $p = 0.57$), indicating the absence of an overall difference between the groups when averaged across all postoperative time points beginning from week 4. In contrast, a large and statistically significant effect of time was detected ($F = 3.02$, $p = 0.02$), indicating improvement of motor function over the course of the experiment. Therefore, to further evaluate the effect of time within each group, separate one-way RM-ANOVA analyses were performed for the period from week 4 to week 24.

This analysis revealed a significant effect in the ketamine/xylazine anesthesia group ($F(5,20) = 6.745$, $p = 0.0008$), indicating substantial recovery of motor function throughout the experimental period. In contrast, no significant recovery was detected in the isoflurane anesthesia group ($F(5,25) = 1.27$, $p = 0.31$). Importantly, the "Group" × "Time" interaction demonstrated a clear and statistically significant difference in recovery dynamics between the groups throughout the entire experimental period ($F = 3.80$, $p = 0.006$). However, post hoc Mann–Whitney U tests revealed no significant between-group differences at any individual observation

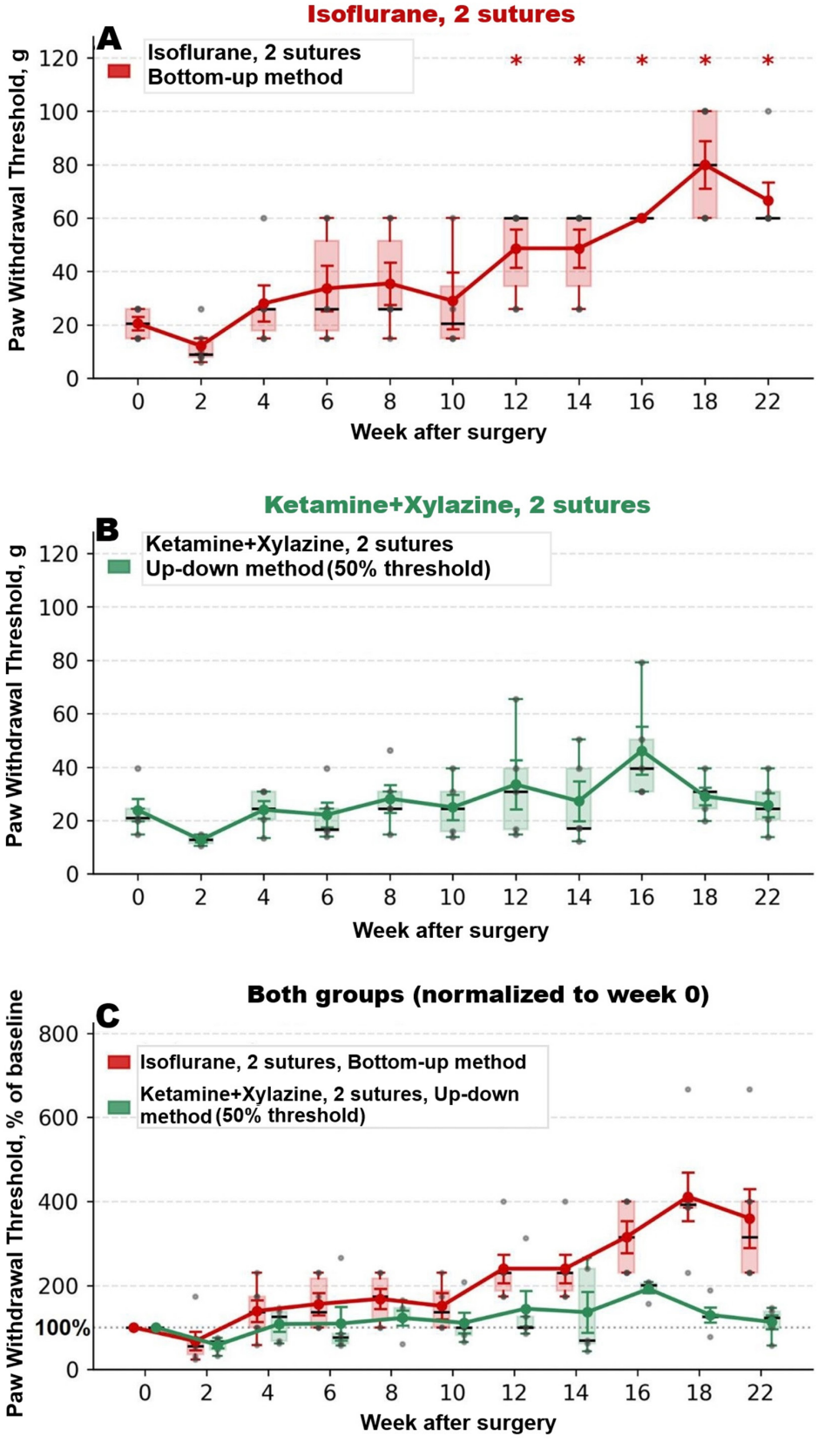

Fig. 3. Dynamics of PWT (von Frey test) throughout the experimental period in the isoflurane and ketamine/xylazine anesthesia groups (2 sutures). Changes induced by neurorrhaphy performed under isoflurane anesthesia (A) and ketamine/xylazine anesthesia (B). Normalized response curves (C), derived from the data presented in panels A and B, demonstrate qualitative differences in recovery dynamics.
