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Assessment of an Electronic Mechanical Sensory Threshold Testing Device (RatMet) in Wistar rats (*Rattus norvegicus*)

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von Frey (vF) monofilaments are used to quantify mechanical hypersensitivity and nociception in rodents; however, this method of testing has been criticized due to inconsistencies in testing methods, filament properties, and nonlinearity. This study compared withdrawal thresholds measured by using vF monofilaments with those of a novel mechanical threshold testing device currently in development (RatMet) in a carrageenan inflammatory model in 9- to 11-wk-old male Wistar rats. Rats were randomly assigned to assessment of mechanical hypersensitivity after intraplantar carrageenan injection by using either vF monofilaments (n = 10) or the RatMet device equipped with 3 sizes of probe tips (0.9 mm [RM0.9], n = 15; 0.5 mm [RM0.5], n = 11; and 0.09 mm [RM0.09], n = 11). All RatMet probe sizes and vF monofilaments identified a reduction in withdrawal threshold after treatment. Systematic differences in threshold were identified between vF and both RM0.9 and RM0.5 groups; RM0.09 did not differ from vF. Withdrawal thresholds showed linear relationships with probe diameter, square root of probe diameter, and area of the RatMet probes. In contrast, exponential relationships were observed with the vF monofilaments. Furthermore, none of the RatMet probe results differed in accuracy when comparing a single test with the averages of 3 or 5 tests per time point. Overall, the RatMet device measurements have construct validity even when the number of testing replicates is low. These data indicate that the RatMet device produces data comparable with those from vF monofilaments, with the potential for a shortened testing period without a decrease in accuracy.

Abbreviations: RM, RatMet device; vF, von Frey

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Correctly quantifying pain and nociception are core components of pain and analgesia research. Mechanical sensory threshold testing is a widely accepted and commonly used method to evaluate hypersensitivity. This testing method continues to play a role within the evolving armamentarium of pain and nociception assessment tools.[18] Developing valid, reliable, and practical mechanical sensory threshold devices are crucial for nociception studies and analgesia development.[15] vF monofilaments (correctly termed Semmes–Weinstein filaments) remain the 'gold standard' for quantifying mechanical nociception in animal pain models despite numerous limitations.[3,4,11]

von Frey monofilaments are used to quantify mechanical sensory thresholds by identifying the force required to stimulate a withdrawal response, most commonly measured on cutaneous surfaces such as the plantar surface of the hind paw or the tail of rodents.[11] These monofilaments are commonly used because they are convenient, simple to use, have good intrarater consistency, and are well-established in the literature as an acceptable mechanical hypersensitivity assay.[3,8]

Despite their popularity, vF monofilaments have drawbacks, including nonuniform surface area when applied,[3,4] augmented hypersensitivity of the animal due to a training effect or tissue damage,[5,6,11] estimated withdrawal thresholds, inconsistency in testing methodology,[4] sensitivity to operator hand tremor, and interrater inconsistency.[2,3] Multiple attempts have been made to standardize the application protocol of vF monofilaments to mitigate these limitations.[2] The 50% withdrawal threshold technique[5] is often cited. However, variations in this technique, including the time between applications, speed of filament application, range of filaments used, and pattern of application, are frequently applied and inconsistently reported in the literature.[2,3] A standardized protocol would address some shortcomings of the vF monofilaments, but the inherent properties of the standard monofilament kit pose important limitations. The buckling nature of vF monofilaments, combined with variation in tissue characteristics, is likely to affect the applied force, and uneven force distribution may confound the actual withdrawal threshold.[3,4,17]

In an attempt to mitigate some of the limitations of traditional mechanical threshold testing with vF monofilaments, electronic vF devices have been developed. These devices typically use a single, nonbending probe. The probe is applied to the testing site (for example, plantar surface of the hind paw), and the force is steadily increased until a withdrawal response occurs. As a result, electronic vF devices provide a continuous force measurement, shorter testing time, and a reduced number of probe applications.[9]

Our study evaluated a novel electronic vF device (RatMet, Topcat Metrology). This device uses a single, slightly flexible probe (plastic material, polypropylene or nylon) that does not buckle, and generates a force–time graph that compares the

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actual force curve to the recommended force application rate. Importantly, the RatMet device obtains an accurate, measured withdrawal threshold, unlike the discrete data and calculated estimate of vF monofilaments.[3,4] These properties have the potential advantages of reducing the effect of hand tremor, presenting a constant probe area to the tissue surface, and providing real-time user feedback on probe application. A standardized, continuous-force application may result in greater consistency within and between experimenters, greater accuracy in measuring withdrawal thresholds, and less likelihood of developing of exaggerated hypersensitivity.[3,5,6]

The objectives of this study were 1) to establish whether the RatMet device has construct validity by comparing the withdrawal thresholds and fundamental probe characteristics from 3 sizes of RatMet probe tips with those of vF monofilaments in Wistar rats and 2) to determine if reducing the number of replicate applications of the RatMet device alters the withdrawal threshold data. The hypotheses were: 1) RatMet would detect changes in mechanical withdrawal thresholds after treatment of rats with carrageenan; 2) reducing the diameter of RatMet probe tips would lower the mechanical withdrawal thresholds; and 3) reducing the number of replicate applications of RatMet would not change the withdrawal thresholds.

