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Comparative Efficacies of Calmare Therapy and Transcutaneous Electrical Nerve Stimulation in Randomized Peripheral Neuropathy Subjects with Resting State fMRI Monitoring

Ty A. Bodily
BYU, bodilyty@gmail.com

Logan Edvalson
BYU, logan.edvalson@gmail.com

Jordan B. Peacock
BYU, jordanbpeacock@gmail.com

Zack M. Bradshaw
BYU, zackmbradshaw@gmail.com

Carson Hart
BYU, carsonhart3@yahoo.com

See next page for additional authors
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Authors

Ty A. Bodily, Logan Edvalson, Jordan B. Peacock, Zack M. Bradshaw, Carson Hart, Cameron Ernst, Samuel Biggs, and David D. Busath

Comparative efficacies of Calmare® therapy and transcutaneous electrical nerve stimulation in randomized peripheral neuropathy subjects with resting state fMRI monitoring

Ty A Bodily, BS, MS, Logan Edvalson, BS, Jordan B Peacock, BS, Zack M Bradshaw, BS, Carson Hart, BS, Cameron Ernst, BS, Samuel Biggs, BS and David D Busath, BA, MS, MD

Department of Physiology and Developmental Biology and Center for Neuroscience, Brigham Young University, Provo, Utah, United States of America

Abstract: Clinical reports of Calmare® protocol efficacy suggest enhanced durability compared to TENS and the possibility changes in resting fMRI connectivity. The objective was to compare peripheral neuropathy pain relief and resting fMRI changes with Calmare and TENS treatments. Randomized double-blind trials performed in August 2015 and between August 2016 and November 2017, with 18 and 20 human peripheral neuropathy subjects, respectively. The initial trial examined effects of a single session while the latter trial examined the effects of a course of ten treatment sessions on consecutive weekdays. fMRI scans were examined for changes in blood flow correlations and connectivity. Subjective pain scores were similarly reduced by both Calmare and TENS treatments. In the ten-session study, the relief was prolonged, again similarly for both groups. The pain area relieved, which may be a more important metric than residual pain score, was reduced significantly more in the Calmare group than in the TENS group in *post hoc* analysis. After treatment, resting fMRI showed an increase in global efficiency and in correlation of activity between left hippocampus and right amygdala for both treatment modalities. Treatment with Calmare or TENS yields prolonged pain reduction and correlates with reproducible fMRI changes.

Correspondence: David D Busath, MD, Professor of Physiology and Biophysics, Department. of Physiology and Developmental Biology, LSB 4005, College of Life Sciences, Brigham Young University, Provo, UT 84602, United States. Email: david_busath@byu.edu

Introduction

Multiple studies have shown that transcutaneous electrical nerve stimulation (TENS) effectively reduces hyperalgesia and allodynia in neuropathy patients (1,2) as well as subjects with spinal cord injury (3,4). Scrambler therapy, using the Calmare® device

(Calmare Therapeutics Inc, Fairfield, CT, USA) and referred to here as Calmare treatment, is a transcutaneous electrical treatment that has also demonstrated significant ability to alleviate neuropathic pain according to one review (5). Subsequently, reduction of chronic post-mastectomy pain (6) and chemotherapy-induced peripheral neuropathy (CIPN) (7-9), and neuromyelitis optica (10) were also reported with Calmare. However, to our knowledge, no reports of efficacy comparison between Calmare and TENS nor of fMRI correlates of pain reduction have been published.

We evaluated whether Calmare efficacy was greater or more durable than TENS in subjects with peripheral neuropathy. Randomized prospective double-blind studies of one-session and ten-session treatment regimens were conducted with resting state fMRI before and after treatment periods and upon later return. Subjects scored their burning superficial pain, which, together with paresthesia and numbness, is a common symptom in peripheral neuropathy (11,12).

The neural network that processes acute pain includes regions of the insular, secondary somatosensory, and anterior cingulate cortices, as well as thalamus, and hypothalamus (13). There is less consensus on brain regions involved with chronic pain. One metastudy found that low back pain, post-herpetic neuralgia, and osteo-arthritic knee pain have different fMRI signatures in spontaneous and evoked pain paradigms (14). Similarly, gray-matter volumes change differentially in chronic back pain, complex regional pain syndrome, and knee osteoarthritis (15). Nevertheless, fMRI with chronic pain often shows aberrant connectivity involving the anterior cingulate gyrus, insula, medial prefrontal cortex, precuneus, amygdala, striatum, hippocampus, thalamus, tegmentum, nucleus accumbens, and periaqueductal gray (16-22). Placebo and sham effects are well-known to affect perception of acute (23) and chronic (24) pain and central mechanisms mediating placebo effects have been hypothesized (25-27). Here, we take advantage of analgesia from Calmare and TENS to examine changes in resting state network activation for idiopathic peripheral neuropathy subjects.

Methods

Subject selection: Subject anonymity was protected by the protocol reviewed and approved by the Brigham Young University Internal Review Board, Protocol F15130 and registered as NCT04242797 at clinicaltrials.gov. Eighteen subjects for the one-session study and twenty for the ten-session study were chosen from a pool of interested candidates with chronic pain conditions and gave written informed consent (see figure 1). Sample size for the ten-session study was based on treatment efficacy observations from the one-session study. Inclusion criteria included a diagnosis by a medical doctor of peripheral neuropathy, preferably idiopathic, a pain rating higher than 4 (on a scale of 1-10), and minimal medication history subject to exclusion criteria (see table S1). Candidates taking pregabalin, topiramate, gabapentin or tramadol were asked to consult with their physician prior to discontinuing the medications two weeks before the study to reduce medication variability and because they are suspected to inhibit Calmare efficacy. They were not excluded from the trial if they were unable, such that a few subjects were included who took these medications up to a few days before beginning the study and, in one case, during the study. Subjects were assigned to groups by the blind-manager for each study using a random number generator and an algorithm to assign similar gender blocks for each treatment block, and assignments were well-matched between groups for their gender, age,

diagnosis, and drug history (see tables S1 and S2). Pain was most frequently described as burning and sharp, starting in the feet, and moving proximally as the disease progressed. This manuscript adheres to Consort 2010 guidelines.