* – Statistically significant difference ($p < 0.05$) between the corresponding time point and baseline values within the same group (post hoc Wilcoxon signed-rank test).

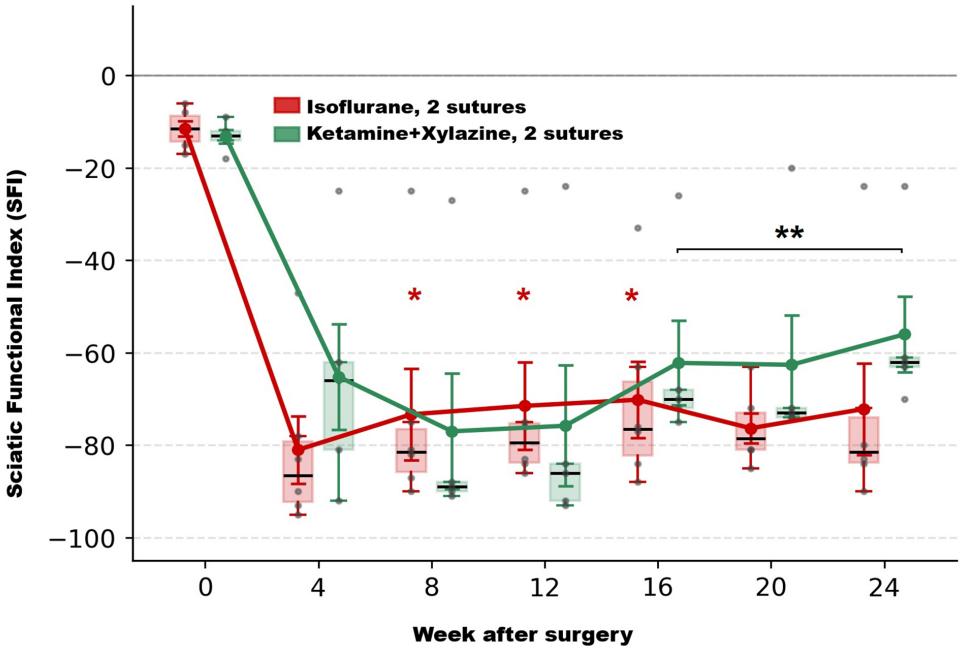

Fig. 4. Dynamics of SFI throughout the experimental period in the isoflurane and ketamine/xylazine anesthesia groups (2 sutures).
* – Statistically significant difference ($p < 0.05$) between the corresponding time point and baseline values within the same group (post hoc Wilcoxon signed-rank test). Horizontal bar indicates significant differences between the pooled SFI values at weeks 16, 20, and 24 (Mann–Whitney U test, **$p < 0.01$)

time point, despite a pronounced visual divergence in this measure during the later stages of the experiment (***Fig. 4***). Since indicator values remained stable within each group during the final three observation periods (weeks 16, 20, and 24), these data points were pooled for each group. Analysis of the pooled datasets revealed a significant between-group difference ($p = 0.004$, Mann–Whitney U test). This finding, together with the results of the preceding tests, indicates significant recovery of motor function when sciatic nerve injury was modeled and immediately reconstructed using two epineurial sutures under ketamine/xylazine anesthesia.

