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A Calibrated Method of Massage Therapy Decreases Systolic Blood Pressure Concomitant With Changes in Heart Rate Variability in Male Rats

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Abstract

Objective: The purpose of this study was to develop a method for applying calibrated manual massage pressures by using commonly available, inexpensive sphygmomanometer parts and validate the use of this approach as a quantitative method of applying massage therapy to rodents.

Methods: Massage pressures were monitored by using a modified neonatal blood pressure (BP) cuff attached to an aneroid gauge. Lightly anesthetized rats were stroked on the ventral abdomen for 5 minutes at pressures of 20 mm Hg and 40 mm Hg. Blood pressure was monitored noninvasively for 20 minutes following massage therapy at 5-minute intervals. Interexaminer reliability was assessed by applying 20 mm Hg and 40 mm Hg pressures to a digital scale in the presence or absence of the pressure gauge.

Results: With the use of this method, we observed good interexaminer reliability, with intraclass coefficients of 0.989 versus 0.624 in blinded controls. In Long-Evans rats, systolic BP dropped by an average of 9.86% ± 0.27% following application of 40 mm Hg massage pressure. Similar effects were seen following 20 mm Hg pressure (6.52% ± 1.7%), although latency to effect was greater than at 40 mm Hg. Sprague-Dawley rats behaved similarly to Long-Evans rats. Low-frequency/high-frequency ratio, a widely-used index of autonomic tone in cardiovascular regulation, showed a significant increase within 5 minutes after 40 mm Hg massage pressure was applied.

Conclusions: The calibrated massage method was shown to be a reproducible method for applying massage pressures in rodents and lowering BP. (J Manipulative Physiol Ther 2017;40:77-88)

Key Indexing Terms: *Musculoskeletal Manipulations; Sprague-Dawley Rat; Long-Evans Rat; Diastolic Blood Pressure; Stress; Sympathetic Nervous System; Parasympathetic Tone*

Introduction

Approximately 30% of adults in the United States have high blood pressure (BP),[1] resulting in an annual cost of more than $76.6 billion in health care services and missed work.[2] The etiology of essential hypertension is still unclear, although both the renin-angiotensin system[3-5] and the sympathetic nervous system (SNS)[6-8] appear to play important roles the development of high BP.

Although pharmacologic approaches to the management of hypertension via the renin-angiotensin system and the SNS have proven to be effective, side effects, such as angioedema, headache, hypotension, and dizziness, are common.[9,10] There are relatively few well-studied, nonpharmacologic options for the prevention and treatment of hypertension.[11-13] One potential nonpharmacologic treatment option, massage, has been associated with a significant impact on BP and autonomic nervous system activity. In humans, moderate pressure massage can decrease heart rate and BP while increasing vagal afferent activity as measured by heart rate variability.[14,15] Lumbar spine manipulation has been shown to increase lumbar parasympathetic nervous system output in patients with pain.[16] Moderate pressure massage in humans has also been shown to decrease self-reported stress while increasing delta activity and decreasing alpha and beta activity

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on electroencephalography, suggesting a relaxation response after only 10 minutes of moderate pressure massage.[17] Massage-like stroking of the skin in rats has been reported to increase withdrawal latency to noxious stimuli,[18] which is a sedative response,[19] indicating benefit for pain modulation. Similar manipulation was shown to produce an inhibitory effect on the cardiovascular excitatory response[20] and a reduction in both BP and heart rate.[21,22] Studies on the effects of manual therapies have emphasized that the responses vary significantly, depending on the type, location, and strength of the manual stimulation procedures.[20-23] Importantly, negative side effects from manual therapy are exceedingly rare.[24]

Relatively few studies have utilized animal models to examine the specific mechanisms underlying how massage therapies impact BP. One possible mechanism is revealed from studies using related stimulation (cutaneous brushing of the chest in rats), which significantly decreases adrenal efferent nerve activity and catecholamine secretion.[20,23] The lack of mechanistic studies performed in animals may be attributed to the lack of an inexpensive, precise, and repeatable technique for applying calibrated massage pressures in small rodents. Several studies reported in the early 1990s had performed massage-like stroking of the skin in rats. Unfortunately, the massage pressures used were not calibrated[18] or were estimated and compared with pressures subsequently applied to a balloon connected to a pressure gauge.[21,22] These past studies produced interesting results and demonstrated significant differences between the applications of mild and moderate estimated pressures. However, we recognized the need to develop a technique for applying calibrated massage pressures to rodents with improved interexaminer reliability for our own studies.

The purpose of this study was to develop a method for applying calibrated manual massage pressures using commonly available, inexpensive sphygmomanometer parts and validate the use of this approach as a quantitative method of applying massage therapy to rodents. In this study, we tested the hypothesis that this simple method would provide for good interexaminer reliability, as determined via the Generalizability Theory method. We further hypothesized that this calibrated method of applying massage pressures in rats would decrease BP and increase heart rate variability, which is an index of autonomic tone in cardiovascular regulation. It is our hope that this technique can be employed easily and reliably to quantify the amount of pressure applied during manual massage in small animals and should enable a wider range of animal laboratories to examine mechanisms underlying the effects of massage therapy in rodents.

Methods

Animals

All animals were maintained in accordance with the guidelines in National Institutes of Health Guide for the Care and Use of Laboratory Animals. Thirteen male Sprague-Dawley and 8 Long-Evans rats (300-500 g) were group-housed 2 to 3 per cage in standard polycarbonate plastic cages with heat-treated pine shavings as bedding. Food pellets (Purina Lab Diet; Nestlé Purina Petcare, St. Louis, MO) and water were provided *ad libitum* except during the experimental period. Temperature was maintained at 21 ± 2°C and relative humidity at 50% ± 10% under a 12/12-hour light/dark cycle (lights on from 7:00 to 19:00 hours). All experiments were designed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee, University of California (Riverside, CA).

Massage Therapy

Massage therapy was performed under light anesthesia (1.75% isoflurane, 1 L/min oxygen [O_2]) after a brief induction (5% isoflurane: 95% O_2; rate of air flow = 2 L/min). Each rat was laid on its back inside a ventilated fume hood and under a heat lamp, which was placed 20 inches above the rat. A neonatal BP cuff (Trimline Tempa-Kuff, Welch Allyn, size 1) was modified via a midline cut that retained only the inflatable bladder (Fig 1A). The cuff was attached to a standard clinical aneroid gauge (Tycos, Skaneateles Falls, NY) via a Luer connector (Fig 1B). These components were chosen because of their durability, ease of cleaning, and ready availability. The bladder was inflated to 100 mm Hg and held lightly by the edges. The hook-and-loop side of the neonatal cuff was positioned at the distal index finger to provide appropriate friction in anesthetized rats, and the smooth side of the cuff was used to make contact with the rats' fur (Fig 2A). An investigator could wrap his or her index finger with a "loop surface" that would attach to the hook surface on the neonatal cuff to secure the cuff to the index finger, although this was not necessary in this study.