Study protocols: In the one-session study, the subjects received an initial fMRI, the double-blind treatment, and a second fMRI on day one, then returned for a third fMRI on day two. The nine subjects in the TENS group were invited two weeks later for a non-blind Calmare treatment as a crossover group. Seven did so and were treated identically to the original two groups except for the elimination of the blind. In the ten-session study, the subjects received an fMRI followed by treatment on day one, treatment only on days two through nine, then treatment followed by an fMRI on day 10. They were scheduled to return for a third fMRI approximately six weeks from the second fMRI. Two subjects were completely relieved of pain before the end of the set of 10 treatments. They were not treated further and did not experience a return of pain before day 10. They also received the second fMRI on day 10. Subjects in the ten-session study were interviewed by phone approximately 12 weeks after the end of treatment (~6 weeks after the third fMRI) about the overall results of the study.

Nerve stimulations instrumentation characteristics: Treatments were administered using a Calmare (Model MC-5A, Competitive Technologies Inc., Seoul, Korea) with two TENS (TENS7000, Current Solutions, LLC, Austin, Texas) units mounted to it on a plexiglass frame (see figure S1). The amplitude knobs of the TENS units were ganged with those of the Calmare with O-rings and the outputs selected under Bluetooth control (see figure S2). The TENS unit continuously generates rectangular, unidirectional 300 µs-wide pulses at 43 Hz. The Calmare unit generates distorted sine waves (see figure S3) with a base frequency varying between 46 and 56 Hz from one random duration period (RDP) to the next, and harmonics that vary in intensity from one RDP to the next (see figure S4). The harmonics distort the sine wave, and the variation in base frequency and harmonic intensities assure that the distortion changes from one RDP to the next. The RDPs lasted ~2 s on average.

Double-blind management and treatment protocol: Subjects were randomly assigned to therapy groups and the instrument switch was set for each subject by the double-blind supervisor (see figure S1). The therapist applied the electrodes proximal to the affected area on the seated subject, typically with a pair on each affected limb, and raised the signal amplitude by turning the ganged knob for each lead pair. Subjects were asked to report the level of pain relief as the amplitude was increased to an optimal level. Sometimes the electrodes or the amplitude were further adjusted during the 30-minute treatment period. Subjects rated their pre- and post-treatment pain apart from the therapist either in writing (preliminary study) or using a laptop (ten-session study) using the Washington Neuropathic Pain Scale (WNPS) (11,12) and the Visual Analog Scale (VAS) (28) each day of treatment, with superficial burning pain reduction pre-specified as the primary outcome. Following therapy, the therapist took notes on electrode placement, area in pain, and other observations.

Post-treatment MRI and follow-up: The subjects returned after one day (one-session study) or approximately six weeks (ten-session study) and again rated their pain using the WNPS and VAS and had MRI scans. In the ten-session study, subjects were also called ~12 weeks after the therapy to verbally rate their pain with the WNPS and other follow-up qualitative questions about the duration of effects, feedback for the study, life events that may have affected the study, and their guess regarding their modality group assignment.

MRI scan parameters: All MRI scans were performed on a Siemens 3T Tim Trio scanner with a 32-channel head coil at the BYU MRI Research Facility. Structural images were acquired using a T1-weighted MPRAGE sequence with the following parameters: TR = 20 ms, TE = 4.92 ms, slices = 192 interleaved, voxel size = 1.0 × 1.0 × 1.0 mm, FOV = 256 mm, flip angle = 25°, total acquisition time = 8:55 min. Resting state BOLD data were acquired using a multi-band EPI sequence with the following parameters: multi-band factor = 8, TR = 867 ms (1125 TRs), TE = 44.2 ms, slices = 72 interleaved, voxel size = 2 × 2 × 2 mm, FOV = 208 mm, flip angle = 52°, total acquisition time = 12:06 min. The first five volumes acquired were discarded to allow for T1 equilibration.

MRI data preprocessing: Preprocessing was performed using the Analysis of Functional Neuroimages (AFNI) suite of programs and Advanced Normalization Tools (ANTs) (29,30). The following steps were performed: 1) structural and functional image co-registration, 2) de-spiking, 3) motion correction by censoring of image in which there was a significant motion event (>.5 mm translation), 4) noise regression of a) white matter, b) CSF, and c) motion-related noise. Cleaned fMRI time series were segmented according to the Automated Anatomical Labeling Atlas (31) as updated with additional subdivisions of the orbitofrontal cortex, AAL2, (32) after which the 94 region-of-interest (ROI) time signals were averaged within regions and correlated between regions for the creation of a scan-specific correlation matrix.

Analysis of subjective pain reports: Student's t-tests were performed on treatment group averaged VAS and WNPS pain scores. Also, three lines of evidence were combined to assess the pained areas *post hoc* using the definitions in Table 1: 1) patient drawings on the initial interview, 2) therapist drawings at the beginning of treatment sessions, and 3) electrode positioning notes by the therapist. These were used for comparison of proportions and odds-ratio hypothesis testing (details in Supplemental Data: Statistical Methods). To reduce the likelihood of false positive findings due to multiple comparisons, statistical significance for a given test was only considered relevant if it appeared in at least four of the five groups.

Objective fMRI pain analysis methods (correlation and graph theory analysis): Weighted correlation matrices were produced by correlating the time courses of all pairs of ROIs. For statistical tests performed on these raw correlation matrices, Fisher's R-to-Z transformation was first applied. Then, the treatment effects on ROI-pairing functional connectivity were examined. A subset of 13 paired ROIs commonly implicated in chronic pain, i.e. superior medial prefrontal cortex, orbital medial prefrontal cortex, insula, anterior cingulate gyrus, middle cingulate gyrus, posterior cingulate gyrus, hippocampus, amygdala, precuneus, caudate, putamen, globus pallidum, and thalamus, with n x (n-1) / 2 = 325 correlations,

was selected for analysis. As pain score changes were largest between scans 1 (pretreatment) and 2 (post treatment in the first study, and post final treatment in the second study), we focused on treatment effects from scan 1 to 2. We performed paired T-tests of treatment effect on the correlation coefficient for each treatment group (Calmare vs TENS vs crossover in the first study; Calmare vs TENS in the second study). Results were considered relevant when the P value was below 0.05 for a given pairing in multiple groups, again out of consideration for possible false positives in any given test in a single group. Additionally, we examined treatment effects on network properties of subject brain correlation-coefficient graphs (i.e. sets of linked nodes, where the nodes are ROIs and the links are correlation coefficients across scans). Weighted correlation matrices were given a threshold at 10% sparsity. Graph measures of connectivity were taken from the Brain Connectivity Toolbox (33) and were implemented in MATLAB (MathWorks Inc, Natick, MA). For each scan, the global efficiency and global modularity were calculated. Then, for each ROI within each scan, the regional metrics of clustering coefficient, degree, and betweenness centrality were determined. Treatment effects on global graph metrics were examined with paired T-tests between scans 1 and 2 in both studies with a significance level set at $\alpha=0.95$. Graph metrics with significant changes from scan 1 to scan 2 were further examined in a linear mixed-effects regression (the R language LME4 package) as predictors of pain score. fMRI observables were pre-specified as secondary outcome measures.