Effect of nerve repair configuration on sensory recovery. To determine whether sensory recovery following sciatic nerve transection and immediate reconstruction depends on the number of epineurial interrupted sutures, animals were allocated into two experimental groups according to the number of epineurial sutures used to reconnect the transected nerve ends. As in the previous part of the analysis, Group 1 consisted of animals that underwent sciatic nerve transection followed by immediate restoration of nerve continuity using two epineurial interrupted sutures under inhalational isoflurane anesthesia ($n = 6$). Group 3 consisted of animals in which the transected nerve ends were repaired using four epineurial interrupted sutures under the same anesthetic regimen ($n = 6$).

Thermal sensitivity was assessed using the Hargreaves test on the day before surgery and every 2 weeks after the intervention for 12 weeks, as well as at weeks 18, 20, and 22 of the experiment. The results are presented in ***Fig. 5*** and were analyzed using repeated-measures analysis of variance (RM-ANOVA). For methodological consistency, values normalized to the preoperative baseline (week 0) were analyzed because baseline PWL measurements differed slightly but significantly between the groups.

A significant main effect of group was identified ($F(1,10) = 9.23$, $p = 0.013$), indicating that throughout the observation period the PWL remained higher when four sutures were used to repair the transected nerve compared with repair using two sutures. Significant effects of time ($F(9,90) = 4.34$, $p < 0.001$) and a significant "Group" × "Time" interaction ($F(9,90) = 3.57$, $p < 0.001$) were also detected, indicating that PWL changed over time in both groups and that the temporal pattern of these changes differed between them.

Integration of the findings from Groups 1 and 3 with the results of the post hoc analyses demonstrated that, following repair with two sutures, the PWL fluctuated around baseline values (97–156%) without significant deviations, indicating relatively rapid recovery of thermal nociception. In contrast, after repair with four sutures, the PWL remained elevated throughout the period from week 2 to week 22 of the experiment (145–173%; $p = 0.031$ at each observation time point). Thus, neurorrhaphy performed with four sutures was associated with pronounced and persistent hypoalgesia lasting for 22 weeks, as well as substantially slower recovery of thermal nociception. The significant "Group" × "Time" interaction ($p < 0.001$, $\eta^2p = 0.263$), together with the post hoc analysis, confirmed that the differences between the groups were primarily attributable to the early postoperative time points (***Fig. 5***).

Post hoc analysis did not reveal significant differences between Groups 1 and 3 at the final stage of the study. However, the "Group" × "Time" interaction across the entire postoperative period demonstrated a trend toward different recovery dynamics between the groups, characterized by a gradual deterioration of thermal

sensitivity parameters throughout the experiment in the two-suture group and progressive improvement in the four-suture group (***Fig. 5***).

Significant hypoalgesia was detected in Group 3 at week 2 after surgery (***Fig. 5***). It is likely that hypoalgesia would also have reached statistical significance in the two-suture group if a larger number of animals had been included, as both experimental groups experienced substantial denervation of the plantar region following sciatic nerve transection.

Taken together, these findings indicate that the number of epineurial sutures used to restore continuity of a transected sciatic nerve has a substantial influence on the dynamics of thermal sensitivity recovery in the paretic limb.

Changes in mechanical sensitivity were also evaluated in experimental Groups 1 and 3.

Because different algorithms were used to determine PWT in the experimental groups, direct between-group comparisons of absolute values were not performed. Instead, for each group, changes relative to baseline (week 0) were analyzed using one-way repeated-measures ANOVA (one-way RM-ANOVA) followed by the Wilcoxon signed-rank test for paired comparisons.