When applying the study method in the rats, the animals were stroked on the ventral abdomen rostrally to caudally from just below the sternum to the pelvis. The bladder was then removed from the animal and positioned just below the sternum for the next stroke. Care was taken to ensure that when using the technique only the bladder surface contacted the animal and that neither the experimenter's hand nor the sphygmomanometer tubing came in contact with the animal during the procedure. A digital timer was used to monitor stroke rate, and massage was applied at a rate of 60 strokes per minute (1 stroke per second) for 5 minutes (Fig 2B). The neonatal cuff was cleaned with 75% ethanol between therapy sessions.

Massage pressures were monitored as net pressure above 100 mm Hg baseline setting, and therapy was delivered at pressures of 40 mm Hg and 20 mm Hg. A no therapy session was performed 5 to 7 days prior to manipulation of the treated rats to remove any effect of anesthesia and the experimental environment. Therefore, each rat received 3

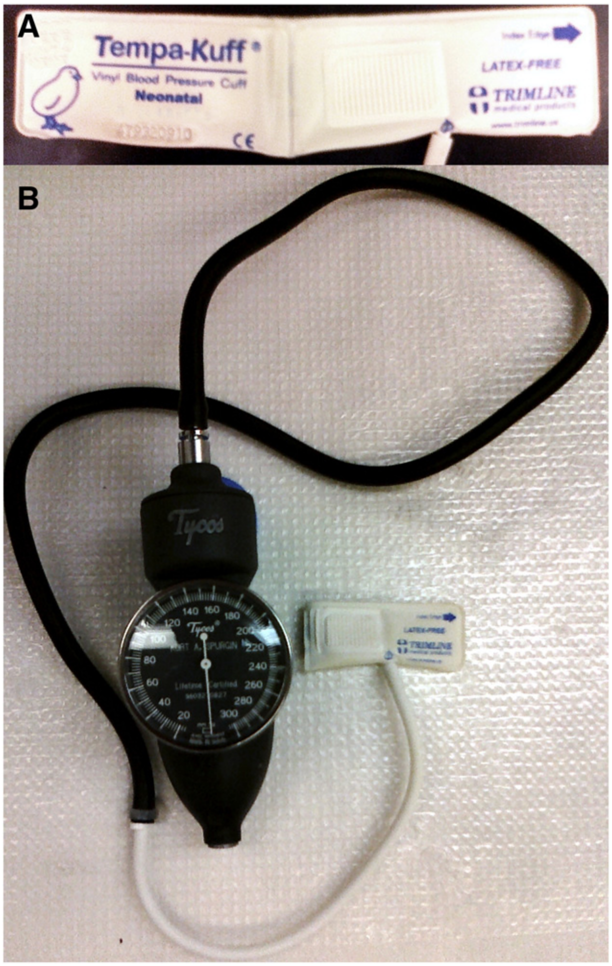


Fig 1. *Apparatus used for applying calibrated massage therapy to rodents was developed using a modified neonatal blood pressure cuff. A neonatal blood pressure cuff (Trimline Tempa-Kuff, size 1) was modified via a midline cut that retained only the inflatable bladder (A). The neonatal cuff was attached to a standard clinical aneroid gauge (Tycos) via Luer connector (B).*

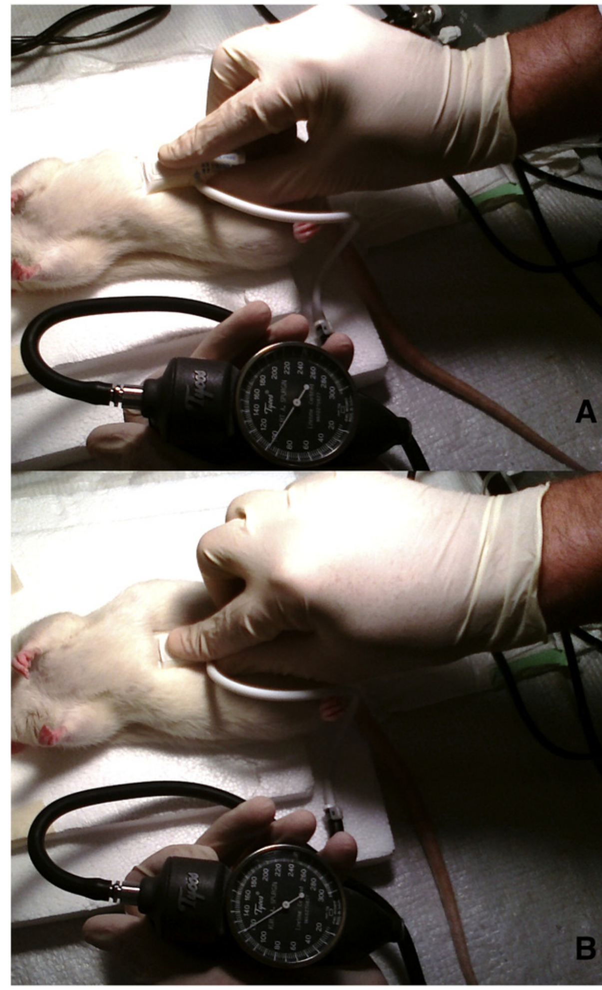


Fig 2. *Demonstration of use. The blood pressure cuff was inflated to 100 mm Hg, held lightly by the edges, and positioned mid-abdomen in preparation for massage therapy procedure (A). The hook and loop side of the neonatal cuff was positioned at the distal index finger to provide appropriate friction while the smooth side of the cuff was used to contact the rats' fur. During each stroke, pressure is precisely monitored relative to 100 mm Hg baseline. Representative image of a lightly anesthetized Sprague-Dawley rat receiving treatment at a massage pressure of 20 mm Hg over the 100 mm Hg baseline (B).*

sessions (no therapy, massage therapy at 20 mm Hg, and massage therapy at 40 mm Hg) on 3 separate days at the same time of day to control for circadian fluctuations in body temperature and other physiological parameters. The massage pressure used for the pressure application sessions for each animal was determined in randomized order via a coin toss. Each session lasted 20 minutes, and subsequent therapy sessions were spaced 2 days or more apart. Baseline systolic BP, mean arterial pressure, and electrocardiography (ECG) values were established after 5 minutes of maintenance anesthesia. Post-massage data collection was performed on each subject for 20 minutes. Blood pressure was monitored via a noninvasive blood pressure (NIBP) method, using sphygmomanometry at 5-minute intervals. Data are expressed as percentage change from the baseline measurement obtained at the beginning of each session. No rats were removed from the study; however, NIBP values were omitted in the rare case of a very low signal-to-noise ratio.