Results

Subjective pain score results: The group average VAS and WNPS residual pain scores were substantially reduced relative to the initial scores after the ten-session treatment (see table 2). The two treatment modalities resulted in similar pain reductions for all pain types, with hot and surface pain being the most reduced types. Average pain scores reported at six weeks after the therapy remained reduced, especially for hot pain and surface pain in both groups, but also for cold pain in the Calmare group and sharp pain and sensitivity in the TENS group. No subject reported increased pain or other unintended effects.

The WNPS hot pain scores in the two studies (see figure 2), dropped significantly (P<0.01) after the first treatment, with one exception (TENS in the one-session study). There were also significant drops after the first treatment in the WNPS intense pain score in the ten-session study and the VAS scores in both studies (see figures S5-S7). Pain reductions were not significantly different between TENS and Calmare. The crossover Calmare treatment during the one-session study also produced statistically significant drops in both VAS (P<0.01) (see figure S6) and WNPS hot pain (P<0.01) (see figure 2C).

In the ten-session study, the WNPS hot pain scores dropped upon the first treatment (see figure 2, D and E) and then stayed low for the ten-session period for both Calmare and TENS treatments (see figure 3), with each treatment producing a small effect that was lost by the next day. Both methods of treatment were also successful in reducing pain by the final day (post 10) compared to day 1, (P<0.005). There was no significant difference between Calmare and TENS in total pain reduction (Post 10 vs Pre 1, P> 0.05). The return of pain at 6 weeks (pre W6) was modest, the same for the two groups (P>0.05).

Post hoc subjective pain region results: As shown in figure 4, the total numbers of pain-affected regions (summed over all the subjects in a group) before the ten-session study

were 80 (Calmare) and 51 (TENS). A comparison of proportions test showed the number of pain areas was more effectively reduced by Calmare than by TENS (Z=3.19, p<0.005). Using the same data set, the odds ratio test for treatment success (reduction in number of pained areas to one or zero in a given quadrant) was 22.67 [95% CI 4.71 to 109], with Calmare treatment more effective than TENS (P<0.0001).

fMRI results: T-tests on the Pearson's R Correlation Coefficients, Fisher R-to-Z transformed: The left hippocampus-right amygdala correlation was increased upon treatment (P<0.05) in three of the groups, i.e. Calmare (0.015±0.13,n=9 to 0.23±0.22,n=9) in the one-session study, and both TENS (0.047±0.13,n=8 to 0.25±0.18,n=8) and Calmare (0.15±0.16,n=9 to 0.26±0.14,n=9) groups from the ten-session study, and increased but possibly underpowered in the TENS one-session study (0.097±0.24,n=8 to 0.23±0.11,n=8, P<0.1) and decreased slightly in the Crossover study (0.17±0.20,n=7 to 0.14±0.24,n=7, P<0.3).

Graph theory analysis: No significant differences were observed in any regional graph theory metrics from scan 1 to 2 in either study. We tested for changes in functional brain graph global efficiency from scan 1 to 2. In the one-day study, the crossover group and all groups when combined had increased global efficiency (p<0.05) after treatment. In the two-week study, both treatment groups, when combined, also had significantly increased global efficiency as a result of treatment (p<0.05). In a mixed effects model with global efficiency as a fixed effect predictor of pain score and with subject ID included as a random effect, we found that global efficiency significantly predicted neither VAS nor WNPS intensity pain scores (p>0.05).

Evaluation of the blind: In a perfect blind, the subjects and therapists would predict the assigned treatment group correctly 50% of the time. In the one-session study, 56% of the subjects predicted correctly the day after the treatment along with 72% of the therapists. In the ten-session study, the subjects were 83% correct after the treatments and 69% correct 12 weeks later, while the therapists were 94% correct after the treatments.

Discussion

To our knowledge, this is the first report of a Calmare study of peripheral neuropathy with a double-blind, active TENS control. A similar randomized non-blind study using a TENS control group was carried out during the same period as this study, which showed superiority in Calmare treatment effects compared to TENS treatment effects (34). Here, the Calmare was effective in reducing reported pain scores for a prolonged period in the ten-session paradigm, as was TENS. Calmare was more effective in reducing the number of pained areas. This is plausible because animal and human clinical studies (35) have shown that high frequency (>100 Hz) and low frequency (<10 Hz) TENS have differential effects on descending pain regulation pathways in the spinal cord, suggesting that a mix of non-traditional frequencies, such as found in the Calmare harmonics, could optimize efficacy.

The subject blind was moderately successful, but the therapist blind was quite unsuccessful (see figure S1 for further discussion). In future studies, single-blind with comparison of traditional TENS and Calmare would be equally successful and easier to

administer. The recent study of neuromyelitis optica (10) compared Calmare to a vibrator single-blind and showed significant effects of Calmare and no effect of the vibrator on pain, with good statistical blinding of the subjects. The observation that traditional TENS was equally effective at prolonged block of pain suggests that generic electrical nerve stimulation, but not the vibratory nerve stimulation parameters used there, mediates the pain relief.