Materials and Methods

Animals. Male Wistar rats ($n = 47$; age, 8 wk; median mass, 381 g; mass range, 339 to 461 g) were obtained from Charles River Laboratories (Senneville, Quebec, Canada). Rats were housed in pairs in a controlled-temperature and -humidity environment (23 °C, 22% humidity) with a 12:12-h light:dark cycle (lights on, 0700). All experimentation occurred during the light phase. Acrylic cages (47 × 25 × 21 cm) contained wood chips, shredded paper, and a plastic tube. Tap water and laboratory rat food (Prolab 2500 Rodent 5P14, LabDiet, PMI Nutrition International, St Louis, MO) were provided ad libitum. Husbandry and welfare checks were performed twice daily. Sentinel rats were in use and negative for rat parvoviruses, Toolan H1 virus, Kilham rat virus, rat minute virus, protoparvovirus NS1, rat sialodacryoadenitis virus, rat theilovirus, *Pneumocystis carnii*, Sendai virus, pneumonia virus of mice, reovirus, *Mycoplasma pulmonis*, lymphocytic choriomeningitis virus, adenovirus, hantavirus, *Encephalitozoon cuniculi*, cilia-associated respiratory bacillus, rat rotavirus, *Bordetella bronchiseptica*, *Corynebacterium kutscheri*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Rodentibacter pneumotropicus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, β-hemolytic *Streptococcus* spp., *Streptococcus pneumoniae*, *Proteus mirabilis*, *Salmonella* spp. and other bacteria, and endo- and ectoparasites. All rats were 9.5 to 11 wk old at the start of the experiment, after habituation, which included handling by the experimenter for 3 to 5 d and habituation to the mechanical threshold testing chamber for 10 to 15 min 24 h prior to the experiment. Rats were randomized by using a random list generator (https://www.random.org/lists/) to one of the following testing groups: vF monofilaments (n = 10); 0.09-mm RatMet probe tip (RM0.09, n = 11); 0.5-mm RatMet probe tip (RM0.5, n = 11); and 0.9-mm RatMet probe tip (RM0.9, n = 15). Rats were tested in the order in which they arrived from the supplier. Ethics approval was provided by the University of Calgary Health Sciences Animal Care Committee, which operates under the auspices of the Canadian Council on Animal Care (protocol number AC13-0161).

Anesthesia and treatment injection. General anesthesia was induced in each animal with 5% isoflurane carried in oxygen at 1 L/min in an acrylic induction chamber. After rats lost the righting reflex, anesthesia was maintained at 2% isoflurane in oxygen at 0.5 to 1 L/min via a nose cone, and body temperature was supported with an electric heating pad (Equate, Mississauga, Ontario, Canada). Body mass and rectal temperatures were recorded. The plantar surface of the left hind paw was cleaned by using 70% ethanol. Once a negative pedal withdrawal reflex was confirmed, a 25-gauge 5/8-in. needle was advanced subcutaneously for 6 mm, and 150 µL of 1% (w/v) λ-carrageenan (Sigma–Aldrich, St Louis, MO) was deposited in the midplantar region, at a single site equidistant from the base of each digit. The injection site was compressed while the needle was removed and isoflurane terminated. Rats recovered on 1 L/min oxygen while the paw was gently massaged, and rectal temperature was measured again. Rats were returned to their home cage after they achieved sternal recumbency. These procedures were completed between 0700 and 0900. Rats were tested in the assessment chamber at 3, 6, 9, and 24 h after injection. Baseline testing was completed at least 1 h before anesthesia. We selected a carrageenan model because it is a commonly used, reliable, induced-inflammation model in nociception studies and one with which we had experience.[11]

Mechanical threshold testing. A single operator performed all testing. The operator was not blind to treatment, given that the carrageenan injection results in gross swelling. Before testing, rats were given approximately 5 min to habituate to the assessment chamber. vF monofilaments (TouchTest sensory evaluator, North Coast, Gilroy, CA) were applied by using a modified version of the 50% withdrawal threshold technique.[5,7] Testing began with the 1-g filament on the injected paw. The filament was touched on to the midplantar surface (filament perpendicular to surface) and held in a buckled position for 3 s. A withdrawal response was recorded when the rat withdrew upon advance or withdrawal of the filament. Filaments were applied individually and in ascending order until paw withdrawal occurred or a maximal filament size of 15 g was applied. After the first positive withdrawal response, filaments were applied in an up–down manner: the next smaller filament was applied, and if there was no reaction, the next larger filament was used. This pattern was continued until 4 filaments were tested after the initial withdrawal. Testing alternated between the left (injected) and right (uninjected) hind paws, with 30 s between tests. Withdrawal thresholds were calculated by using a previously derived formula.[5,9]

The RatMet device was used according to the manufacturer's guidelines. The probe was applied to the plantar surface of the paw (probe perpendicular to the surface) and advanced to increase the applied force. Once the withdrawal threshold was reached or when the 'out of range' light illuminated (triggered by a force in excess of 100 g), the measurement was recorded, and a force–time curve was generated. Testing alternated between the left and right hind paw with a 30 s interval between applications. Each paw was tested 5 times. The 5 withdrawal thresholds from each time point for each paw were averaged. To ensure consistency, each force–time curve was visually assessed against the recommended force application slope of 20 g/s. If the generated curve was outside the recommended zone, the graph was discarded and the paw retested.

Statistical analysis. The dataset was assessed for normality by using an Anderson–Darling test; because most comparisons were normally distributed and ANOVA is robust to small deviations from normality, ANOVA was used as described. Within-group data between time points were analyzed by using one-way ANOVA for repeated measures and Dunnett posthoc testing to detect differences between baseline and each postinjection time point. Differences between treatment groups

