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A calibrated blunt-clamp refinement of the cutaneous trunk muscle reflex assay for standardized assessment of nociceptive responses and local anesthetic effects in rats

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Abstract

Background

The cutaneous trunk muscle reflex (CTMR) is a spinally mediated nociceptive reflex that can be used to evaluate mechanically evoked responses in experimental animals. However, the traditional needle puncture stimulation has inherent limitations, including tissue trauma, stress interference, and high variability. This study aimed to establish a calibrated blunt-clamp stimulation approach to improve the reproducibility of CTMR assessment and investigate its applicability for detecting pharmacological suppression of nociceptive reflex responses by local anesthetics.

Methods

Twenty-four female rats were divided into the needle puncture group and the calibrated blunt-clamp group. Without any pharmacological intervention, skin stimulation was applied at the T13 segment. CTMR response rate, response intensity and behavioral stress were recorded. Another group of 24 rats was randomly divided into the same two groups. 15 minutes prior to stimulation, they received a subcutaneous injection of 2% lidocaine. Changes in the CTMR inhibition rate were dynamically observed at 15, 30, 45, 60, 75, 90, 105, and 120 minutes to investigate the time-dependent nature of CTMR inhibition induced by calibrated blunt-clamp stimulation, as well as its safety.

Results

Compared with needle puncture group, the calibrated blunt-clamp group produced higher CTMR response rate (97.9 ± 3.8% vs 81.2 ± 9.5%; $P < 0.001$), with reduced inter-animal variability (3.9% vs 11.7%) and lower behavioral stress scores ($P < 0.01$). In the local anesthesia model, lidocaine administration induced a significant time-dependent suppression of clamp-induced CTMR (Repeated-measures ANOVA: main effect of time ($F(2.464,54.217) = 514.784$, $P < 0.001$) and group by time interaction ($F(2.464,54.217) = 4.945$, $P = 0.007$)), with faster recovery in the clamp group at 105 and 120 minutes (both $P < 0.01$).

Conclusion

The calibrated blunt-clamp CTMR assay provides a standardized refinement of mechanical nociceptive stimulation in rats. This method may improve reproducibility in preclinical studies investigating nociceptive reflex modulation and local anesthetic effects.

Introduction

Pain-related behavioral assays in rodents are widely used for investigating nociceptive processing and evaluating the pharmacological effects of analgesic agents, including local anesthetics[1]. Reliable assessment of nociceptive responses is essential for preclinical anesthesia research because variations in stimulation procedures may influence behavioral outcomes and reduce reproducibility[2]. Therefore, standardized experimental approaches that minimize procedure-related variability are important for improving the translational value of animal models used in analgesic and anesthetic studies.

The cutaneous trunk muscle reflex (CTMR) is a polysynaptic, spinally mediated nociceptive reflex characterized by rapid contraction of lateral trunk musculature and visible displacement of the overlying dorsal skin following noxious cutaneous stimulation[3]. CTMR responses are generated through activation of cutaneous sensory afferents and subsequent spinal motor pathways, providing an observable behavioral endpoint for assessing mechanically evoked nociceptive reflex activity. Therefore, CTMR has been widely used as an experimental model for investigating nociceptive processing and pharmacological modulation of reflex responses in preclinical studies[4].

However, conventional CTMR induction methods commonly rely on needle puncture stimulation, which may introduce variability due to differences in applied force, penetration depth, and procedure-related stress[5,6]. In addition, differences in stimulation intensity between operators may compromise reproducibility. These limitations highlight the need for a standardized, non-penetrating mechanical stimulation method that can provide consistent activation of CTMR while reducing procedure-related confounding factors.

A calibrated blunt-clamp stimulation approach may represent a potential refinement of CTMR assessment by providing controlled mechanical compression without skin penetration[7]. Compared with puncture-based stimulation, this approach may improve consistency of stimulus delivery and reduce stress associated with invasive stimulation procedures. However, whether a calibrated clamp-based CTMR assay can provide reproducible measurements and sensitively detect pharmacological suppression of nociceptive reflex responses remains unclear.