In the fMRI studies, global efficiency and left-hippocampus/right-amygdala correlations were increased by treatment. This neural correlate to decreased perception of chronic pain can be interpreted in terms of the "learned pain" model, in which the central circuits subserving acute pain are inappropriately prolonged by learning and memory based in the hippocampus (14,20,36). These central circuits include both analytical localization in the somatosensory cortex and affective evaluation in the limbic system (e.g. cingulate gyrus and amygdala). With the expectation that the most likely treatment-effect changes in central function would be in the limbic system (37), we initially searched for reduced activity (blood flow) or connectivity (correlation in the time series of blood flow fluctuations) in these regions. Because the subjective pain reports for the two Calmare, two TENS and one crossover group were similar, we evaluated their fMRI results both independently and jointly. As expected for resting fMRI, the blood flow variations were not statistically significant, and the correlation coefficients showed many possible statistically significant (at $\alpha=0.05$) interactions between the 13 regions of interest, but none except the left-hippocampus/right-amygdala increase was reproducible between the five groups. The involvement of the amygdala may relate to the recent observation that the mouse central amygdala contains GABAergic neurons that project to numerous pain regions in the brain and inhibit pain-associated behaviors (38). Interestingly, the left hippocampal morphology has been recently implicated in biasing memories of pain (39) in ways that can affect personality-dependent placebo responses in chronic pain. Nevertheless, the increased correlation between the left hippocampus and right amygdala upon reduction of pain is difficult to rationalize both because of the contralateral connection, which has no obvious direct neurological basis, and because one might expect such an increase in correlation to subserve aberrant memory of pain, rather than a decrease in pain. One is forced to speculate that the increased correlation in this particular circuit is inhibitory rather than excitatory and of sufficient magnitude as to stand out. In an intra-operative corticocortical evoked potential study of medicinally intractable seizure patients, stimulation of the left posterior hippocampus was found to produce recordable activity in the right posterior cingulate gyrus and stimulation of the right posterior cingulate gyrus produced recordable activity in the right amygdala (40). Perhaps this indirect (two-step) connection between the left hippocampus and right amygdala, obscured by network complexities, is responsible for the increased left hippocampus/right amygdala correlation treatment effect reported here.

Regional network analysis using graph theory, in which each region of interest is treated as a "node" and each pairwise correlation with 93 regions as a possible "edge," yielded no consistent change in any of the standard metrics (see methods). But global efficiency, the connectivity of the global network, was increased after treatment for both studies when the treatment groups were combined. This can be interpreted to suggest that an individual in chronic pain reflexively shuts down many connections in the brain, perhaps as one might expect for a defensive posture where the brain limits its interactions in

negative feed-forward preparation for focal risks. Once the risk is perceived as resolved, the brain returns to a more globally free interaction state where it monitors input in a non-selective fashion, prepared for any eventuality of environmental change. After therapy, interactions between regional pairings deemed to be of lower priority for the focal risk are disinhibited and global efficiency returns to a "broad environmental surveillance" state. Correlations between the important limbic regions, the hippocampal "memory" region and the amygdala "fear, reward learning, anxiety" region could reasonably be expected to subserve the learned pain phenotype.

Both the pain and fMRI observations presented here involved multiple measurements. In both cases, the reproducibility of uncorrected statistical significance for given measures between similar groups (TENS and Calmare, one-session and first session of the ten-session) is used as a measure of reliability. It was originally intended that the fMRIs results from the one-session study would allow power estimates for the second study, but ultimately it became expedient to proceed with the ten-session study before that could be accomplished.

In future studies, perhaps connections with the localization regions responsive to the individual subject's specific pained areas (i.e. in "foot region" of the postcentral gyrus) and/or with the error detection regions of the cingulate gyrus (41) could also be identified. Normal (pain-free) treated controls would be valuable to distinguish pain reduction effects from pain-unrelated treatment effects and a single-treatment paradigm would be sufficient.

Conclusions

In conclusion, Calmare and TENS both produced prolonged, substantial pain relief for subjects with idiopathic peripheral neuropathy and fMRI showed increased correlations between blood flow vacillations in left-hippocampus and right-amygdala as well as increased global efficiency after a single treatment session and after a series of ten daily treatments.

Acknowledgements

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41.   Averbeck BB. Unbelievable: Neural correlate of the feedback negativity in the anterior cingulate. Neuron 2017;95(2):237-9.

Table 1. Definitions of pain areas

Pain Regions[a]	Lower Quadrants	Upper Quadrants
Region 1	Toes and either tops or bottoms of feet	Fingers
Region 2	Whole foot up to ankle	Whole hand up to wrist
Region 3	Ankle to knee	Wrist to elbow
Region 4	Knee to groin	Elbow to shoulder dermatomes (C4-T3)
Region 5	Superior to groin (left or right)	Torso (left or right) except shoulder dermatomes

[a]A region was considered "in pain" if a subject reported pain in any portion of that region.

Table 2. Average relative[a] pain remaining

	Calmare		TENS	
	10 Day Tx	6 Week Return	10 Day Tx	6 Week Return
VAS Pain	38%	93%	15%	69%
WNPS Intense Pain	35%	78%	32%	66%
WNPS Sharp Pain	31%	70%	28%	53%
WNPS Hot Pain	30%	52%	27%	48%
WNPS Dull Pain	51%	73%	39%	70%
WNPS Cold Pain	28%	59%	46%	61%
WNPS Sensitivity	35%	67%	31%	57%
WNPS Itchy Pain	61%	82%	43%	81%
WNPS Unpleasant Pain	43%	84%	33%	71%
WNPS Deep Pain	39%	81%	35%	78%
WNPS Surface Pain	30%	64%	27%	56%

[a]Average pain after ten-session therapy and upon follow-up 6 weeks later relative to the original score. N=8-10 in each case (all subjects completed the initial surveys, but in some cases one survey answer was missed at the end of treatments and two were missed at the 6-week return).

Figure Legends

Figure 1. CONSORT 2010 Flow Diagram: Randomized clinical trial selection for both the one-treatment and the ten-treatment study. The number of subjects in the two studies are designated as one-session count + ten-session count where appropriate. Of 376 subjects that volunteered for study in response to advertisements, the first 18 meeting the study criteria were selected for the preliminary one-treatment study, nine randomly assigned to each group (Calmare vs. TENS). Seven of those assigned to the TENS group opted to return three weeks later for unmasked Calmare treatment as a crossover group. The following year, all of the original volunteers that were qualified and had current contact information, except for the eighteen in the one-treatment study, were called back and invited to participate in the ten-treatment study. The twenty that were qualified and willing were identified and randomly assigned to each group (Calmare vs. TENS). Exclusions from the fMRI analysis were due to data loss.

Figure 2. Upper: Average subject hot pain (WNPS) ratings for one-session study before treatment (blue), after treatment (red), and next day (purple) for A) Calmare group (N=9), B) TENS group (N=9), and C) Crossover group (N=7). Error bars are ±2 S.E.M. *P<0.01 (treatment effect, Post Tx vs Pre Tx, paired, one-tailed T test). Lower: Average subject hot pain (WNPS) ratings for ten-session study before treatment 1 (after fMRI, blue), after treatment 1 (red), and (before treatment) next day (day 2, purple) for D) Calmare group (N=10), E) TENS group (N=9). Error bars are ±2 S.E.M. *P<0.01 (treatment effect, Post Tx vs Pre Tx, paired, one-tailed T test).