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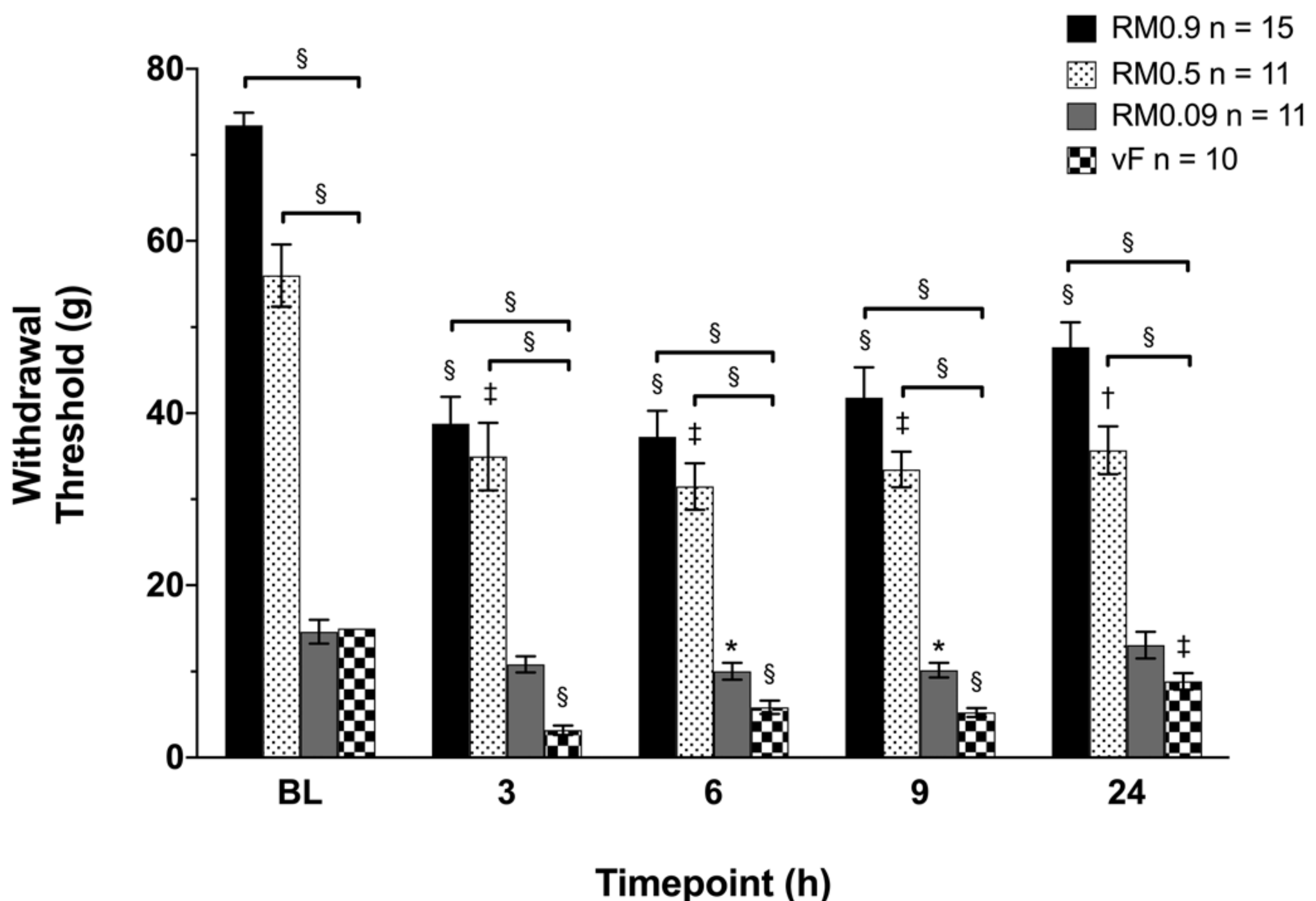

Figure 1. Average withdrawal threshold (grams) for RM0.9 (0.9-mm RatMet probe tip), RM0.5 (0.5-mm RatMet probe tip), RM0.09 (0.09-mm RatMet probe tip), and von Frey (vF) monofilaments at baseline and 3, 6, 9, and 24 h after injection on the treated left hindpaw. Significant differences between vF monofilaments and both the RM0.9 and RM0.5 probes were present at all time points ($P < 0.0001$, § accompanied by horizontal bar). Significant differences within groups, between baseline and other time points, are indicated as *, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$; and §, $P < 0.0001$.

were tested by using 2-way ANOVA for repeated measures and Dunnett posthoc testing to compare withdrawal thresholds at every time point. The effect of testing frequency was analyzed by comparing the first test to the average of first to third tests and to the average of first to fifth tests by using 2-way ANOVA and Tukey posthoc testing. To evaluate the relationship between withdrawal thresholds and probe or filament properties, the baseline withdrawal thresholds (in g) were plotted against the probe area (in mm^2), probe diameter (mm, measured by using digital calipers), and the square root of probe diameter ($mm^{0.5}$). Lines of best fit were fitted to the data by using a curve-fitting function and least-squares regression. A sample size of 12 animals per group was estimated to provide power of 80%, α value of 0.05, and effect size of 1.5. Statistical analysis and curve fitting were performed by using commercial software (Prism 6.0f, GraphPad Software, San Diego, CA). A P value of less than 0.05 was considered significant. All data are presented as mean ± SEM. Data supporting the results are available in an electronic repository: https://doi.org/10.7910/DVN/GSDRUM.

Results

Comparing withdrawal thresholds between RatMet and vF monofilaments. All 47 rats enrolled in the study were included in analysis. In the injected left hind paw, testing with RM0.9, RM0.5, and vF monofilaments revealed significantly lower withdrawal thresholds at all time points after injection as compared with baseline (Figure 1; RM0.9: main effect, $F_{3.1,43.3} = 38.0$, $P < 0.0001$; 3 h, $P < 0.0001$; 6 h, $P < 0.0001$; 9 h, $P < 0.0001$; 24 h, $P < 0.0001$; RM0.5: main effect, $F_{2.1, 20.9} = 19.3$, $P < 0.0001$; 3 h, $P = 0.0003$; 6 h, $P = 0.0005$; 9 h, $P = 0.0009$; 24 h, $P = 0.002$; vF monofilaments: main effect, $F_{1.7,15.2} = 49.4$, $P < 0.0001$; 3 h, $P < 0.0001$; 6 h, $P < 0.0001$; 9 h, $P < 0.0001$; 24 h, $P = 0.0004$). RM0.09 resulted in significantly lower withdrawal thresholds (main effect, $F_{2.5,24.5} = 5.0$, $P = 0.007$) at 6 h ($P = 0.035$) and 9 h ($P = 0.041$) but not at 3 h ($P = 0.07$) or 24 h ($P = 0.76$). Between-groups comparison showed a significant interaction effect ($F_{12,172} = 10.1$, $P < 0.0001$) and a significant main effect ($F_{3,43} = 137$, $P < 0.0001$). The multiple comparisons revealed no significant difference in thresholds between vF monofilaments and RM0.09 at any time point (baseline, $P = 0.99$; 3 h, $P = 0.09$; 6 h, $P = 0.51$, 9 h, $P = 0.38$; 24 h, $P = 0.51$), whereas a significant difference between vF monofilaments and RM0.9 ($P < 0.0001$) and the RM0.5 probe ($P < 0.0001$) was present at every time point (Figure 1).