Interexaminer Reliability

To test interexaminer reliability, 12 students from the University of California, Riverside (UCR) were randomly assigned to 2 groups by using a coin toss. One group of students was allowed to monitor the amount of pressure they were applying by observing the pressure gauge (nonblinded). In the other group, students utilized the same apparatus but were not permitted to look at the gauge (blinded). Both groups were briefly trained to accurately apply 20 mm Hg or 40 mm Hg pressure while monitoring the aneroid gauge. Each student was then shown a card with the number 20 or the number 40 printed on it. The order of card presentation was randomized by using a coin flip, and each student was shown 5 cards of each pressure for a total of 10 cards. After being shown the card, the student would then apply the appropriate 20 or 40 mm Hg pressure to a digital scale for 3 seconds. A blinded observer recorded the mass applied during the test in grams. Data were then expressed in Newtons (unit of force) by using the conversion factor: 1 kg = 9.80665002864 N.

Two methods were used to calculate interexaminer reliability[1]: 95% limits of agreement were used to assess the agreement among 7 examiners in both blinded and nonblinded experiments. Therefore, a modified method[25] was used to extend the Bland-Altman method to the case with more than 2 examiners. The systematic differences between examiners were investigated using an analysis of variance (ANOVA) test.[2] Generalizability theory was based on ANOVA, in which multiple sources of measurement error are recognized and estimated.[26,27] Potential sources of measurement error included examiner (e), pressure (p), and their interaction. For each factor and interaction, associated variance components were estimated for both experiments. For each factor and interaction, associated variance components were estimated for both blinded and nonblinded experiments. In the equation used for interexaminer reliability coefficient below the variance component σ_p^2 stands for the factor pressure, the variance component σ_e^2 stands for the factor examiner, and the variance component $\sigma_{e\times p}^2$ stands for the interaction between pressure and examiner.

Interexaminer reliability coefficients were calculated as follows:

$$\frac{\sigma_p^2}{\sigma_e^2 + \sigma_p^2 + \sigma_{e\times p}^2}$$

In separate experiments, we sought to determine the relationship between the amount of pressure being monitored via the aneroid gauge and the force equivalent. A blinded observer recorded the mass applied to a digital scale for 3 different applications of pressure at 10-mm Hg intervals from 10 mm Hg through 80 mm Hg.

Noninvasive BP

Noninvasive blood pressure was measured by tail cuff plethysmography by using a manual BP monitoring system with manual deflation (IITC Inc., Woodland Hills, CA), as described previously.[28] Each NIBP reading was recorded on digital video and systolic BP (highest pressure at which the heart pulses return) and mean arterial pressure (MAP; peak amplitude pulses) were measured over a minimum of 20 cardiac cycles for each recording. Diastolic BP was calculated using the formula MAP = diastolic BP + 1/3 pulse pressure, where pulse pressure is the difference between systolic BP and diastolic BP. All measurements were obtained with the animals under light anesthesia (1.75% isoflurane, 1 L/min oxygen) after a brief induction (5% isoflurane: 95% O_2; 2 L/min). A heat lamp positioned 20 inches from the rat inside the ventilated fume hood was used to maintain the thermoneutrality of the animal. Compared with simultaneous measurements with an invasive catheter and transducer method, NIBP values vary by no more than 7%. Moreover, previous studies have determined a near-perfect correlation (0.98) between radiotelemetry measurements and the pulse-based tail cuff method.[29]

Heart Rate Variability/Autonomic Tone

Electrocardiography was performed with the animals under light anesthesia (1.75% isoflurane, 1 L/min O_2) following a brief induction (5% isoflurane: 95% O_2; rate of air flow = 2 L/min). Electrocardiography recordings were obtained by using adhesive gel electrodes placed on the paws of the rats, and data were recorded by using PowerLab 15T data acquisition hardware (ADInstruments, Dunedin, New Zealand) running *LabChart8* software (ADInstruments). Data from the ECG recording was divided into 5-minute intervals and were exported and postprocessed and smoothed with a Savitzky-Golay filter[30] using the Python software developed in our laboratory (https://github.com/drcgw/BASS). Data were analyzed via the open source *gHRV* software (Milegroup) and our custom Python software, using time-domain parameters, nonlinear analysis, and frame-based analysis. Band limits for spectral analysis of ECG R-R intervals were 0.050 to 0.149 for low frequency (LF) and 0.150 to 0.400 for high frequency (HF). Normalized power spectral density was calculated to evaluate the LF/HF ratio and reported as a percentage of pretreatment baseline plotted over time.

Statistical Analysis

Noninvasive blood pressure and LF/HF ratios were analyzed via 2-way repeated-measures ANOVA. Multiple group comparisons were made post hoc by using the Bonferroni test or Tukey's test. Two methods were used to calculate interexaminer reliability[1]: 95% limits of agreement and Generalizability Theory (see above). Linear regression analysis was used to examine the relationship between the desired pressure applied to the bladder and the actual force registered on a weighing scale in Newtons. Statistical significance was acknowledged at an alpha level of 0.05 unless otherwise stated.