Figure 3. Average subject hot pain (WNPS) ratings for ten-session study before (pre) and after (post) treatment each day and before fMRI at the 6-week post-treatment follow-up for Calmare group (orange, N=8-10) and TENS group (blue, N=8-10).

Figure 4: The pre-treatment number of pained regions as compared to the post-treatment total for the Calmare group (red) and the TENS group (blue) in the ten-session study.
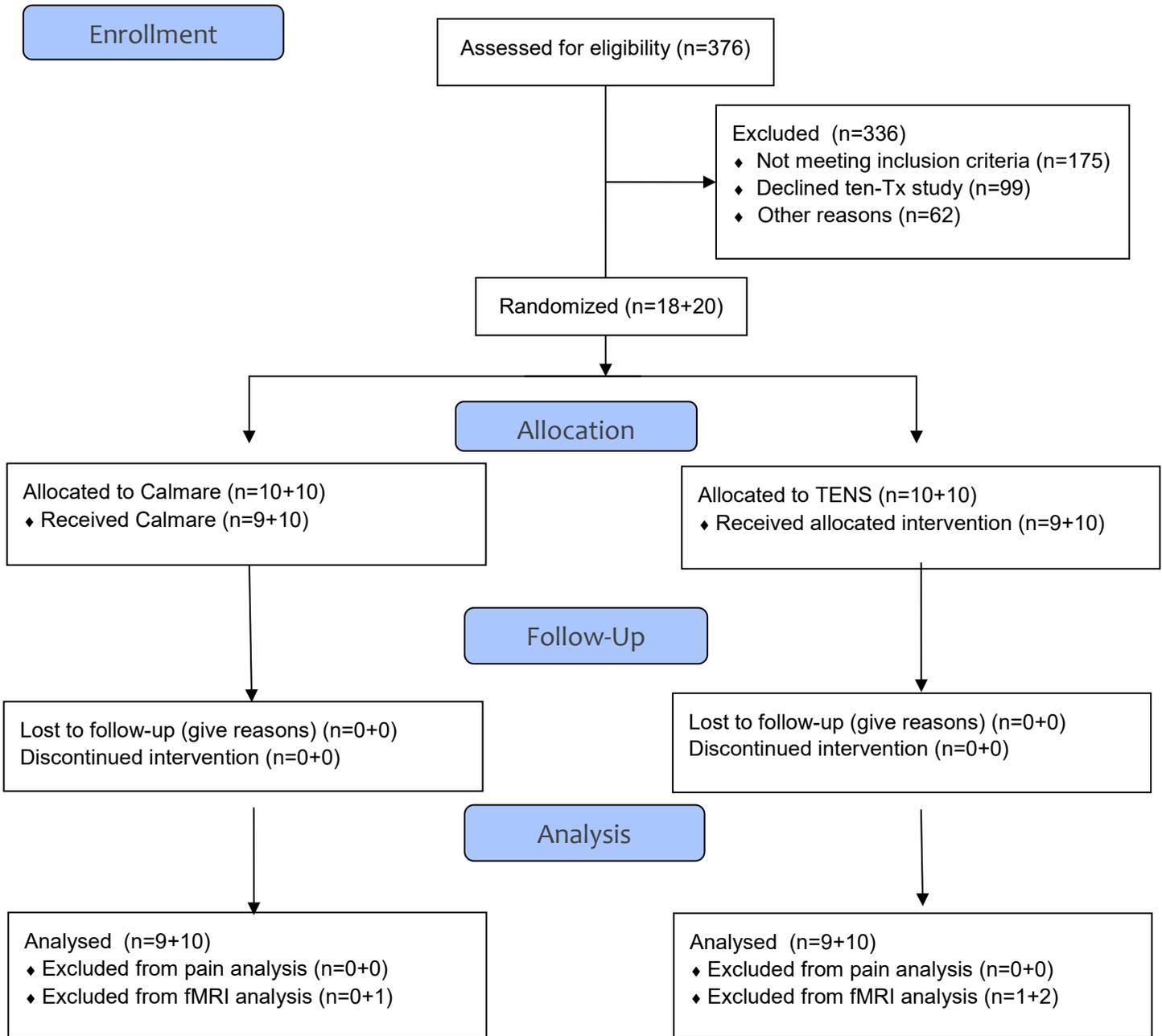
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Enrollment

                    Assessed for eligibility (n=376)

                                          Excluded  (n=336)
                                          ♦  Not meeting inclusion criteria (n=175)
                                          ♦  Declined ten-Tx study (n=99)
                                          ♦  Other reasons (n=62)

                    Randomized (n=18+20)


                         Allocation

Allocated to Calmare (n=10+10)            Allocated to TENS (n=10+10)
♦ Received Calmare (n=9+10)               ♦ Received allocated intervention (n=9+10)


                         Follow-Up

Lost to follow-up (give reasons) (n=0+0)  Lost to follow-up (give reasons) (n=0+0)
Discontinued intervention (n=0+0)         Discontinued intervention (n=0+0)