The average withdrawal threshold (grams) of the noninjected right hind paw showed no significant differences within groups across all time points (Figure 2): RM0.9 (main effect, $F_{2.52,35.08} = 2.62$, $P = 0.075$; 3 h, $P = 0.94$; 6 h, $P = 0.54$; 9 h, $P = 0.24$; 24 h, $P = 0.86$); RM0.5 (main effect, $F_{2.91,29.11} = 2.67$, $P = 0.068$; 3 h, $P = 0.13$; 6 h, $P = 0.26$; 9 h, $P = 0.10$; 24 h, $P = 0.21$), RM0.09 (main effect, $F_{2.02,20.16} = 2.58$, $P = 0.10$; 3 h, $P = 0.14$; 6 h, $P = 0.22$; 9 h, $P = 0.07$; 24 h, $P = 0.13$), vF monofilaments (main effect, $F_{1.0,9.0} = 1.0$, $P = 0.34$; $P > 0.99$, all time points). Between-

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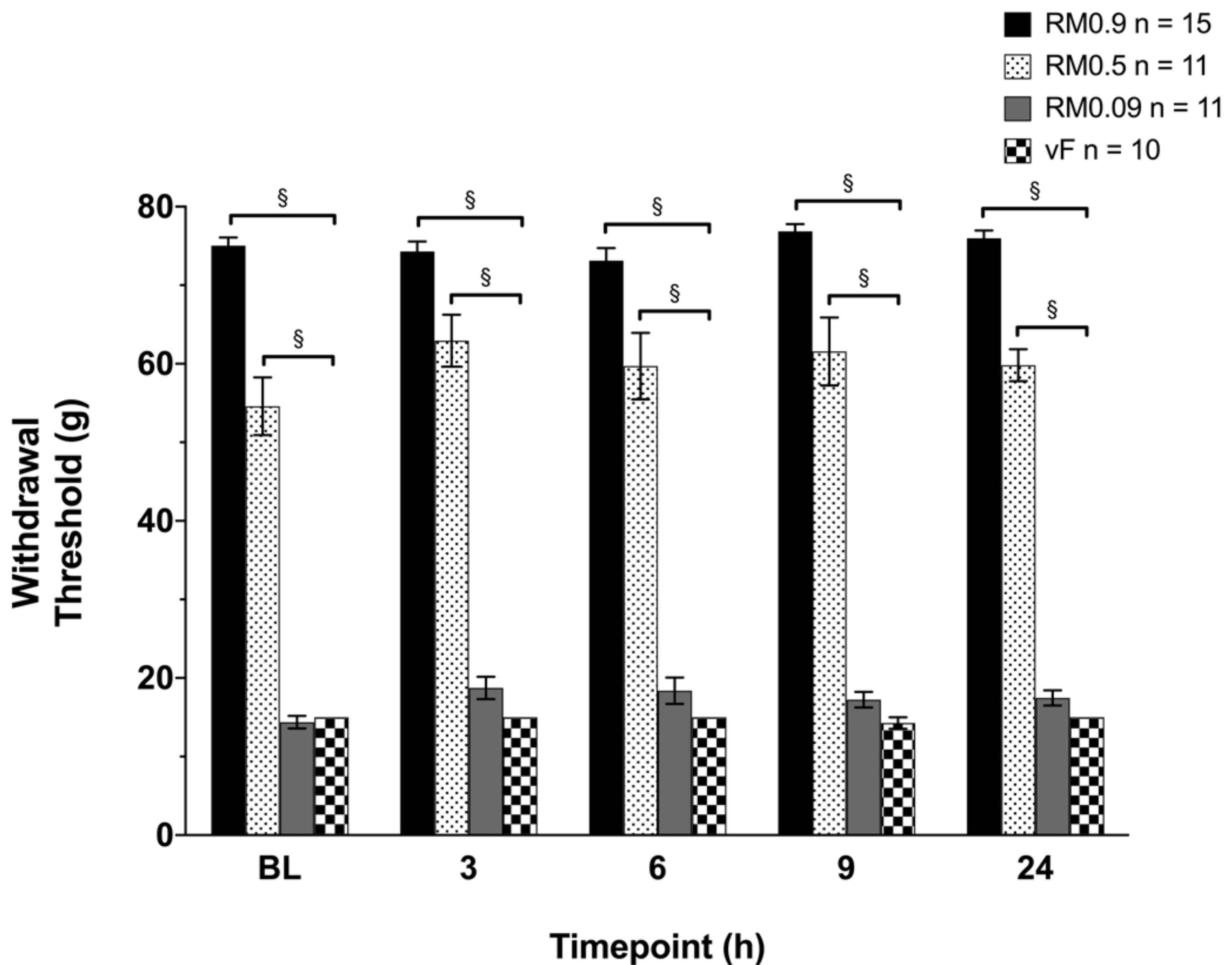

Figure 2. Average withdrawal threshold (grams) for RM0.9 (0.9-mm RatMet probe tip), RM0.5 (0.5-mm RatMet probe tip), RM0.09 (0.09-mm RatMet probe tip), and von Frey (vF) monofilaments at baseline and 3, 6, 9, and 24 h after injection on the untreated right hindpaw. Significant differences were not recorded within groups at any time point compared with baseline ($P > 0.99$). A systematic difference was observed between vF and both the RM0.9 and 0.5 probes at all time points ($P < 0.0001$, § accompanied by horizontal bar).