The present study aimed to establish a refined CTMR assay using a calibrated blunt-clamp stimulation method in rats. We compared the clamp-based approach with conventional needle puncture regarding CTMR response consistency, behavioral stress responses, and sensitivity to lidocaine-induced suppression. We hypothesized that standardized mechanical stimulation would improve the reproducibility of CTMR assessment and provide a useful methodological approach for preclinical studies evaluating local anesthetic effects on nociceptive reflex responses.

Materials and Methods

Animals and Ethics

All adult female Sprague-Dawley rats were purchased from Vital River Laboratory (Beijing, China). All animal procedures were approved by the Animal Ethics Committee of Xiamen University (Approval No. XMULAC20240337) and were conducted in accordance with institutional guidelines, the Guide for the Care and Use of Laboratory Animals, and the principles of replacement, reduction, and refinement. This study was reported in accordance with the ARRIVE guidelines for animal research.

Adult female Sprague-Dawley rats weighing 220–280 g were used. Animals were housed in a specific pathogen-free barrier facility under controlled temperature (22 ± 2 ℃), humidity (50 ± 5%), and a 12-hour light/dark cycle, with free access to standard chow and water. Rats were acclimatized for 7 days before the experiment. All rats used in the experiments will be sacrificed by cervical dislocation after being anesthetized with isoflurane at the end of the experiments.

Study Design

This study is a prospective, randomized controlled animal experiment, which was designed to evaluate the reliability, reproducibility, and safety of the calibrated blunt-clamp in comparison with the needle puncture in the context of measuring the CTMR in rats. This study employed two models.

Direct Stimulation Model

A total of 24 female SD rats were randomly divided into the calibrated blunt-clamp group (n = 12) and needle puncture group (n = 12). This division was conducted using a random number table. In the absence of pharmacological intervention, rats in both groups received stimulation at the T13 skin site using either the calibrated blunt-clamp or needle puncture to induce the CTMR. The primary objective of this experiment was to compare the CTMR response rate, response intensity, and behavioral stress levels under the two stimulation methods. This was done in order to evaluate the detection sensitivity and repeatability of the calibrated blunt-clamp under routine conditions.

Local Anesthesia Model

A total of 24 additional female SD rats were randomly assigned to receive either the calibrated blunt-clamp group (n = 12) and needle puncture group (n = 12). 15 minutes prior to CTMR stimulation, 500 μL of 2% lidocaine was injected subcutaneously into the T13 region to reversibly block peripheral sensory input. Subsequently, alterations in the CTMR inhibition rate were meticulously documented at multiple predetermined time points (15, 30, 45, 60, 75, 90, 105, and 120 minutes). The purpose of this experiment was to investigate the time-dependent nature of the inhibition of CTMR induced by calibrated blunt-clamp stimulation following lidocaine administration and the safety of calibrated blunt-clamp stimulation.

Randomization and Blinding

The experiment utilized a simple randomized design. In the direct stimulation model, 24 female SD rats were randomly divided into two equal groups following the completion of the acclimatization period. This randomization was conducted using a random number table. The rats were then separated into two groups: the calibrated blunt-clamp group (n = 12) and the needle puncture group (n = 12). The local anesthetic block model followed the same randomization method and was divided 24 female SD rats into two groups, as the calibrated blunt-clamp group (n = 12) and the needle puncture group (n = 12).

To minimize observer bias during behavioral assessments, a two-person blinding protocol was implemented for all CTMR scoring and stress evaluations. All mechanical stimulations were delivered by a trained operator who was aware of the group assignments. An independent observer, completely blinded to the experimental design, group allocation, and stimulation tools, conducted the behavioral scoring. To prevent the observer from identifying the stimulation method, a custom opaque cardboard shield was placed between the operator’s hands and the rat's dorsum, ensuring that the observer could view only the rat's dorsal skin and trunk musculature without seeing the stimulation device. The blinded observer scored the CTMR response intensity and behavioral stress in real time.