As shown in ***Fig. 6A***, neurorrhaphy with 2 epineurial sutures under isoflurane anesthesia was associated with a pronounced and significant effect of time ($F(10,30) = 8.022$, $p < 0.001$), with the PWT remaining substantially above baseline values (49–80 g versus approximately 21 g at baseline). This persistent hypoalgesia indicated a lack of recovery of mechanical sensitivity in Group 1. Significant deviations from preoperative values (week 0) persisted at weeks 12, 14, 16, 18, and 22 ($p = 0.031$). As noted above, these findings most likely reflect the gradual development of persistent mechanical hypoalgesia and the absence of complete recovery of mechanical sensitivity by the end of the experiment. In contrast, the use of four epineurial sutures under the same anesthetic regimen produced a significant overall effect of time ($F(10,50) = 2.14$, $p = 0.04$), but did not result in significant deviations from baseline values (week 0) at any observation time point (***Fig. 6B***). The PWT fluctuated within a range of approximately 16–44 g without a systematic increase, and mechanical sensitivity remained close to preoperative levels. This finding indicates recovery of mechanical sensitivity following an initial tendency toward mechanical hypoalgesia, which, as in the case of thermal sensitivity, was most likely associated with the initial denervation of the paw.

Although direct comparison between the groups cannot be considered methodologically appropriate because of differences in the measurement algorithms, a normalized plot (***Fig. 6C***) is presented for illustrative purposes. This graph demonstrates that repair with two epineurial sutures under isoflurane anesthesia resulted in a progressive and significant increase in normalized PWT values (approximately 200–400% of baseline), whereas repair with four epineurial sutures under the same anesthetic conditions maintained values close to baseline throughout the entire experimental period.

Thus, the von Frey test results are consistent with the findings of the Hargreaves test, supporting the conclusion that sensory recovery differs significantly depending on the number of epineurial sutures used to

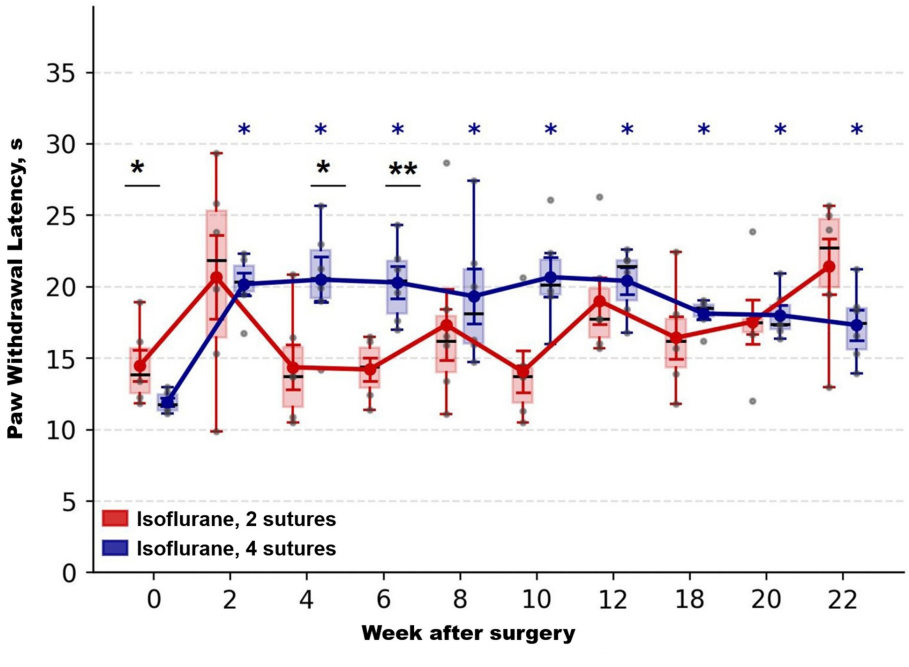

Fig. 5. Dynamics of PWL to a thermal stimulus throughout the experimental period in the isoflurane anesthesia groups repaired with 2 or 4 epineurial sutures. Data are presented as the median with the interquartile range. Statistical analysis was performed using RM-ANOVA followed by planned post hoc comparisons. Asterisks indicate statistically significant differences between the corresponding time points and baseline values (post hoc Wilcoxon signed-rank test; *$p < 0.05$, **$p < 0.01$), whereas horizontal bars indicate significant differences between the groups at the corresponding time points (post hoc Mann–Whitney U test)