Results

The Described Apparatus Provided for an Accurate and Reproducible Method for Applying Pressure

In this experiment, we tested the hypothesis that our simple method of manual massage therapy provided high interexaminer reliability. Figures 3A and 3B show values of the force, as measured on the massage apparatus, produced by different examiners under both nonblinded and blinded conditions. Using the 95% limits of agreement method for

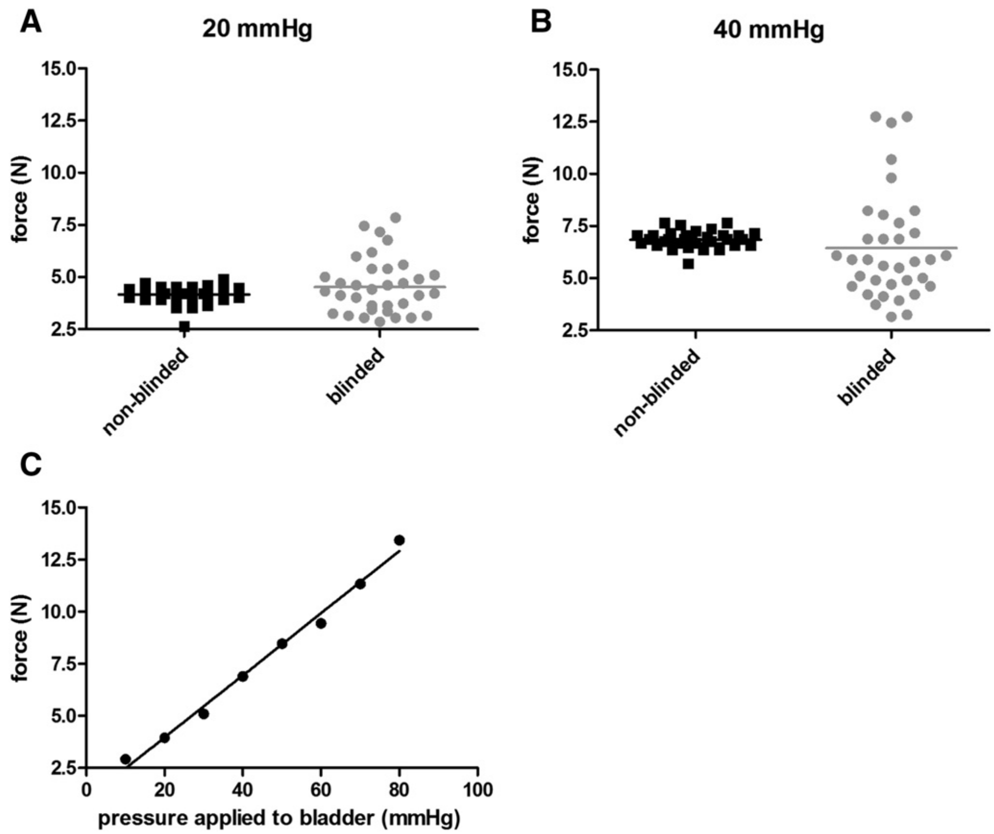


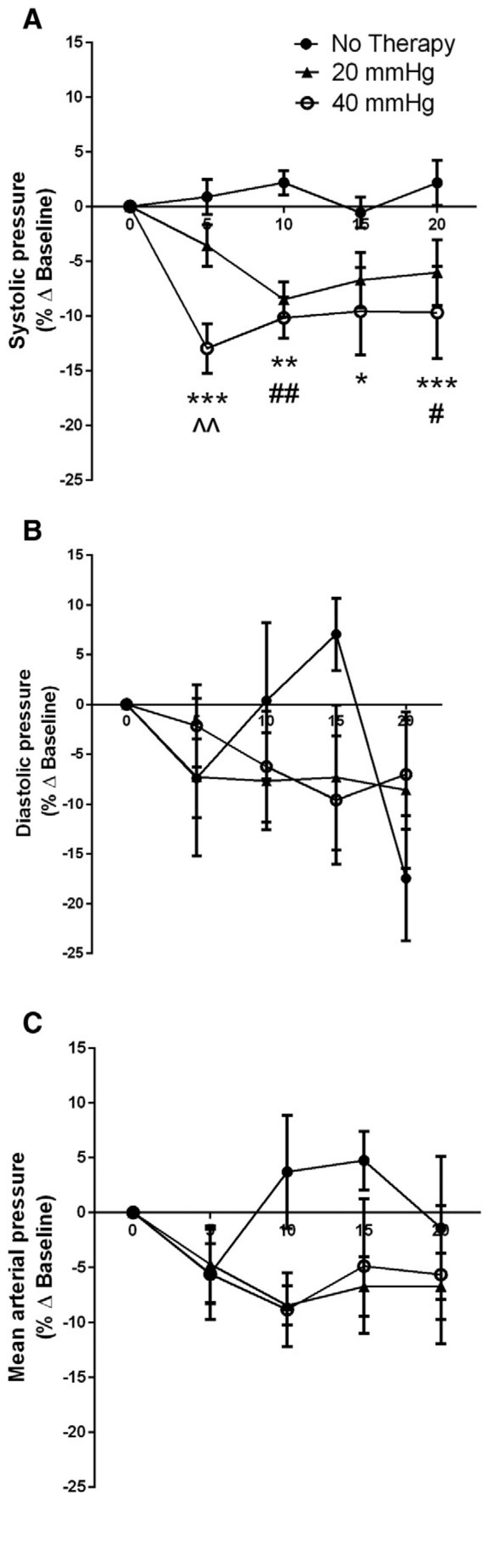
Fig 3. *The calibrated massage therapy method provided an accurate and reproducible method for applying massage therapy pressures in rodents. The massage apparatus shown in Figures 1 and 2 produced reliable applications of force between examiners. When subjects used the apparatus while monitoring the pressure gauge (nonblinded), interexaminer reliability was excellent, as measured by Limits of Agreement and Generalizable Theory methods (A and B). In contrast, when the subjects attempted to reproduce the appropriate massage pressures while blinded to the aneroid gauge, interexaminer reliability was low. Relationship between the pressure measured on the aneroid gauge and calculated force applied to a digital scale converted to Newtons shows a strong positive correlation (C). This analysis showed that an aneroid gauge reading of 20 mm Hg is equivalent to 3.9 N of applied force wherein 40 mm Hg is equivalent to an applied force of 6.9 N.*

the nonblinded experiment (examiners could see the gauge), ANOVA showed significant evidence of a systematic difference among the examiners ($F_{6,62} = 2.51$; $P < .05$) as expected. However, the 95% limits of agreement with the mean, estimated as –0.279, 0.279, were very good, indicating high reliability. For the blinded experiment, ANOVA showed strong evidence of a systematic difference among the examiners ($F_{6,62} = 35.69$; $P < .0001$) as expected. The 95% limits of agreement with the mean are estimated as –2.083, 2.038, suggesting that individual examiners (they could not see the pressure gauge) can be discordant in the mean force generated by as much as 2.083 N.