Selection and Marking of the Stimulation Area

The T13 region was selected for the study (Fig. 1A). The thoracolumbar dorsal cutaneous stimulation has been reported to elicit robust CTMR responses, and the T13 level can be localized using the last rib as a surface landmark[8]. The dorsal hair of each rat was shaved (6 cm × 8 cm) along the midline 24 hours prior to the experiment. Under isoflurane anesthesia (3–4% induction, 2% maintenance), a circular area with a radius of 2 cm was marked on the dorsal skin of each rat. The center was delineated as the intersection of the sagittal midline and a horizontal line through the T13 level. The circular field was subdivided into six equal sectors, designated 1–6[9,10]. After full recovery from anesthesia, rats were placed on a custom restraint platform to minimize confusion between spontaneous movement and CTMR responses (Fig. 1B).

Mechanical Stimulation Devices and Force Calibration

The refined stimulation device was a small straight Glover bulldog clamp with blunt tips (Shanghai Jinzhong Company, Shanghai, China) (Fig. 1C). Before each experimental session, the clamping force was calibrated using a force measurement device and adjusted within a predefined range of 140–180 gf (approximately 1.4–1.8 N). The clamp opening was adjusted to compress approximately 10 mm² of dorsal skin during each stimulation. This design provided standardized non-penetrating mechanical stimulation while avoiding skin penetration[11,12].

The estimated average pressure was calculated by dividing the applied force by the contact area of the clamp tip. The calculated pressure represents an average value and does not directly reflect local peak

pressure distribution at the tissue–clamp interface. Because of possible edge-related stress concentration, local peak pressure may exceed the calculated average value[13].

Needle-Puncture Stimulation

For comparison, conventional needle-puncture stimulation was performed using a 25-gauge needle connected to a 26-gauge von Frey filament. Needle stimulation was performed according to the established CTMR induction protocol. The same operator performed all stimulations following the predefined sequence to minimize procedural variability.

Weighted CTMR Response and Inhibition Rate Assessment

CTMR responses were evaluated using a semi-quantitative scoring system based on the magnitude of trunk muscle contraction following stimulation. Each of the six predefined dorsal sectors was stimulated once in clockwise order starting from the 12 o-clock position, resulting in six stimulations per animal. Each stimulation site was assigned a score according to the observed reflex intensity: 1, strong trunk muscle contraction (Supplementary Video 1); 0.5, mild contraction (Supplementary Video 2); and 0, no detectable contraction.

In the direct stimulation model, the weighted CTMR response rate was used for assessment. The weighted CTMR response rate (%) was calculated by the following formula:

$$\text{Weighted CTMR Response Rate}\,(\%) = \frac{1 \cdot\ n_1 + 0.5 \cdot\ n_{0.5}}{6} \times\ 100\%$$

Where n_1 represents strong trunk muscle contraction; $n_{0.5}$ represents mild contraction.

In the local anesthesia model, to quantify the efficacy of local anesthesia without altering the underlying scoring system, we used the weighted CTMR inhibition rate for evaluation. The weighted CTMR inhibition rate (%) was calculated by the following formula:

$$\text{Weighted CTMR Inhibition Rate}\,(\%) = \left(1 - \frac{1 \cdot\ n_1 + 0.5 \cdot\ n_{0.5}}{6}\right) \times\ 100\%$$

Where n_1 represents strong trunk muscle contraction; $n_{0.5}$ represents mild contraction.

Behavioral Stress Assessment

Behavioral stress during stimulation was scored using a 4-point scale: 0, no observable stress response; 1, mild stress such as alerting, mild agitation, or a brief escape attempt; 2, moderate stress such as persistent agitation, repeated escape attempts, brief vocalization, or piloerection; and 3, severe stress such as sustained escape behavior, loud vocalization, defecation or urination, or marked agitation[14,15] (Table 1).

Table 1
Behavioral stress scoring system used during CTMR testing

Score	Observable stress response during CTMR testing
0	No observable stress response
1	Mild stress: alerting, mild agitation, brief escape attempt, or increased attention
2	Moderate stress: persistent agitation, repeated escape attempts, brief vocalization, or piloerection
3	Severe stress: sustained escape behavior, loud vocalization, defecation/urination, or marked agitation

CTMR, cutaneous trunk muscle reflex.