group comparisons showed a significant interaction effect ($F_{3,43} = 362$, $P < 0.0001$) and a significant main effect ($F_{12,172} = 10.1$, $P < 0.0001$). Multiple comparisons revealed no significant differences in withdrawal thresholds from vF monofilaments compared with the RM0.09 probe at any time point (baseline, $P = 0.99$; 3 h, $P = 0.42$; 6 h, $P = 0.50$; 9 h, $P = 0.60$; 24 h, $P = 0.72$). Significant differences were observed between vF monofilaments and the RM0.9 probe ($P < 0.0001$) and RM0.5 probe ($P < 0.0001$) at all time points (Figure 2).

To identify the assessment group that was the most robust in detecting changes in withdrawal thresholds, percent change from baseline was calculated for the injected left hind paw. The RM0.9 and RM0.5 probes had the largest decrease at 6 h (decreases of 49% and 44% from baseline, respectively), whereas RM0.09 had a peak that was identical at 6 and 9 h (decrease of 31% from baseline). The greatest reduction in withdrawal threshold occurred at 3 h with vF monofilaments (decrease of 79%; Table 1).

Relationship between withdrawal threshold, area, and diameter of RatMet probes and vF monofilaments. For the RatMet device, the withdrawal threshold approximated a linear relationship with probe diameter, the square root of diameter, and probe area (Figure 3). In contrast, vF monofilaments exhibited an exponential relationship (Figure 3).

Table 1. Percentage change of mechanical withdrawal thresholds from baseline

	Time after injection			
Group	3 h	6 h	9 h	24 h
vF	**–79%**	–61%	–65%	–41%
RM0.9	–47%	**–49%**	–43%	–35%
RM0.5	–38%	**–44%**	–40%	–36%
RM0.09	–26%	**–31%**	**–31%**	–11%

Withdrawal thresholds measured by the RatMet device with lower numbers of replicate applications. To investigate the accuracy of the RatMet device when fewer test applications are used, withdrawal thresholds from the first, the average of the first to third, and the average of the first to fifth withdrawals from the injected left hind paw were calculated and used to assess whether the quality of the data was maintained with reduced testing. The withdrawal thresholds from these 3 application conditions were not significantly different at any time point among all 3 RatMet assessment groups (Table 2, Figure 4).

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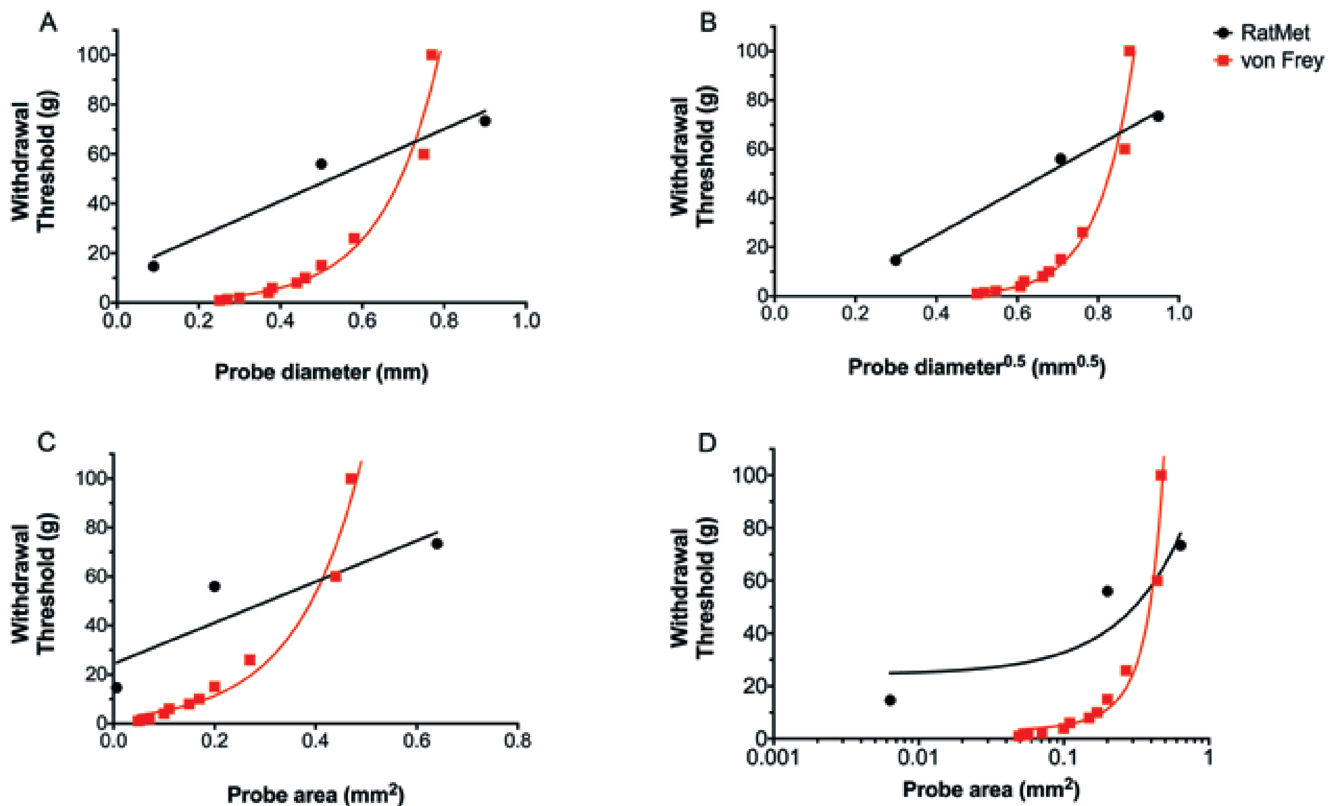

Figure 3. Baseline withdrawal thresholds (grams) plotted against (A) probe diameter (mm), (B) square root of probe diameter (mm$^{0.5}$), (C) probe area (mm^2) on a linear scale and D) probe area (mm^2) on a log scale. Withdrawal thresholds measured by RatMet best approximated a linear relationship with diameter, square root of diameter, and area. vF filaments exhibited an exponential relationship in all 3 cases.