Using the Generalizability Theory method, the interexaminer reliability coefficients were 0.956 and 0.260 for the nonblinded and blinded experiments, respectively. Thus, the nonblinded experiment had a very good interexaminer reliability coefficient close to 1, whereas the blinded experiment yielded a poor interexaminer reliability coefficient (Figs 3A and 3B). In a separate experiment, we tested the hypothesis that the manual therapy apparatus can yield predictable force when an examiner applies calibrated pressures as measured on a gauge. Figure 3C shows that there is a predictable relationship between the pressure measured on the aneroid gauge and the calculated force applied to a digital scale converted to Newtons. Linear regression analysis gave an R square of 0.99 for "goodness of fit." This analysis showed that an aneroid gauge reading of 20 mm Hg is equivalent to an applied force of 3.9 N, wherein 40 mm Hg is equal to an applied force of 6.9 N.

Calibrated Massage Therapy Reduced Post-Massage Systolic BP in Male Long-Evans Rats in a Dose-Dependent Manner

In these experiments, we tested the hypothesis that our calibrated method of massage therapy could decrease systolic BP up to 20 minutes following massage. Figure 4 shows that under control conditions (ie, no therapy), BP did not change significantly for the duration of the experimental procedure. This finding confirms that the experimental apparatus and the application of anesthesia did not significantly alter BP. A

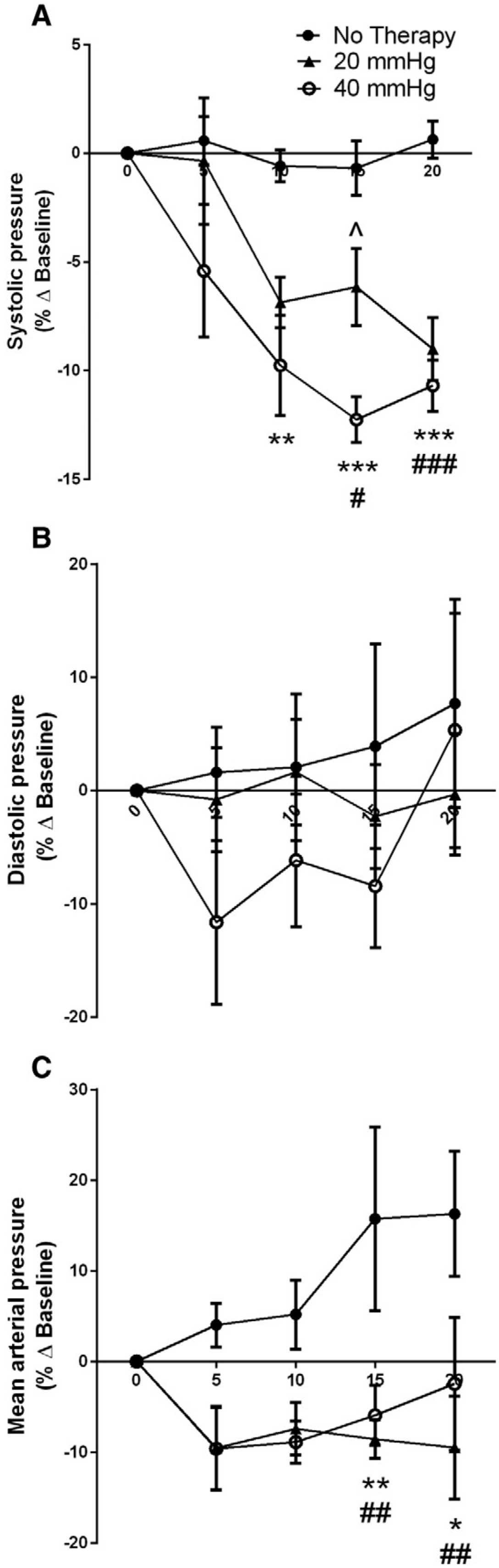


decrease in systolic BP (Fig 4A) was observed in male Long-Evans rats following massage of the lower abdomen at 20 and 40 mm Hg. A repeated measures ANOVA for systolic BP revealed the significant main effects of time ($F_{4,28}$ = 6.77; $P < .001$; $\eta^2 = 0.49$), and therapy ($F_{2,14}$ = 9.34; $P < .01$; $\eta^2 = 0.57$) and for the interaction between time and therapy ($F_{8,56}$ = 3.58; $P < .05$; η^2=0.34). Bonferroni post-tests revealed significant differences between no pressure and 20 mm Hg at 10 minutes (t = 3.53; df = 56; $P < .01$; 95% confidence interval [CI] 3.21-18.15) and 20 minutes (t = 2.71; df = 56; $P < .05$; 95% CI 0.74-15.68). Significant differences were also seen between no pressure and 40 mm Hg at all time points: 5 minutes (t = 4.58; df = 56; $P < .001$; 95% CI 6.38-21.32), 10 minutes (t = 4.09; df = 56; $P < .01$; 95% CI 4.90-19.84), 15 minutes (t = 2.98; df = 56; $P < .05$; 95% CI 1.56-16.50), and 20 minutes (t = 3.93; df = 56; $P < .001$; 95% CI 4.41-19.35). Bonferroni post-tests also revealed significant differences between 20 mm Hg and 40 mm Hg pressures at 5 minutes (t = 3.1; df = 56; $P < .01$; 95% CI 1.91-16.85), suggesting a dose-dependent effect. Two-way repeated measures ANOVA showed no main or interaction effects on MAP (Figs 4B and 4C). With regard to diastolic BP, there was an apparent, but not significant, effect of time ($F_{4,20}$ = 2.7; $P = .0601$; $\eta^2 = 0.35$) and apparent interaction ($F_{8,40}$ = 2.02; $P = .0693$; $\eta^2 = 0.29$).