                         Analysis

Analysed  (n=9+10)                        Analysed  (n=9+10)
♦ Excluded from pain analysis (n=0+0)     ♦ Excluded from pain analysis (n=0+0)
♦ Excluded from fMRI analysis (n=0+1)     ♦ Excluded from fMRI analysis (n=1+2)
```

Figure 1

A) Calmare
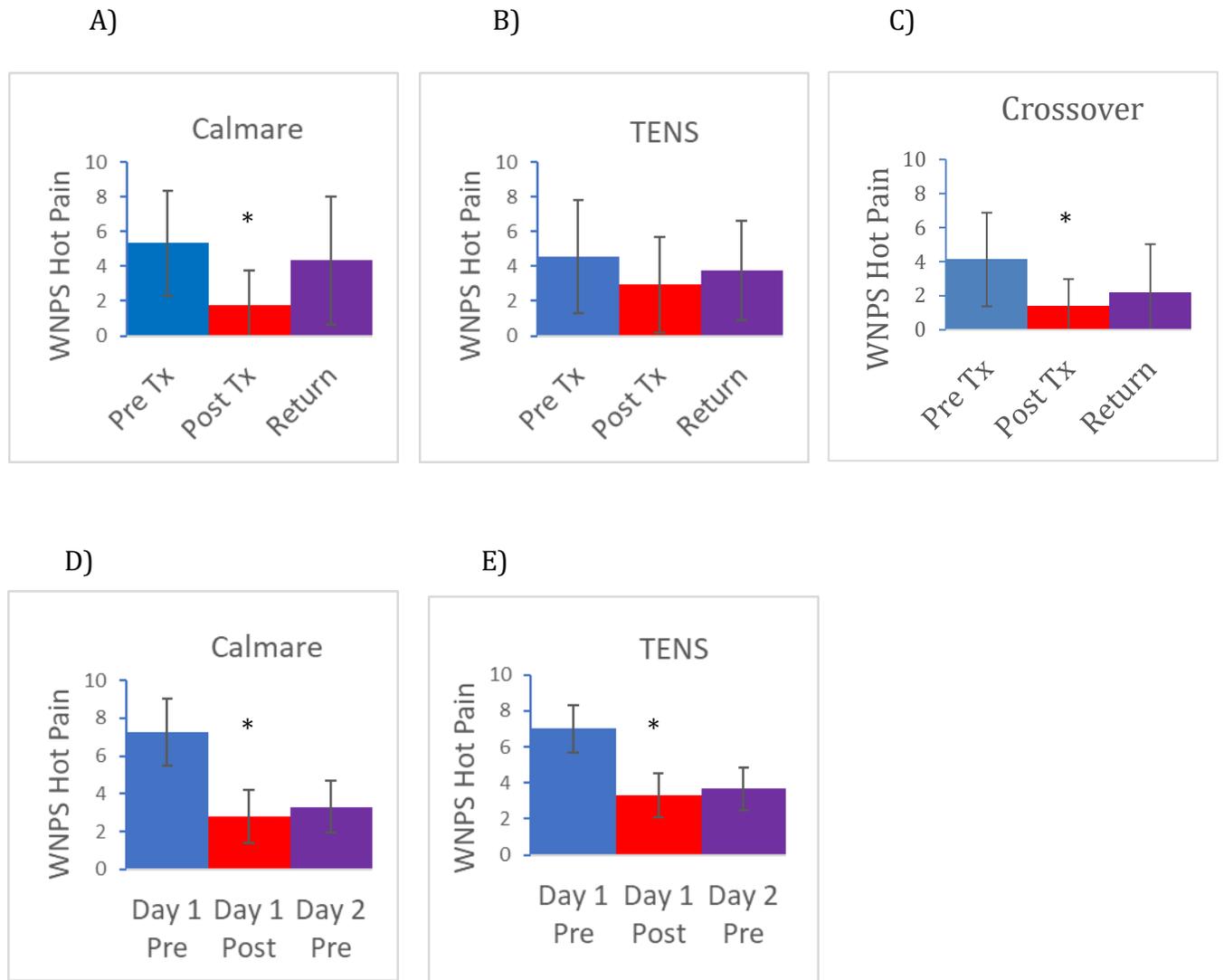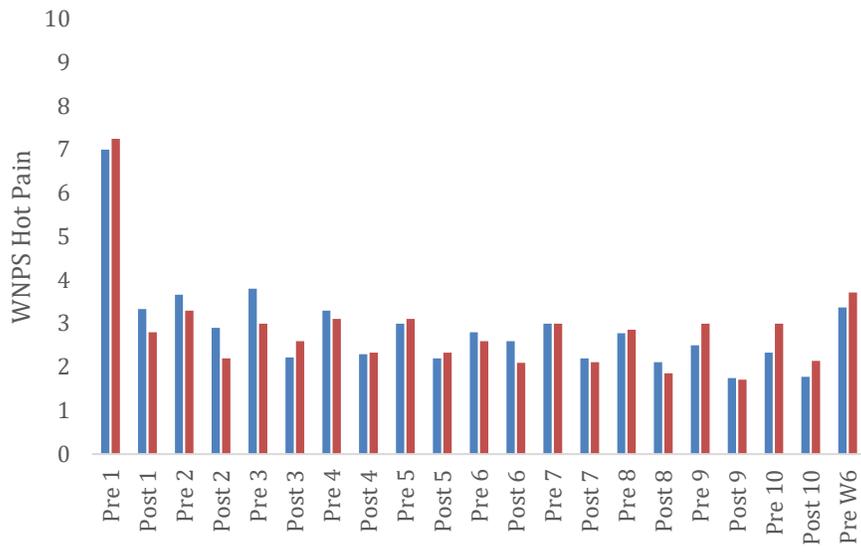
B) TENS

C) Crossover

D) Calmare
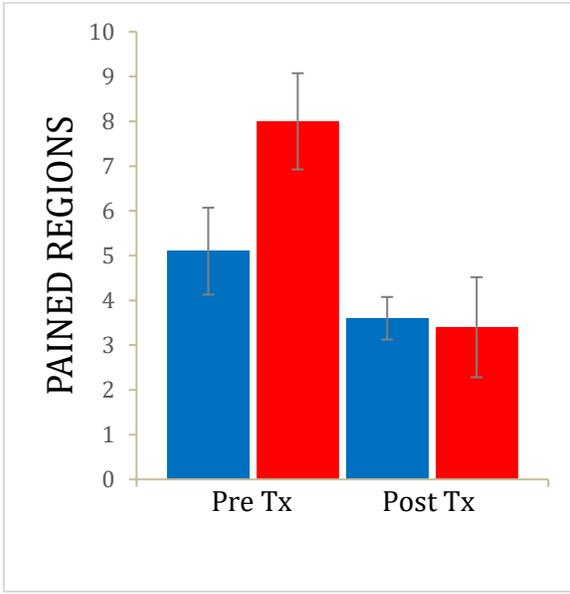
E) TENS

Figure 2

Figure 3

Figure 4

Supplemental Data

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Statistical Methods
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Figure S6. VAS pain in single-treatment study
Figure S7. VAS pain after first treatment in ten-treatment study

Statistical methods

Comparison of Proportions: For each group, the proportion of interest was defined as the number of regions relieved of pain after treatment relative to the total number of pained regions before treatment. The difference in the proportions of relieved pain regions for the Calmare group ($\bar{P}_1$) and TENS group ($\bar{P}_2$) relative to the composite standard error (which is based on the composite proportion of pained regions ($\bar{P}$) and the initial pain region counts for the Calmare group (n_1) and the TENS group (n_2)) was assumed to be Z distributed (Eq. 1) for the hypothesis test.

$$Z = \frac{(\bar{p}_1 - \bar{p}_2)}{\sqrt{\bar{p}(1-\bar{p})\left(\frac{1}{n_1}+\frac{1}{n_2}\right)}} \tag{1}$$

Odds Ratio: The treatment of each affected subject-quadrant (as defined in Table 1) was determined to be "success" if only one region in the quadrant was still affected post-treatment or the quadrant was completely pain free. Otherwise, the treatment was classified as "failure." The odds ratio (OR) is defined in Eq. 2 and its standard error in Eq. 3 where a, b, c, and d represent Calmare "success" count, Calmare "failure" count, TENS "success" count, and TENS "failure" count, respectively. Equation 4 gives the 95% confidence interval for OR.

$$OR = \frac{Calmare\ Success \times Tens\ Failure}{Calmare\ Failure \times Tens\ Success} \tag{2}$$

$$SE(OR) = \sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}} \tag{3}$$

$$CI \equiv e^{(\log(OR)\pm[1.96\times SE(\log(OR))])} \tag{4}$$

Figure S1. Assembled TENS/Calmare double-blind device. The two TENS units are mounted on plexiglass above the Calmare. The white oval encompasses the O-ring assembly that mechanically couples the amplitude control of the TENS to that of the Calmare. The black box in front contains the Arduino-controlled switch for the output, governing whether the TENS input or the Calmare input is put out. The two TENS devices, each with two independent outputs (top) were modified to have output amplitudes governed by one-turn potentiometers controlled by aluminum knobs. The knobs were mounted on the plexiglass rack. The aluminum knobs were mechanically coupled by O-rings to similar aluminum knobs, which, using set screws, replaced the knobs on the Calmare. The output leads from the TENS and Calmare were both fed into a black plexiglass box containing a Bluetooth receiver (Arduino UNO, Arduino, Somerville, Massachusetts). The receiver was controlled by a computer kept out of the treatment room, or out of sight in the treatment room, to switch all inputs from either the Calmare or the TENS to the four outputs from the black box. Then, each of the knobs was set at the counterclockwise extreme to eliminate any accumulation of slippage of the O-rings between sessions. A trained therapist, blind to the treatment group, then placed up to 4 electrode pairs on the subject's skin proximal to the region

of pain. Generally, one pair was applied transversely to each leg or ankle. But in a few cases of foot and hand neuropathy, additional pairs were applied to the wrists. Then, while asking the subject about the level of sensation, the therapist or assistant would turn up the amplitude slowly and gently until the subject reported feeling a mild shock sensation, and then proceeded a bit further until the subject reported cessation of the chronic pain, or until the knob reached its maximum position, which always occurred below the muscle contraction induction level. As the therapist turned up a Calmare knob, the TENS knob controlling the same output lead was rotated an equal amount by the O-ring, producing a signal of similar amplitude, subjectively speaking, whichever of the two modalities was being applied. This double-blind design benefits from simplicity, but suffers from a few unavoidable tells that may become evident to experienced therapists, but still could be easily hidden from subjects. The most apparent tell is that the Calmare signal varies while TENS is steady, which therapists may realize and subjects may inadvertently comment on during electrode placement. A more subtle tell is that the Calmare instrument has, in addition to the broken circuit fault detection, (which this design overcomes, see Figure S2), an undocumented overdrive fault detection which causes it to enter into fault mode occasionally when the amplitude knob is turned nearly all the way up. It seems to be related to circuit resistance, as it seemed to occur more often with some types of electrodes than others and could often be alleviated by replacing the electrodes. We inferred from this behavior that it might be related to a feedback overdrive, such as might be used in a current clamp configuration. The TENS instrument used does not have any fault mode, so a knowledgeable technician might consciously or subconsciously associate Calmare faults with a Calmare group assignment for a given subject.
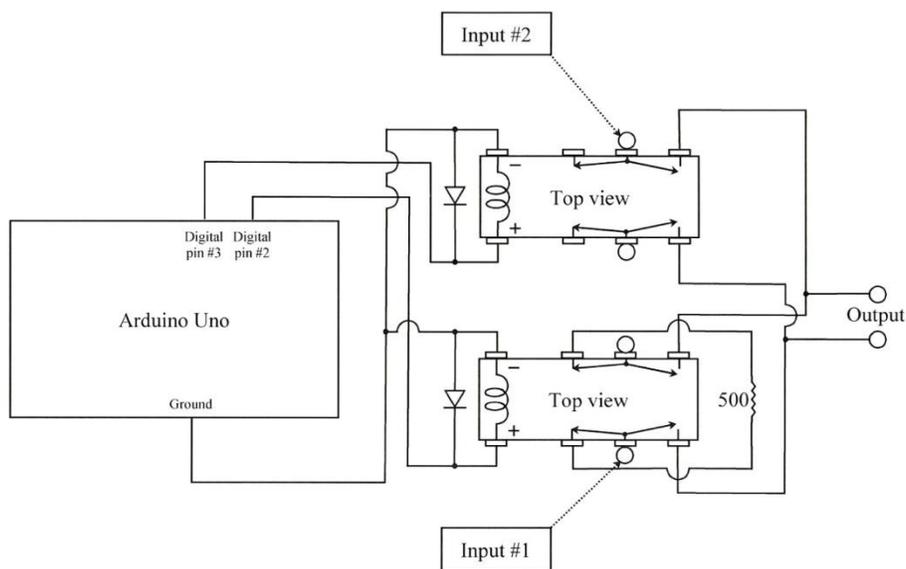
Figure S2. Switching circuitry diagram for assembled TENS/Calmare double blind device. Leads from Calmare and TENS attached to Arduino Uno bluetooth receiver to modulate output through standard electrode pads. The diagram shows the circuitry for one of the four pairs of output leads, all four being controlled simultaneously by the Arduino Uno. Each pair exits the black box in a single cable, which later bifurcates. Circuitry in the switching circuit allowed for each of the four pairs of Calmare leads to be connected through a 500-ohm resistor (simulating