Table 2. Comparisons of withdrawal thresholds recorded by using RM0.09 at different time points before (baseline) and after injection of carrageenan into the left hindpaw

	RM0.9 *P* (95% CI)	RM0.5 *P* (95% CI)	RM 0.09 *P* (95% CI)
baseline			
1st compared with 1st–3rd average	>0.99 (–11.0 to 11.1)	0.83 (–13.6 to 8.3)	0.25 (–1.7 to 8.9)
1st compared with 1st–5th average	0.98 (–10.3 to 11.9)	0.65 (–15.0 to 6.9)	0.08 (–0.4 to 10.2)
1st–3rd compared with 1st–5th average	0.99 (–10.4 to 11.8)	0.95 (–12.4 to 9.6)	0.84 (–4.0 to 6.7)
3 h			
1st compared with 1st–3rd average	0.96 (–9.8 to 12.4)	0.95 (–9.5 to 12.4)	0.98 (–4.9 to 5.8)
1st compared with 1st–5th average	0.95 (–9.6 to 12.5)	0.87 (–8.6 to 13.3)	0.96 (–4.7 to 5.9)
1st–3rd compared with 1st–5th average	>0.99 (–11.0 to 11.2)	0.98 (–10.1 to 11.8)	>0.99 (–5.1 to 5.5)
6 h			
1st compared with 1st–3rd average	0.93 (–9.4 to 12.7)	0.95 (–9.6 to 12.3)	0.94 (–4.6 to 6.0)
1st compared with 1st–5th average	0.96 (–9.8 to 12.3)	0.86 (–8.5 to 13.4)	0.81 (–3.9 to 6.7)
1st–3rd compared with 1st–5th average	>0.99 (–11.5 to 10.7)	0.97 (–9.9 to 12.0)	0.95 (–4.6 to 6.0)
9 h			
1st compared with 1st–3rd average	0.94 (–9.5 to 12.7)	>0.99 (–11.0 to 10.9)	0.99 (–5.0 to 5.6)
1st compared with 1st–5th average	0.94 (–9.5 to 12.7)	0.96 (–9.7 to 12.2)	0.99 (–4.9 to 5.7)
1st–3rd compared with 1st–5th average	>0.99 (–11.0 to 11.1)	0.96 (–9.7 to 12.2)	>0.99 (–5.3 to 5.4)
24 h			
1st compared with 1st–3rd average	0.99 (–10.6 to 11.6)	>0.99 (–11.0 to 10.9)	0.99 (–5.1 to 5.5)
1st compared with 1st–5th average	0.90 (–9.0 to 13.1)	0.99 (–10.8 to 11.1)	0.88
1st–3rd compared with 1st–5th average	0.94 (–9.5 to 12.6)	0.99 (–10.7 to 11.2)	0.92

Discussion

This study had 3 major findings: 1) the RatMet device can discriminate changes in withdrawal threshold after treatment of rats with carrageenan; 2) with the RatMet device, withdrawal thresholds are directly related to probe diameter, the square root of probe diameter, and area, whereas vF exhibit an exponential relationship

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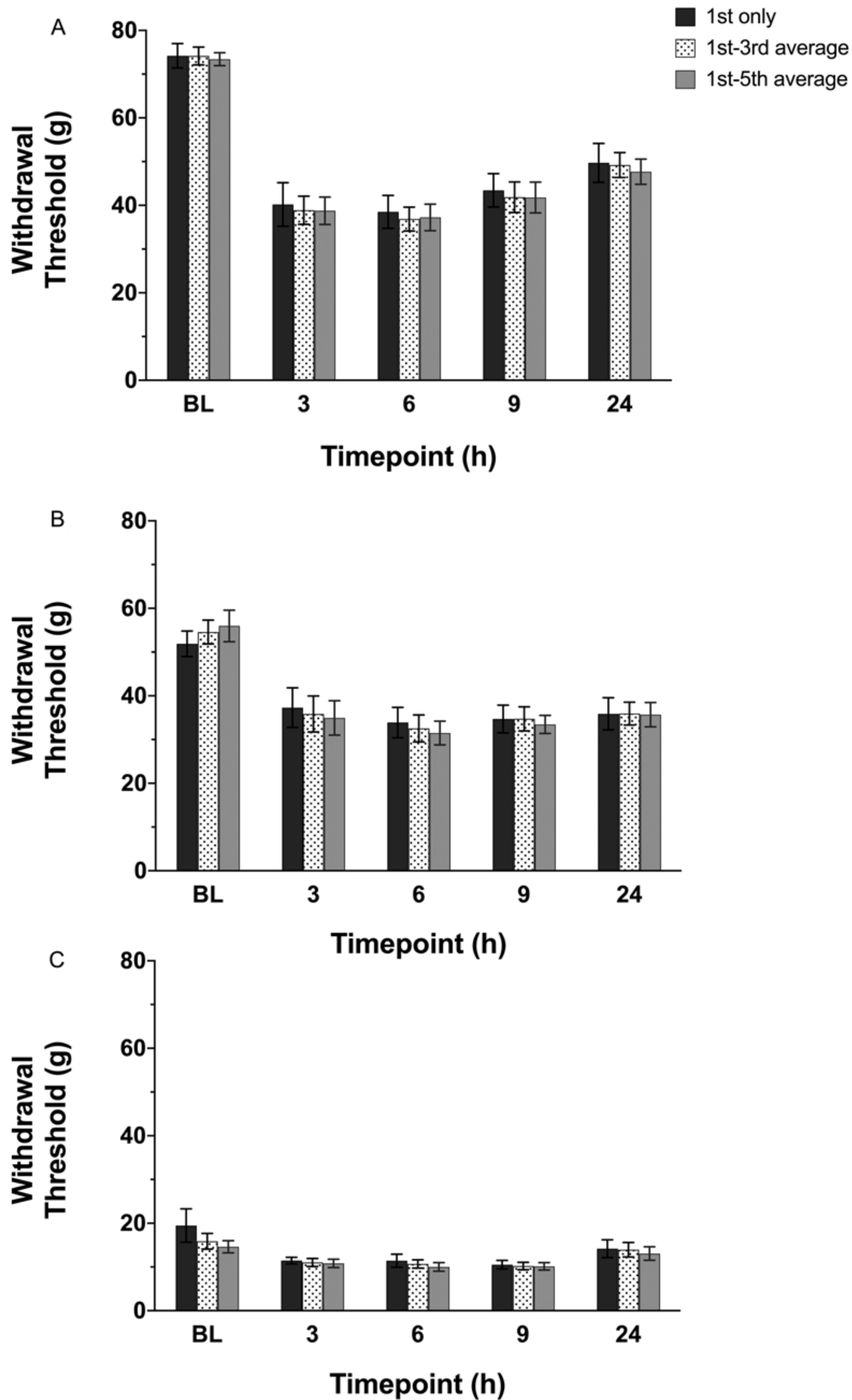