Sample Size

Sample size was calculated using a two-sample comparison of mean weighted CTMR scores. Based on a pilot study with 3 rats per group, the mean weighted CTMR scores were 0.90 ± 0.20 in the calibrated blunt-clamp group and 0.65 ± 0.20 in the needle puncture group. Assuming a two-sided alpha of 0.05 and 80% power, at least 10 rats per group were required for the main comparison. To allow for biological and procedural variability, 12 rats per group were included in the primary comparison. For the lidocaine validation experiment, 24 additional rats were randomly assigned equally to the calibrated blunt-clamp group and needle puncture group.

Statistical Analysis

Statistical analyses were performed using IBM SPSS (version 25.0) and GraphPad Prism (version 8.0.2). Data distribution was assessed before selecting statistical tests. Continuous variables with normal distributions were analyzed using parametric tests, whereas non-normally distributed variables were analyzed using non-parametric tests.

Comparisons between two independent groups were performed using an unpaired t-test or Mann–Whitney U test as appropriate. Categorical response distributions were analyzed using Fisher's exact test. Time-dependent changes in CTMR responses following lidocaine administration were analyzed using repeated-measures analysis of variance (ANOVA). A two-sided P value < 0.05 was considered statistically significant.

Results

Calibrated blunt-clamp stimulation improves the consistency of CTMR

Compared with the needle puncture group, the weighted CTMR response rate was significantly higher in the calibrated blunt-clamp group (97.9 ± 3.8% vs. 81.2 ± 9.5%, P < 0.001). In the calibrated blunt-clamp

group, 9 of 12 rats achieved a response rate of 100%, with a minimum individual rate of 91.7%, whereas response rates in the needle puncture group ranged widely from 66.7% to 100%, indicating greater inter-animal dispersion (Fig. 2A).

Calibrated blunt-clamp stimulation reduces variability of CTMR

The coefficient of variation for the weighted response rates was 3.9% in the calibrated blunt-clamp group, in comparison to 11.7% in the needle puncture group. This demonstrated reduced inter-animal variability following calibrated blunt-clamp stimulation, in contrast to the needle puncture stimulation. The findings indicated that the implementation of a standardized mechanical compression technique may serve to reduce the variability observed in relation to manually applied puncture stimulation.

Calibrated blunt-clamp stimulation improves the response intensity of CTMR

A significant difference in the distribution of response intensity was observed between the two groups ($P < 0.001$). The experiment involved the administration of a total of 72 stimuli, with each group receiving the same number of stimuli. In the calibrated blunt-clamp group, all stimuli provoked a response, with strong contractions accounting for 95.8% (69/72) and mild contractions for 4.2% (3/72). Conversely, the response intensity exhibited significant variability within the needle puncture group. It was found that strong contractions accounted for only 62.5% (45/72) of the total stimuli, mild contractions for 36.1% (26/72), and 1.4% (1/72) produced no detectable response (Fig. 2B). This finding suggested that the noxious stimuli generated by the calibrated blunt-clamp method were more uniform and distinct than those produced by traditional acupuncture. As a representative observation, macroscopic examination immediately following the test revealed punctate marks left by the needle and slight localized erythema in the needle puncture group, whereas the skin in the calibrated blunt-clamp group remained macroscopically intact (Figs. 2D, E).

Behavioral stress responses during stimulation

Behavioral responses elicited by the various stimuli were compared across groups (Table 2). In comparison with rats exposed to needle stimulation, those exposed to stimulation with calibrated blunt-clamp exhibited lower stress-related behavior scores ($P < 0.01$). In the calibrated blunt-clamp group, only 1 rat (8.3%) exhibited mild stress, while the remaining 11 rats (91.7%) showed no observable stress response. In contrast, in the needle puncture group, 2 rats (16.7%) exhibited severe stress, 3 rats (25.0%) exhibited moderate stress, 1 rat (8.3%) exhibited mild stress, and only 6 rats (50.0%) showed no observable stress response.