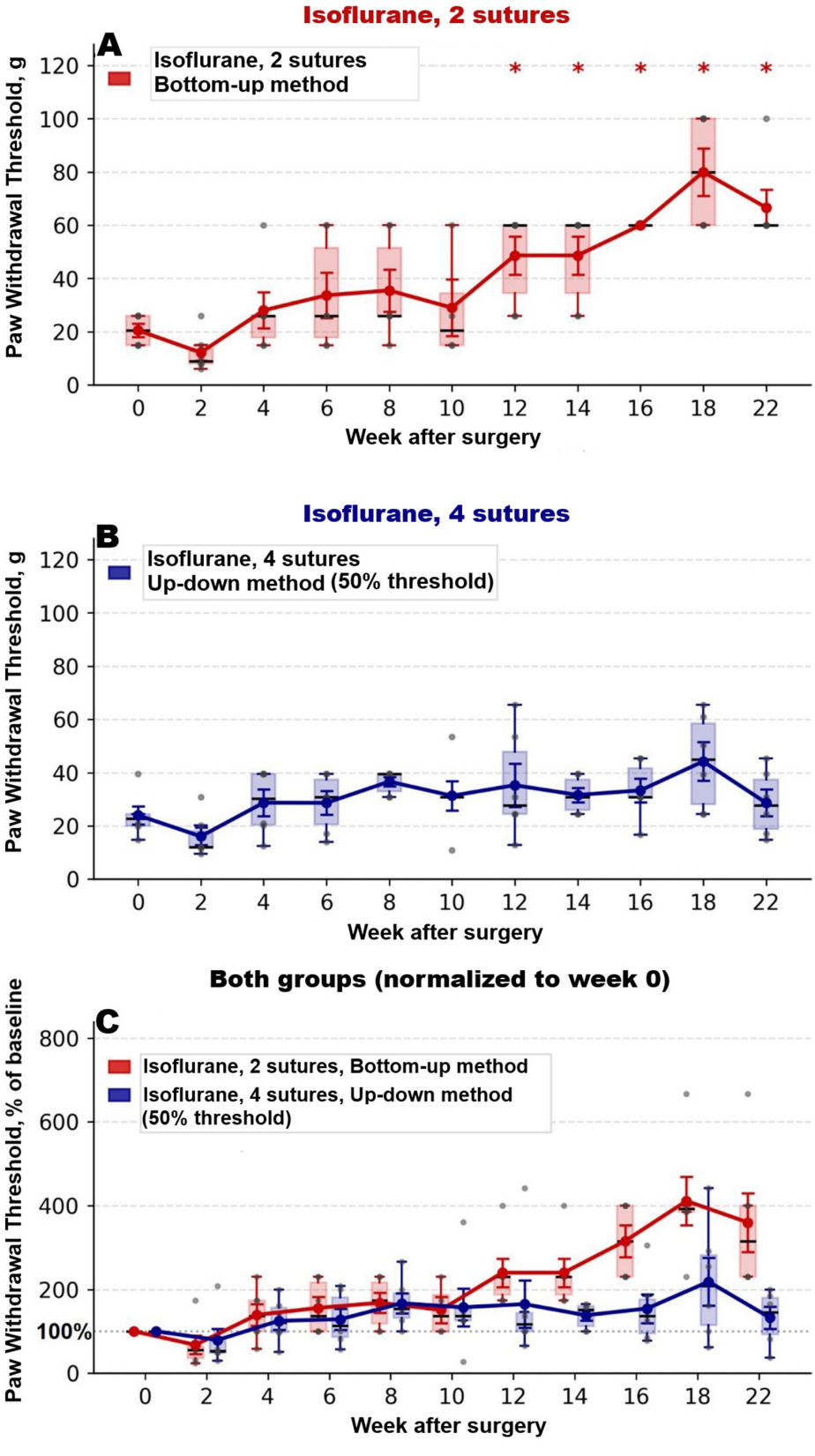

Fig. 6. Dynamics of PWT (von Frey test) throughout the experimental period in the isoflurane anesthesia groups repaired with 2 and 4 epineurial sutures. Changes induced by neurorrhaphy performed with 2 sutures (A) and 4 sutures (B). The normalized response curves (C), derived from the data presented in panels A and B, demonstrate qualitative differences in recovery dynamics.