Figure 4. Withdrawal thresholds (grams) for the first application only, an average of the first to third application, and an average of the first to fifth application in the injected left hindpaw measured by using the (A) 0.9-mm, (B) 0.5-mm, and (C) 0.09-mm RatMet probes. Withdrawal thresholds are not significantly different between the 3 application conditions at each time point ($P > 0.05$, Table 2).

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with these measures; and 3) using fewer replicate applications with the RatMet device did not compromise data quality.

Each RatMet probe was able to detect a reduction in withdrawal threshold after injury was introduced. With the exception of the RM0.09 probe, the other 2 probes showed good agreement in identifying the peak reduction at 6 h after injection, whereas the peak was identified at 3 h when using vF monofilaments. The sensitivity of detecting changes appeared to decline as the RatMet probes became smaller, with less dramatic and fewer significant changes from baseline over time. vF monofilaments had a greater sensitivity to changes in nociception than RatMet when the absolute force required for withdrawal was compared at baseline and after injection.

When examining probe and filament characteristics, the RatMet device showed a linear relationship between withdrawal threshold and probe diameter, the square root of probe diameter, and probe area. These findings are in contrast to previous studies that found variable relationships: a linear relationship with diameter but not with area,[10] a sigmoidal relationship with the square root of diameter, and a logarithmic relationship with area.[15] The conclusions from our current study are limited due to the few (n = 3) probes used to characterize these relationships. Defining the relationship on the basis of 3 probes could be an oversimplification. The inconsistency of reporting of these results in the literature[13] confounds the relationships between withdrawal thresholds and probe and filament characteristics, with some papers reporting log10 scales and drawing conclusions about linear relationships,[10] whereas others use linear scales[15] with vast differences in withdrawal threshold ranges. However, when the type of scale is matched (linear or log10) and analogous probe sizes are compared, our results broadly agree with the findings of others.[10,15] This agreement suggests that inconsistent reporting is an important contributor to some of the disparate results in the literature.

The shape of the tip,[1,10] material,[12] length of the probe,[12] and the speed of reaching maximum load[3] may all contribute to the difference of the probe and threshold relationships observed with RatMet compared with vF. In addition, these factors may affect the type of sensation that is produced (touch, sharpness, pressure, or pain),[10] which further complicates interpretation of data from nonhuman or nonverbal scenarios. These factors should be considered in future studies seeking to determine the relationship between withdrawal thresholds and probe characteristics.

Repeated testing with vF monofilaments can decrease withdrawal thresholds in healthy, uninjured rats.[5,6,11] We found that withdrawal thresholds from the untreated paw were not significantly different at any time point as compared with baseline for vF monofilaments and all sizes of RatMet probe tips. This finding indicates a lack of training effect or tissue damage due to stimulus application for any method. The testing frequency and duration of this experiment may have been short enough to avoid this training effect. However, studies that require prolonged testing periods need a device that can accurately quantify nociception with a low number of stimulus presentations. By comparing the first, an average of the first to third, and an average of the first to fifth RatMet probe applications, we were able to determine that less testing is not detrimental to the quality of the data. Our data support the accuracy of RatMet down to a single application per paw. This need for a single test reduces the amount of time needed to test the animal, enabling greater efficiency for the experimenter, less discomfort for the animal, and potentially improved repeatability.[16] Fewer applications also reduces the risk of inadvertently inducing tissue damage or altering behavioral responses due to repetitive testing.[5,6,11] Considering that testing with vF monofilaments required 8 to 11 filament applications per testing session, the RatMet device is notably time-sparing. A comparison of the RatMet device with other electronic vF testing devices would provide a complete picture of its performance. This evaluation was beyond the scope of the current study, whose goal was to make an initial comparison with standard vF monofilaments. After a literature search and contacting several manufacturers of electronic vF testing devices, we have found that direct comparisons between standard vF monofilament testing and electronic vF devices are seldom performed in rodents. One study in rats, using neuropathic pain models, reported that both standard vF monofilaments and an electronic vF device (Dynamic Plantar Aesthesiometer, Ugo Basile SRL, Gemonio, Italy) were able to identify mechanical hypersensitivity in 3 models (partial sciatic nerve ligation, chronic constricted injury, spinal nerve ligation).[14] The study found the most consistent response with the spinal nerve ligation model.[14] The authors suggested that this difference among models could be due to difficulties in applying the electronic vF device probe if the model causes changes in paw conformation and posture. Handheld devices, such as RatMet, may confer an advantage in probe positioning when postural changes are present.