Table 2
Behavioral stress during CTMR testing

Group	0 (no stress)	1 (mild stress)	2 (moderate stress)	3 (severe stress)
Clamp group	11 (91.7%) **	1 (8.3%)	0 (0.0%)	0 (0.0%)
Puncture group	6 (50.0%)	1 (8.3%)	3 (25.0%)	2 (16.7%)

Data are presented as number of rats (%). (** indicates Clamp compared with Puncture, **: $P < 0.01$; statistical significance was assessed by Fisher's exact test)

Lidocaine suppresses calibrated blunt-clamp-induced CTMR

Following the subcutaneous injection of 500 μL of 2% lidocaine hydrochloride, the CTMR inhibition rates in both groups exhibited a time-dependent pattern characterized by an initial peak followed by a gradual decline. The primary effect of time was found to be significant ($F (2, 464, 54.217) = 514.784$, $P < 0.001$), and the interaction effect between group and time was also found to be significant ($F (2, 464, 54.217) = 4.945$, $P = 0.007$). The finding was consistent with the pharmacological characteristics of lidocaine, which reversibly block peripheral nerve conduction. However, throughout the entire time course, the calibrated blunt-clamp group demonstrated significantly higher consistency and accuracy. The weighted CTMR inhibition rates in both groups reached their peak 15 minutes after injection, but in the calibrated blunt-clamp group, the level was significantly higher than that in the needle puncture group. (99.3 ± 2.4% vs. 91.7 ± 8.7%, $P = 0.008$). Subsequent to this, the inhibitory effects in both groups gradually diminished over time. The mean inhibition rates of the two groups did not differ significantly ($P > 0.05$) between 30 and 75 minutes. It is noteworthy that between 90 and 120 minutes, a marked difference in the suppression rate became evident between the two groups. Following a 90-minute period of observation, the suppression rate exhibited by the subjects in the calibrated blunt-clamp group was found to be significantly lower than that observed in the acupuncture group (4.8 ± 4.3% vs. 14.6 ± 11.9%, $P = 0.014$). In the calibrated blunt-clamp group, the CTMR inhibition rate had fully recovered to baseline levels by 105 minutes and remained stable at 120 minutes. Conversely, the needle puncture group demonstrated a persistent degree of residual inhibition at the same time points (105 minutes: 12.5 ± 13.5%; 120 minutes: 11.8 ± 14.0%). There was a statistically significant differences between the two groups (105 minutes, $P = 0.004$; 120 minutes, $P = 0.008$), indicating that CTMR recovery was significantly faster in the calibrated blunt-clamp group than in the needle puncture group (Fig. 2C).

Discussion

This study proposed an improved CTMR detection method, with the objective of improving the reliability of preclinical skin analgesia testing. In comparison with the use of a needle puncture, stimulation with a calibrated blunt-clamp resulted in a higher CTMR response rate, stronger reflex contractions, lower variability, and a significant reduction in observable pressure. Furthermore, CTMR responses in the calibrated blunt-clamp group were almost inhibited by lidocaine and exhibited time-dependent inhibition.

CTMR is defined as a spinal-mediated nociceptive reflex, which is triggered by the activation of cutaneous sensory pathways. Although traditional needle puncture stimulation is an effective method for inducing CTMR responses, the process is subject to operator-dependent factors, including stimulation force and penetration depth[16,17]. Furthermore, the phenomenon of tissue penetration has the potential to induce localized tissue damage and procedure-related pressure, which in turn can affect behavioral measurements[18]. Consequently, enhancing the standardization of mechanical stimulation is imperative for optimizing the reproducibility of preclinical anesthesiology research.

The primary application of CTMR in the field of anesthesiology is the evaluation of the pharmacological effects of local anesthetics. In this study, the administration of lidocaine was found to significantly inhibit the CTMR response induced by calibrated blunt-clamp stimulation. The clamp group exhibited a higher inhibition rate at 15 minutes, suggesting that the sensory afferents activated by the calibrated blunt-clamp may be more concentrated on lidocaine-sensitive Aδ and C-type afferent fibers[19]. Conversely, the needle puncture, in addition to activating the aforementioned fibers, may also activate non-nociceptive mechanoreceptors that are insensitive to lidocaine or trigger local axon reflexes, resulting in a comparatively lower inhibition rate. This discrepancy further underscored the hypothesis that the blunt-clamp provides a purer signal for inducing noxious reflexes. In the time frame spanning 90 to 120 minutes, the inhibition rate exhibited by the blunt-clamp group was found to be significantly lower in comparison to that observed in the needle-prick group. This finding suggested that the recovery period following reflex stimulation was faster in the clamp group. The micro-injury instigated by needle puncture stimulation has been demonstrated to elicit local tissue exudation and a mild inflammatory response. These non-neural tissue repair processes have been shown to interfere with the normal recovery of reflex function[20]. Conversely, the calibrated blunt-clamp, as a non-penetrating mechanical stimulus, does not breach the skin barrier or cause local bleeding. Therefore, reflex recovery is unlikely to be hindered by tissue repair processes. From a functional perspective, this result provided direct physiological evidence of the advantages of the blunt-clamp in terms of animal welfare and experimental reproducibility[21]. These findings suggested that the refined CTMR assay had the capacity to detect the inhibtion and reversible recovery of mechanically evoked reflex activity following local anesthesia. Consequently, it may offer a valuable instrument for preclinical anesthesia research.