The Calibrated Massage Therapy Method Reduced Post-Massage Systolic BP and MAP in Male Sprague-Dawley Rats

In a separate strain of rats, we tested the hypothesis that our calibrated massage therapy method significantly decreases post-massage BP. Figure 5 demonstrates that the no-therapy control animals had consistent BP throughout the experimental period. Reduced systolic BP (Fig 5A) was observed in male Sprague-Dawley rats following massage of the lower

Fig 4. *The calibrated massage therapy method reduced systolic blood pressure (BP) in male Long-Evans rats in a dose-dependent manner. Male Long-Evans rats were treated with no therapy, massage therapy at 20 mm Hg, and massage therapy at 40 mm Hg on separate days under light anesthesia (isoflurane, 1.75%) and systolic BP (A), diastolic BP (B), and mean arterial pressure evaluated (C). Each data point represents mean change from baseline ± standard error of mean. Massage therapy at 20 mm Hg reduced systolic BP at 10 minutes and 20 minutes (A), whereas massage therapy at 40 mm Hg reduced systolic BP at all time points following treatment versus no-therapy control sessions. Systolic BP at 40 mm Hg was significantly reduced compared with that at 20 mm Hg at 5 minutes. There was no significant effect of massage therapy on mean arterial pressure or diastolic BP (B and C). Asterisks = A statistically significant difference between 40 mm Hg and no-therapy control sessions via repeated measures analysis of variance (ANOVA) at P < .05 (*), P < .01 (**), and P < .001 (***). Pound symbols = Statistical difference between 20 mm Hg and no-therapy control sessions via repeated measures ANOVA at P < .05 (#) and P < .01 (##). Caret = Difference between 40 mm Hg and 20 mm Hg pressure via repeated measures ANOVA at P < .01 (^).*



abdomen at 20 and 40 mm Hg. ANOVA for systolic BP revealed a significant effect of time ($F_{4,24} = 15.42$; $P < .0001$; $\eta^2=0.72$), therapy ($F_{2,12} = 13.87$; $P < .001$; $\eta^2=0.70$), and interaction ($F_{8,48} = 3.93$; $P < .01$; $\eta^2=0.40$). Bonferroni post-tests revealed significant differences between no pressure and 20 mm Hg at 15 minutes (t = 2.68; df = 48; $P < .05$; 95% CI 0.42-10.84), and 20 minutes (t = 4.65; df = 48; $P < .001$; 95% CI 4.55-14.98). Significant differences were also seen between no pressure and 40 mm Hg at 10, 15, and 20 minutes: at 10 minutes (t = 3.59; df = 48; $P < .01$; 95% CI 2.32-12.75), 15 minutes (t = 5.1; df = 48; $P < .001$; 95% CI 5.50-15.92), and 20 minutes (t = 5.26; df = 48; $P < .001$; 95% CI 5.84-16.26). Bonferroni post-tests also revealed significant differences between 20 mm Hg and 40 mm Hg pressure at 15 minutes (t = 2.42; df = 48; $P < .05$; 95% CI –0.14 to 10.29).

There were no statistically significant main effects or interactions between therapy and time on diastolic BP (Fig 5B). A 2-way repeated-measures ANOVA showed an effect of therapy on MAP (Fig 5C). The effect of massage therapy ($F_{2,10} = 5.25$; $P < .05$; $\eta^2=0.51$) and interaction between therapy and time ($F_{8,40} = 2.22$; $P < .05$; $\eta^2 = 0.31$) was statistically significant. Bonferroni post-tests revealed a significant difference between no therapy and 20 mm Hg at 15 minutes (t = 3.67; df = 40; $P < .01$; 95% CI 7.74-40.83) and 20 minutes (t = 3.89; df = 40; $P < .01$; 95% CI 9.21-42.98). Bonferroni post-tests revealed significant differences between no therapy and 40 mm Hg pressure at 15 minutes (t = 3.27; df = 40; $P < .01$; 95% CI 5.11-38.21) and 20 minutes (t = 2.83; df = 40; $P < .05$; 95% CI 2.18-35.28). There were no significant differences between the 20 and 40 mm Hg therapies.

Fig 5. *The calibrated massage therapy method reduced systolic blood pressure (BP) and mean arterial pressure (MAP) in male Sprague-Dawley rats. Male Sprague-Dawley rats were treated with no therapy, massage therapy at 20 mm Hg, and massage therapy at 40 mm Hg on separate days under light anesthesia (isoflurane, 1.75%). Each data point represents mean change from baseline ± standard error of mean. Massage therapy at 20 mm Hg reduced systolic BP at 15 and 20 minutes following treatment versus no-therapy control sessions (A). Massage therapy at 40 mm Hg reduced systolic BP at 10, 15, and 20 minutes following treatment versus no-therapy control sessions. Significant differences in systolic BP were observed between 20 mm Hg and 40 mm Hg pressures at 15 minutes. There were no statistically significant main effects or interaction between therapy and time on diastolic BP (B). A 2-way repeated-measures analysis of variance (ANOVA) showed an effect of therapy on MAP (C). Bonferroni post-tests revealed a significant difference in MAP between no therapy and 20 mm Hg at 15 minutes and 20 minutes. Significant differences in MAP were noted between no therapy and 40 mm Hg pressure at 15 minutes and 20 minutes. Asterisks = Statistical difference between 40 mm Hg and no-therapy control sessions via repeated measures ANOVA at P < .05 (*), P < .01 (**), and P < .001(***). Pound symbols = Statistical difference between 20 mm Hg and no-therapy control sessions via repeated measures ANOVA at P < .05 (#), P < .01(##), and P < .001(###). Caret = Difference between 40 mm Hg and 20 mm Hg pressure via repeated measures ANOVA at P < .05 (^).*

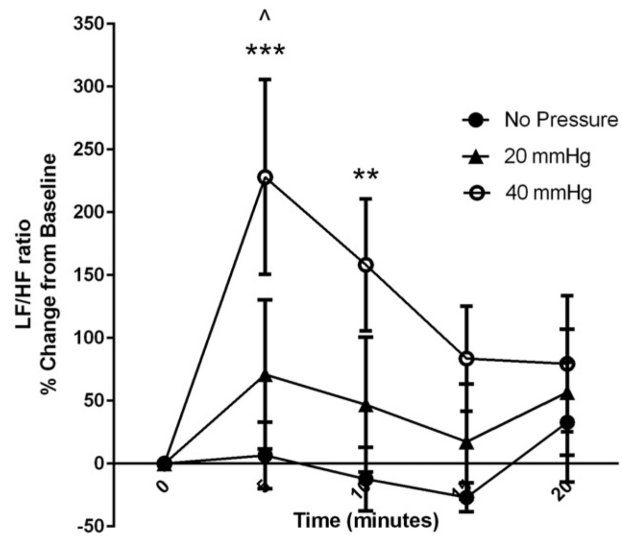


Fig 6. *Calibrated massage therapy altered the low-frequency/high-frequency (LF/HF) ratio. Male Long-Evans rats were treated with no therapy, massage therapy at 20 mm Hg, and massage therapy at 40 mm Hg on separate days under light anesthesia (isoflurane, 1.75%). Pooled data for each treatment group is represented as mean change from baseline ± standard error of mean. A 2-way analysis of variance of LF/HF ratios showed a significant effect of therapy and of the interaction between time and therapy. Bonferroni's multiple comparison test revealed a significant effect of massage therapy at 40 mm Hg on the LF/HF ratio at 5 and 10 minutes following treatment versus no-therapy control sessions. Bonferroni post-tests also revealed a significant difference between changes at 20 mm Hg and 40 mm Hg at 5 minutes. Asterisks = A statistically significant increase in the LF/HF ratio between 40 mm Hg and no-therapy control sessions at P < .05 (*), P < .01 (**). Caret = Difference between 40 mm Hg and 20 mm Hg pressure at P < .05 (^).*