The limitations of our study include the inability to blind the operator to the testing method and the relative time point after injection of carrageenan due to the gross inflammatory reaction that carrageenan induces. Studying additional RatMet probe diameters could more accurately characterize the relationship between withdrawal threshold, probe diameter, and probe area. The injected foot was not randomized to support consistency in performing injections and testing. Generalization of our results are limited by the use of a single strain of rat, a single sex, and a single pain model. These decisions were made due to our focus on comparing methods rather than a detailed exploration of strain-, sex-, or model-associated differences during testing. Future studies are necessary to expand the repertoire and validity for the use of RatMet in various experimental models.

In light of our current findings, we agree with Bove's assertion[3] that vF filaments more closely resemble a tin standard than a gold standard. We have outlined fundamental disadvantages of vF filaments and validated the RatMet device, which overcomes many of these issues. We hope that our results here encourage others to reconsider the context in which vF monofilaments are used.

Acknowledgments

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References

1. **Bishop GH.** 1949. Relation of pain sensory threshold to form of mechanical stimulator. J Neurophysiol **12:**51–57. https://doi.org/10.1152/jn.1949.12.1.51.
2. **Bonin RP, Bories C, De Koninck Y.** 2014. A simplified up-down method (SUDO) for measuring mechanical nociception in rodents using von Frey filaments. Mol Pain **10:**1–10. https://doi.org/10.1186/1744-8069-10-26.

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-02-25

3. **Bove G.** 2006. Mechanical sensory threshold testing using nylon monofilaments: the pain field's "tin standard". Pain **124:**13–17. https://doi.org/10.1016/j.pain.2006.06.020.
4. **Bradman MJG, Ferrini F, Salio C, Merighi A.** 2015. Practical mechanical threshold estimation in rodents using von Frey hairs/ Semmes-Weinstein monofilaments: Towards a rational method. J Neurosci Methods **255:**92–103. https://doi.org/10.1016/j.jneumeth.2015.08.010.
5. **Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL.** 1994. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods **53:**55–63. https://doi.org/10.1016/0165-0270(94)90144-9.
6. **De Rantere D.** [Internet]. 2014. The evaluation of the Rat Grimace Scale and ultrasonic vocalisations as novel pain assessment tools in laboratory rats. Master of Science Thesis presented at the University of Calgary, Calgary, Canada. [Cited 26 June 2017]. Available at: https://prism.ucalgary.ca/handle/11023/1943. http://dx.doi.org/10.11575/PRISM/28137.
7. **De Rantere D, Schuster CJ, Reimer JN, Pang DSJ.** 2015. The relationship between the Rat Grimace Scale and mechanical hypersensitivity testing in three experimental pain models. Eur J Pain **20:**417–426. https://doi.org/10.1002/ejp.742.
8. **de Sousa MV, Ferraresi C, de Magalhaes AC, Yoshimura EM, Hamblin MR.** 2014. Building, testing and validating a set of home-made von Frey filaments: a precise, accurate and cost effective alternative for nociception assessment. J Neurosci Methods **232:**1–5. https://doi.org/10.1016/j.jneumeth.2014.04.017.
9. **Deuis JR, Dvorakova LS, Vetter I.** 2017. Methods used to evaluate pain behaviors in rodents. Front Mol Neurosci **10:**1–17. https://doi.org/10.3389/fnmol.2017.00284.
10. **Greenspan JD, McGillis SL.** 1991. Stimulus features relevant to the perception of sharpness and mechanically evoked cutaneous pain. Somatosens Mot Res **8:**137–147. https://doi.org/10.3109/08990229109144738.
11. **Le Bars D, Gozariu M, Cadden SW.** 2001. Animal models of nociception. Pharmacol Rev **53:**597–652.
12. **McGill M, Molyneaux L, Yue DK.** 1998. Use of the Semmes-Weinstein 5.07/10 gram monofilament: the long and the short of it. Diabet Med **15:**615–617. https://doi.org/10.1002/(SICI)1096-9136(199807)15:7<615::AID-DIA641>3.0.CO;2-P.
13. **Mills C, LeBlond D, Joshi S, Zhu C, Hsieh G, Jacobson P, Meyer M, Decker M.** 2012. Estimating efficacy and drug ED50's using von Frey thresholds: impact of weber's law and log transformation. J Pain **13:**519–523. https://doi.org/10.1016/j.jpain.2012.02.009.
14. **Nirogi R, Goura V, Shanmuganathan D, Jayarajan P, Abraham R.** 2012. Comparison of manual and automated filaments for evaluation of neuropathic pain behavior in rats. J Pharmacol Toxicol Methods **66:**8–13. https://doi.org/10.1016/j.vascn.2012.04.006.
15. **Taylor PM, Dixon MJ.** [Internet]. 2011. The effect of mechanical probe tip diameter. [Cited 08 April 2020]. Available at: http://www.topcatmetrology.com/products/mechanical-threshold-wired-and- wireless/mechanical-probe-tip-diameter/.
16. **Tena B, Escobar B, Arguis MJ, Canterro C, Rios J, Gomar C.** 2012. Reproducibility of electronic von Frey and von Frey monofilaments testing. Clin J Pain **28:**318–323. https://doi.org/10.1097/AJP.0b013e31822f0092.
17. **Treede RD, Rolke R, Andrews K, Magerl W.** 2002. Pain elicited by blunt pressure: neurobiological basis and clinical relevance. Pain **98:**235–240. https://doi.org/10.1016/S0304-3959(02)00203-8.
18. **Turner PV, Pang DSJ, Lofgren JLS.** 2019. A review of pain assessment methods in laboratory rodents. Comp Med **69:**451–467. https://doi.org/10.30802/AALAS-CM-19-000042.

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-02-25