Since calibrated blunt-clamp stimulation does not require skin penetration, this method may offer potential improvements over puncture methods. However, this study did not include histological evaluation of tissue reactions or assessment of the long-term effects of repeated compression. Therefore, further research is needed before drawing definitive conclusions regarding improvements in animal welfare.

Several limitations should be noted. First, the CTMR response was assessed using a semi-quantitative behavioral scoring system. Although this method allows for comparisons between stimulation methods, it was not validated by objective physiological measures, such as electromyography (EMG) recordings[22]. Second, the clamping force was calibrated prior to the experiment; however, the force during stimulation

could not be monitored in real time. Therefore, potential variations in instantaneous pressure distribution cannot be completely ruled out. Third, although average pressure was estimated, peak pressure at the clamp-skin interface was not directly measured. Fourth, no histological evaluation was performed to assess potential tissue effects and long-term consequences of repeated clamping. Finally, this study included only female rats; further research using a wider range of models and both sexes is needed to determine the general applicability of this method.

Conclusion

The calibrated blunt-clamp CTMR assay provides a standardized refinement of mechanical stimulation for evaluating nociceptive reflex responses in rats. This approach improves the consistency of CTMR measurements and enables detection of local anesthetic-induced suppression of reflex activity. Further validation using objective physiological and histological approaches is required to establish its broader applicability in preclinical anesthesia research.

Abbreviations

CTMR
cutaneous trunk muscle reflex
SD
Sprague-Dawley
ANOVA
Analysis of Variance.

Declarations

Ethics Approval

All animal experimental procedures were approved by the Animal Ethics Committee of Xiamen University and followed the ethical guidelines for the investigation of experimental pain in conscious animals. The studies were conducted in accordance with the local legislation and institutional requirements (Approval No. XMULAC20240337).

Clinical trial number

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Author Contribution

Guanhua Qiu contributed to conceptualization, investigation, data acquisition, formal analysis, and drafting of the manuscript. Linhong Wang contributed to methodology, data curation, and revision of the manuscript. Bin Yang contributed to conceptualization, supervision, project administration, funding acquisition, and critical revision of the manuscript. All authors made substantial contributions to the work reported; took part in drafting, revising, or critically reviewing the article; approved the final version to be submitted; agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