The Calibrated Massage Therapy Method Altered Autonomic Tone in Long-Evans Rats in a Dose-Dependent Manner as Measured by the LF/HF Ratio

In our last set of experiments, we tested the hypothesis that manual massage therapy alters the LF/HF ratio in the post-massage period. Heart rate variability, indicating measured changes in power in LF and HF bands from the power spectrum of ECG, was used to estimate changes in the parasympathetic/sympathetic balance provoked by massage therapy. Long-Evans rats were equipped with ECG leads and heart rate monitored for 5 minutes to acquire baseline reading. After sham or massage therapy, ECG readings were taken at 5, 10, 15, and 20 minutes following treatment. Figure 6 shows the changes in the LF/HF ratio over time following massage therapy. A 2-way ANOVA of LF/HF ratios showed a significant effect of therapy ($F_{2,12}$ = 6.16; $P < .05$; $\eta^2 = 0.51$) and interaction between therapy and time ($F_{8,48}$ = 2.44; $P < .05$; $\eta^2 = 0.29$). Bonferroni's multiple comparison test revealed a significant increase in the LF/HF ratio after 40 mm Hg compared with no therapy at 5 minutes (t = –4.25; df = 48; $P < .001$; 95% CI –363.77 to –95.48) and 10 minutes (t = –3.22; df = 48; $P < .01$; 95% CI –308.08 to –39.72). Bonferroni post-tests also revealed a significant difference between changes at 20 mm Hg and 40 mm Hg at 5 minutes (t = –3.06; df = 48; $P < .05$; 95% CI –29.45 to –31.09).

Discussion

This study provides the first description in the literature of an inexpensive, precise, and reliable technique for applying calibrated massage pressures in small rodents. The described technique utilizes readily available and inexpensive sphygmomanometer parts that can be configured in such a way as to provide consistent massage pressures across studies. We have demonstrated that this technique can produce predictable applications of force by monitoring pressure applied via the aneroid gauge. The method has very good interexaminer reliability because of the use of a visible aneroid gauge. Furthermore, this method can be easily used in animal studies to examine the mechanisms underlying the beneficial effects of massage therapy. Other researchers have successfully engineered methods for applying manual therapies in rodents. For example, Budgell et al used a computer-driven manipulator attached to the C2 spinous process of a rat and incorporated a friction-clutch mechanism to prevent unwanted displacement of the C2 vertebra.[31] Reed et al attached a pair of rigid, adjustable, toothed forceps to the lateral surfaces of the L5 spinous process via an incision and then delivered computer-assisted manipulative thrusts.[32] Although these methods are certainly impressive in their engineering design and can apply the appropriate manipulative forces very accurately, these techniques are invasive and rely on motors and computers to calculate and generate force. Many laboratories that want to conduct research in the field of manual therapy may not be capable of reproducing the methods described by these researchers. In contrast, Song et al utilized an Activator III to model spinal manipulative therapy,[33] and Grayson et al applied finger pressure over the spinous process of L5 to produce a Grade II mobilization of the vertebra.[34] Although these methods have the benefit of being noninvasive and easy to reproduce, the methods required for applying massage pressures differ from those needed for spinal manipulation, as massage involves gliding over the surface of the skin. The method we have described here is advantageous for the purposes of studying the effects of massage therapy because it is noninvasive, is easy to perform, uses inexpensive parts, and can provide an accurate and reproducible delivery of force.

Utilizing this technique, we were able to confirm reports that manually stroking the abdomen of rats can lower systolic BP.[21,22] In our study, a 5-minute massage period produced benefits that lasted beyond the therapy period. Long-Evans and Sprague-Dawley rats were both responsive to massage therapy, and the hypotensive effects lasted up to 20 minutes after therapy, suggesting that the beneficial effect results from conserved physiological traits. However, some differences were observed. In Long-Evans rats, the lowest pressure, 20 mm Hg, produced a latency period of 10 minutes to reduced systolic BP, whereas with the higher massage pressure, 40 mm Hg, the latency period was reduced to 5 minutes. In Sprague-Dawley rats, these latencies were 15 and 10 minutes, respectively, suggesting greater responsiveness to therapy in Long-Evans rats. Moreover, a significant main effect of therapy was a greater reduction in systolic BP after 40 mm Hg than after 20 mm Hg massage therapy in both rat strains, again seen relatively earlier in Long-Evans rats (5 minutes) than in Sprague-Dawley rats (15 minutes). Long-Evans rats have been shown to have higher BP in response to stressors, such as psychosocial stress[35,36] and caloric restriction.[37] These findings demonstrate the reproducibility of our calibrated method of applying massage therapy. The differential latency in effect and in dose dependency highlights the need for accurately calibrating the application of massage therapies in rodents to optimize responses and also for comparing results across studies.

Prior studies have demonstrated that massage-like stroking of the abdomen produces a more robust decrease in BP compared with stroking of the sides or back.[22] Thus, it is likely that stroking of the abdomen activates the visceral afferents in addition to activating the somatosensory afferents. It is possible that massaging the abdomen in this manner displaces the diaphragm, thereby influencing tidal volume and carbon dioxide concentrations.[38] This technique likely produces some mechanical compression of the vena cava; however, prior studies have demonstrated that decreased BP following stroking of the abdomen in rats most likely results from a reduction of the efferent sympathetic nerve activity and associated decreases in adrenal catecholamine secretion.[20-22] We found increased LF/HF ratios during massage therapy via heart rate variability analysis, suggesting altered autonomic tone during massage. The LF/HF ratio has been used in the past to identify changes in sympathetic tone, but current views of the LF/HF ratio indicate that these heart rate variables are predominantly under parasympathetic control and may be less representative of the sympathovagal balance.[39,40] Consequently, it is unclear whether significant elevation in the LF/HF ratio represents changes in sympathetic tone in relation to parasympathetic tone. Our findings implicate changes in autonomic tone, but the relative contribution of sympathetic tone versus parasympathetic tone in reducing BP during massage therapy[20,23] remains unclear. Further experiments designed to directly quantify sympathetic activity (via recordings from sympathetic nerves) would provide more insight into the relative role of sympathetic drive in altering BP during massage-like manipulations.