* – Statistically significant difference ($p < 0.05$) between the corresponding time point and baseline values within the same group (post hoc Wilcoxon signed-rank test)

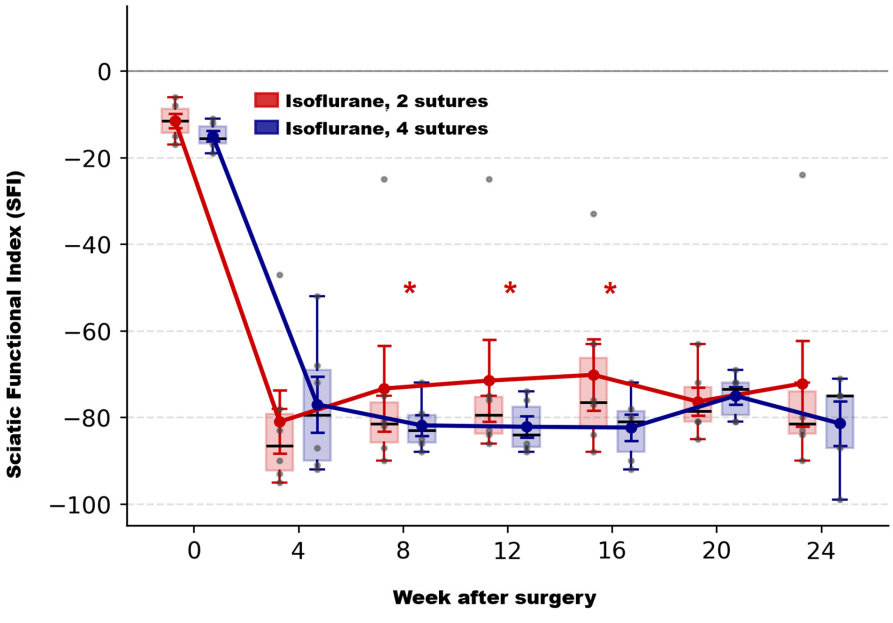

Fig. 7. Dynamics of SFI throughout the experimental period in the isoflurane anesthesia groups repaired with 2 and 4 epineurial sutures. * – Statistically significant difference ($p < 0.05$) between the corresponding time point and baseline values within the same group (post hoc Wilcoxon signed-rank test)

reconnect the transected sciatic nerve. Furthermore, the results indicate more favorable conditions for restoration of sciatic nerve sensory function, particularly mechanical sensitivity, when nerve reconstruction is performed using four rather than two epineurial sutures under isoflurane anesthesia.

Effect of nerve repair configuration on motor function recovery. Motor function recovery was assessed using the SFI [24] before surgery and at 4, 8, 12, 16, 20, and 24 weeks after the surgical intervention. The results are presented in ***Fig. 7*** and were analyzed using repeated-measures analysis of variance (RM-ANOVA).

The analysis revealed no significant main effect of group ($F = 0.36$, $p = 0.57$), indicating the absence of an overall difference between the groups when averaged across all postoperative time points beginning from week 4. Likewise, neither a significant effect of time nor a significant "Group" × "Time" interaction was detected throughout the experimental period. These findings indicate that, under isoflurane anesthesia, the number of epineurial sutures used for end-to-end repair of the transected sciatic nerve (2 versus 4 sutures) did not significantly affect the recovery of motor function. Furthermore, the results demonstrate the absence of significant motor function recovery in either experimental group. This observation may suggest that the use of isoflurane anesthesia is associated with less favorable recovery of motor function following sciatic nerve injury and reconstruction.