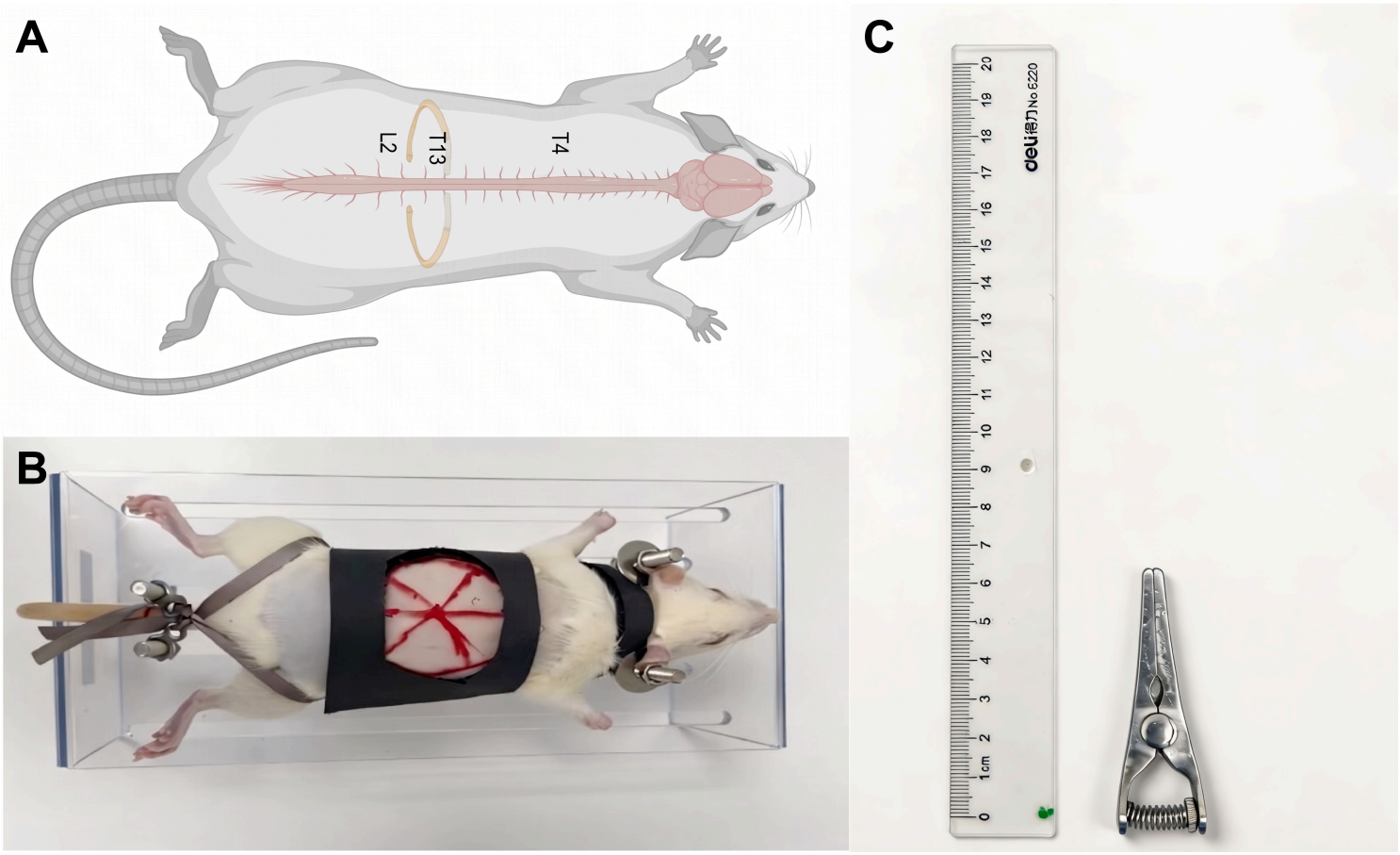


Figure 1

Mechanical stimulation apparatus, dermatomal localization, and behavioral testing configuration for the refined CTMR assay. **(A)** The rat was to be placed on a fixed platform, with the dorsal skin area centered on T13 serving as the stimulation site. (A was created with BioRender.com). **(B)** Adult rat positioned on the customized transparent restraint platform, displaying the circular dorsal testing field centered at T13 and partitioned into six radial sectors (numbered 1–6) for standardized sequential stimulation. **(C)**Small blunt-tipped Glover bulldog clamp used for calibrated mechanical stimulation.