It is likely that endocrine, in addition to neural pathways, contributes to the reduced systolic BP following therapy, since the effect persisted for 20 minutes following massage. Manual stimulation in rats has been shown to significantly increase glucocorticoid receptor gene expression, which can enhance negative feedback inhibition of hypothalamic–pituitary–adrenal (HPA) axis activity and reduces poststress secretion of adrenocorticotropic hormone and glucocorticoid.[35] Reduced HPA axis activity at the level of corticotropin-releasing factor neurons within the paraventricular nucleus of the hypothalamus may, in turn, reduce sympathetic efferent nerve activity and adrenal catecholamine secretion, contributing to reduced systolic BP.[20] Alternatively, long-acting peptide modulatory systems regulated by sympathetic nerves may participate.[41] The purpose of this study was to demonstrate and test the effectiveness of a new calibrated manual massage method. This method should enable future study into the mechanisms underlying autonomic effects of massage as well as how massage impacts the complex relationships between the HPA axis and the SNS.

Considering the broad socioeconomic impact of hypertension and related diseases, there is a need to evaluate the efficacy of safe, widely available, and minimally invasive alternatives to popular pharmacologic treatments. Manual therapies, such as manipulation and massage, show promise in this arena because of the relationship between hypertension and the SNS and the ability of manual therapies to impact the SNS. Importantly, recurring massage therapy could prove to be a valuable tool in the prevention of stress-related disorders. Consider, for instance, that nearly 1 in 5 US soldiers returning from the wars in Iraq and Afghanistan suffer from post-traumatic stress disorder, which has an estimated societal cost of $2 billion to $3 billion per year.[42] There is also a higher incidence of hypertension among combat-deployed soldiers.[43] In this context, a hypertension prevention strategy that includes massage therapy could be incredibly valuable. Further, if a preventive strategy is to be broadly employed, safety is of vital importance. Current treatment strategies carry significant side effects and are not suited for prophylactic treatment. Our novel calibrated method of applying massage therapy offers an improved strategy that is noninvasive and inexpensive. Further improvements could involve automation for application of massage pressures and for measurement of BP. Improvements in the techniques for examining the effects of massage therapy in animal models should assist in providing a better understanding of the mechanisms underlying the effects and the possible long-term persistence of benefits of massage therapy. Further research is needed to develop guidelines for the appropriate use of manual massage strategy in the treatment and/or prevention of stress-related disorders and hypertension.

Limitations

Although it may be possible to mechanize the described procedure in the future, we specifically set out to develop an inexpensive method that uses readily available components and that can be easily and cheaply adopted by smaller laboratories. However, the study has some limitations. We attempted to control for any effects of anesthesia and core temperature changes by using the lightest anesthesia necessary to prevent movement, limiting the experimental period to 20 minutes and the number of sessions on each animal to 3 and performing the procedure on different days. Using a calibrated vaporizer that controlled the mixture of oxygen and isoflurane gas, each animal likely reached the same degree of anesthesia during the no-therapy, 20 mm Hg therapy, and 40 mm Hg therapy sessions. The experimental setup was identical for each session; that is, the rat was in a fume hood with a preset heating lamp overhead. Thus, each animal received the same degree of warming. The effect of the massage therapy technique for each pressure was compared was each animal's no-therapy session. Over the experimental period, systolic BP was unchanged in the no-therapy groups in both rat strains, indicating that our experimental conditions had no significant effect on systolic BP. However, isoflurane may cause a slight increase in MAP measures over time. We have attempted to control for this effect by statistically comparing each animal's parameters with those obtained during a no-therapy session.

Because the massage method involves manual application, there may be some slight variation in each examiner's application of the procedure. However, we have demonstrated that interexaminer reliability is excellent when employing this calibrated manual massage therapy technique as long as the examiner can visually track the pressure applied by using an aneroid gauge.

Furthermore, during each massage stroke, the inflatable bladder glides over the surface of the animal. The internal organs, skeleton, and muscle of the animal provide different degrees of resistance at different points along the stroke path. This is a variable that is inherent in any massage procedure. We tried to minimize differences in "load" by conducting all of the massage procedures on male rats of similar size and weight. There may be differential effects of the pressures when used in female rats or in smaller rats, although this remains to be determined. An important advantage of our technique is that examiners can use increased or decreased pressures as necessary for the animal model of their choosing. The examiner monitoring a pressure gauge has the ability to rapidly adjust the amount of pressure being applied during each stroke to maintain a constant reading on the aneroid gauge. Achieving a similar degree of consistency by using mechanical means would, however, require a level of engineering that is not readily available to most laboratories.

Conclusions

This study demonstrated a reliable and predictable method for applying precise manual massage pressures in rodents, and this technique showed excellent interexaminer reliability. The procedure decreased systolic BP in a dose-dependent manner concomitant with altered LF/HF ratio.

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Contributorship Information

Concept development (provided idea for the research): K.S., M.C.C.

Design (planned the methods to generate the results): K.S., M.C.C.

Supervision (provided oversight, responsible for organization and implementation, writing of the manuscript): M.C.C.

Data collection/processing (responsible for experiments, patient management, organization, or reporting data): K.S., M.C.C., R.G., V.J., A.K.

Analysis/interpretation (responsible for statistical analysis, evaluation, and presentation of the results): K.S., M.C.C., C.G.W., A.K., K.X.

Literature search (performed the literature search): K.S., M.C.C.

Writing (responsible for writing a substantive part of the manuscript): K.S., M.C.C.

Critical review (revised manuscript for intellectual content, this does not relate to spelling and grammar checking): C.G.W.

Practical Applications

- A calibrated method for applying manual therapy to rodents was developed by using a modified neonatal BP cuff.
- The calibrated manual therapy method provided a reproducible method for applying manual pressures in rodents.
- Calibrated massage therapy reduced post-massage systolic BP in male Long-Evans rats in a dose-dependent manner.
- The calibrated manual therapy method reduced systolic BP and MAP in male Sprague-Dawley rats.
- Calibrated manual therapy reduced heart rate variability in Long-Evans rats as measured by the LF/HF ratio.

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