Discussion

Because currently available treatments for PNI, particularly surgical approaches, do not provide complete restoration of nerve function [31], the experimental development of novel therapeutic strategies remains an important biomedical challenge. Progress in this field critically depends on the quality of PNI models and on the baseline surgical procedures used to treat this type of injury. In particular, the influence of the type of general anesthesia on the regenerative process following PNI requires careful investigation, as the two most commonly used anesthetic regimens—injectable ketamine/xylazine anesthesia and inhalational isoflurane anesthesia—differ substantially in the rate at which animals recover motor activity after anesthesia. To reduce animal suffering and improve intraoperative control of anesthesia, modern experimental medicine and biology increasingly employ noninvasive inhalational anesthetic techniques, particularly isoflurane administration [18]. However, our findings suggest that, in models involving peripheral nerve transection followed by immediate repair using a small number of interrupted sutures, isoflurane inhalation may be an unsuitable method of general anesthesia for experimental animals. This assumption is consistent with published data indicating that intraperitoneal administration of anesthetic agents remains the most commonly used method [7] of general anesthesia in studies of transected nerve regeneration.

In our opinion, the rate of postoperative mobilization of the injured limb is a particularly important factor when a limited number (two) of interrupted epineurial sutures are used for end-to-end fixation of the sciatic nerve stumps. According to our observations, animals recover consciousness within several minutes after discontinuation of isoflurane inhalation, and full restoration of general motor activity occurs within the subsequent 10–15 min. In contrast, when injectable ketamine/xylazine anesthesia is used, the interval between completion of neurorrhaphy and restoration of general motor activity is, according to our data, at least 1 hour. This period is likely sufficient to stabilize the

nerve repair site through the action of resident fibrin and other rapid mechanisms of the wound-healing process.

The importance of mechanical factors in the development of nerve repair failure is supported by the use of postoperative immobilization of limbs with injured nerves [20], as well as by partial limb immobilization following restoration of rat sciatic nerve continuity using mechanically weak spot-welded nerve junctions [21].

Among the relatively few reports employing inhalational anesthesia for experimental PNI modeling [7], including isoflurane anesthesia [32–34], two studies described the use of isoflurane in rats undergoing peripheral nerve injury modeling with a minimal number of epineurial sutures applied to each nerve stump. In both cases, satisfactory recovery of SFI values was observed during the first two months of follow-up.

Our findings indicate that the type of general anesthesia may represent an important factor influencing the outcomes of experimental studies on peripheral nerve regeneration.

Study limitations

The widely used rat sciatic nerve transection model has several methodological limitations, including the inherent shortcomings of the analog functional–anatomical method used to assess the condition of the injured sciatic nerve through the SFI, as well as the lack of standardized surgical techniques for neurorrhaphy and anesthetic management [17].

The main limitations of the present study include the relatively small sample sizes and, most importantly, the absence of a morphological assessment of nerve regeneration. Verification of the proposed hypothesis will require studies involving larger animal cohorts and the application of immunohistochemical, morphometric, and electrophysiological methods.

Conclusions

Current approaches to the treatment of peripheral nerve injuries remain insufficiently effective, highlighting the importance of experimental models and the conditions under which they are implemented. One of the key factors is the type of general anesthesia used. Inhalational isoflurane provides rapid recovery of motor activity, which may compromise the stability of the nerve repair site, whereas injectable ketamine/xylazine anesthesia allows a longer period for stabilization of the nerve junction. Mechanical factors, particularly early limb activity or postoperative immobilization, also have a substantial influence on the healing process. Therefore, the choice of anesthetic regimen may significantly affect the outcomes of experimental studies of peripheral nerve regeneration.

Disclosure statement

Conflict of interest

The authors declare that the conduct of this study and the publication of its results were not associated with any conflicts of interest related to commercial or financial relationships, relationships with organizations and/or individuals that could have influenced the study, or relationships among the co-authors.

Ethical approval

All procedures involving experimental animals were conducted in accordance with accepted ethical standards and were approved by the Biomedical Ethics Committee of the O.O. Bogomolets Institute of Physiology, NAS of Ukraine (Protocol No. 3/22, November 16, 2022).

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