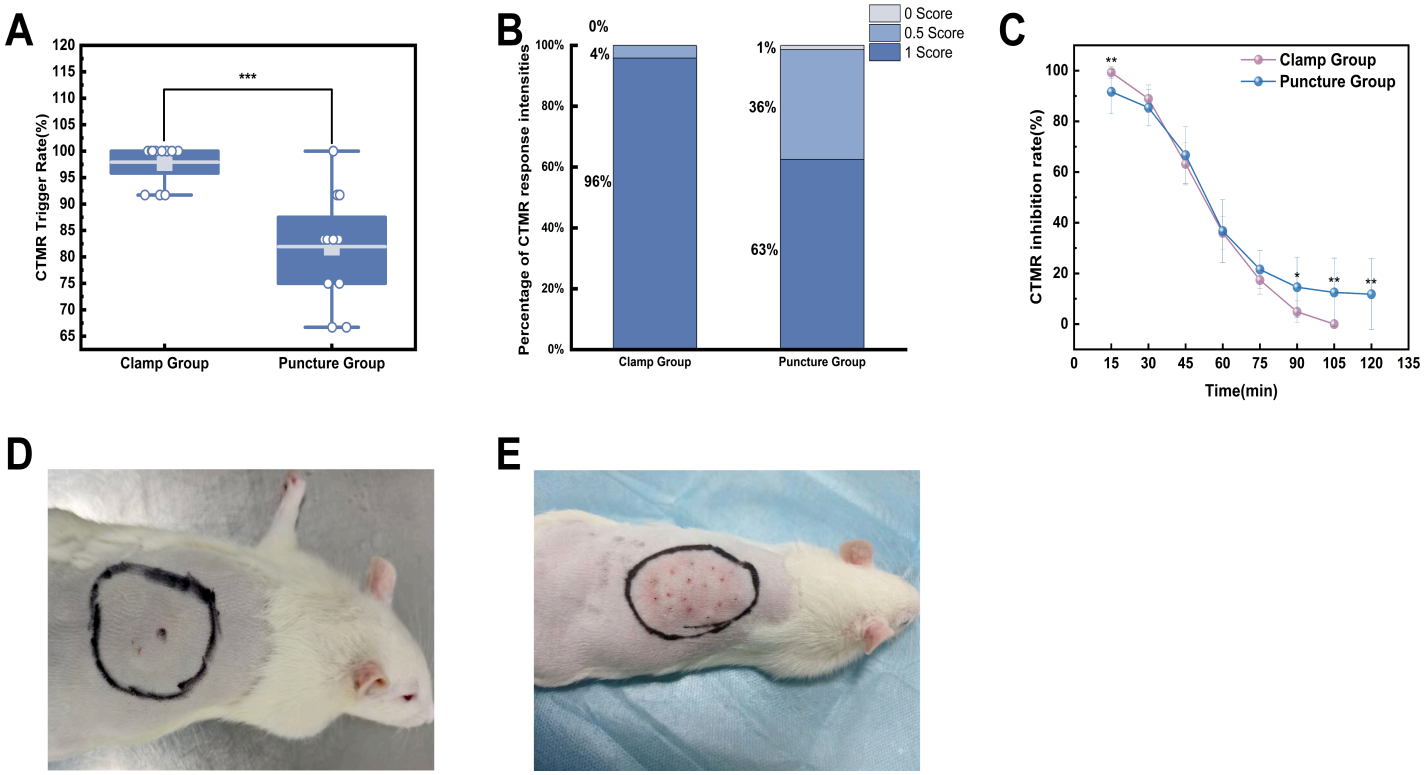


Figure 2

Comparison of reflex efficacy, response intensity, procedural tissue trauma, and local anesthetic pharmacodynamics between clamp and puncture paradigms. **(A)**Weighted CTMR response rates in the clamp and puncture groups (n = 12 per group; statistical significance was assessed by Independent-samples t-test). **(B)**Percentage distribution of CTMR response intensities across 72 mechanical stimulations per group. Responses are categorized into strong contraction (Score 1), mild contraction (Score 0.5), and no detectable contraction (Score 0). (n = 12 per group; statistical significance was assessed by Mann-Whitney U test). **(C)** Time-course curve of CTMR inhibition rates following 2% lidocaine infiltration (n = 12 per group; statistical significance was assessed by Two-way repeated-measures ANOVA by Bonferroni's multiple comparisons test). **(D)**Representative macroscopic photograph of the rat dorsum following needle puncture testing, showing visible punctate erythema and micro-hemorrhages within the T13 testing field. **(E)** Representative macroscopic photograph of the rat dorsum following calibrated clamp testing, demonstrating an intact epidermal barrier without observable tissue trauma. (*: P < 0.05, **: P < 0.01, ***: P < 0.001).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementVideo1.mp4
- SupplementVideo2.mp4