skin resistance) when out of the circuit in order to bypass the Calmare broken-circuit detection warning and shut-off. Each input has an inductor connected to a flyback diode, which prevents sudden spikes in voltage across the inductor with sudden interruptions or changes in current. This allows the Arduino Uno to switch which treatment is being administered without disconnecting either of the treatment inputs. The four output leads coming from the black box (initial segments visible in the lower right of Figure S1) were Calmare-brand leads that had been cut, the back ends being inserted as inputs into the black box. The leads were connected to standard electrode pads (Little PALS, Axelgaard Manufacturing Company LTD, Fallbrooke, California) for application to healthy skin regions in the affected limbs of the subjects.
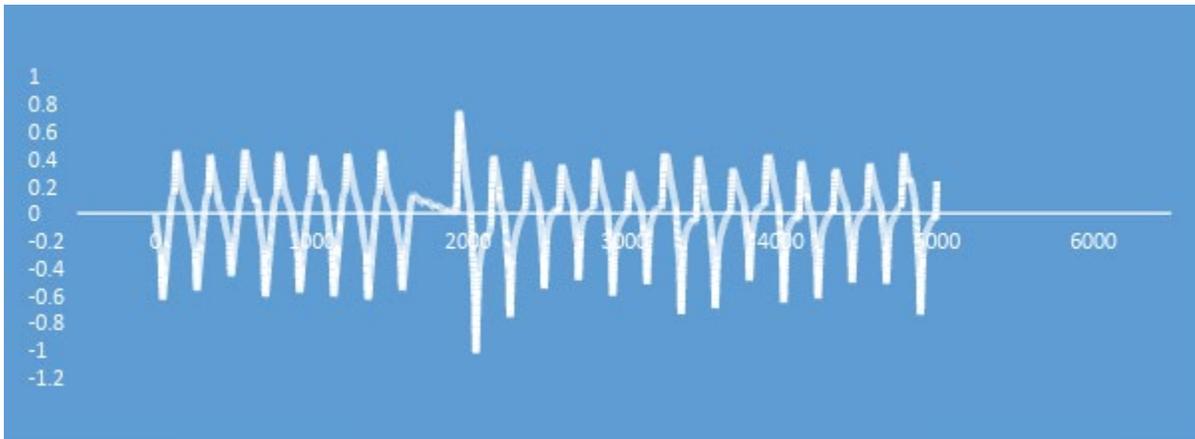
Figure S3. Oscilloscope reading of Calmare output over 0.5 seconds. Analyses of the waveforms generated from the Calmare and TENS units were performed by directly connecting the electrodes to the leads of a digital oscilloscope and spectrum analyzer (Analog Discovery, Discovery Inc., Pullman, Washington). The Calmare output is a randomly distorted sinusoidal curve in contrats to the TENS output, which consists of rectangular pulses with a width of 300 µs initiated and followed by brief double-exponential spikes (not shown) that appear to be designed for charging skin capacitance, such that the ohmic current into the skin is a roughly rectangular pulse.
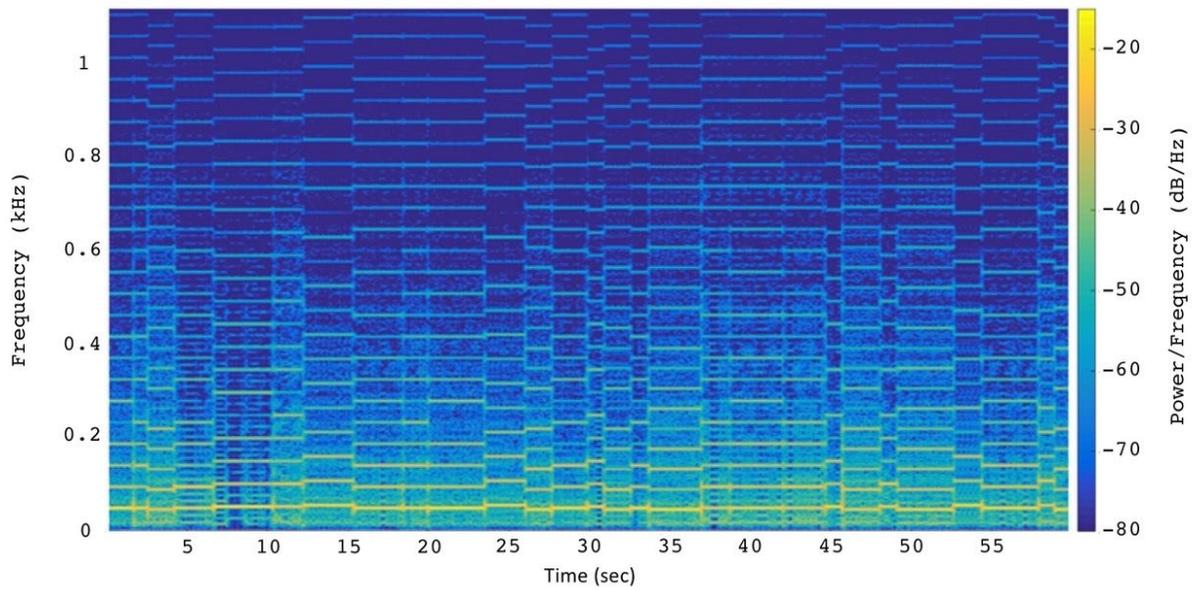
Figure S4. Calmare output spectrogram showing the Fourier transform of Calmare output colorimetrically over a 60 second period. The power amplitude is most intense at the base frequency ~47 Hz) with harmonics of lesser intensity. RDPs are evident as periods of constant base frequency. In each RDP the harmonics of the base frequency have a unique set of intensities.

Table S1. Exclusion criteria

Hospital Anxiety and Depression Scale [1]	Depression score above 15 (severe depression)
MRI criteria	Metal implants or claustrophobia
Maternity status	Pregnancy or breast feeding
Neurological history	History of epilepsy, brain damage, or serious psychiatric disorder (schizophrenia, etc)
Allergies	Skin condition that would preclude placement of electrodes
Therapy history	Neurolytic pain control devices such as implanted spinal cord stimulators used within one month of or during the study

[1] Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand. 1983;67(6):361-370.

Table S2. One-treatment study subject characteristics

Sex	Age	Dx[1]	Yrs of Pain	Rx[2]	Verbal Pain Description	Drugs
F	60+	I	10	T	numbness and tingling, no burning pain	-
M	50+	I	6	C	burning pain	Lyrica BID until 6 weeks ago Ibuprofen PRN (800-1600 mg/day) Flomax & Sudafed PRN.
F	30+	-	2	C	stabbing pain up Achilles tendon	Lyrica BID for 2 w 1 yr ago Gabapentin for 1 m 1.5 yr ago Metanx BID
M	50+	D	20	C	pain in bottom of feet and toes	Gabapentin for a few m 4 yrs ago Oxycodone 30 mg TID Tradjenta Metformin Aspirin 325 mg Supplements
F	50+	I	3	C	neuropathy both feet & hands	Gabapentin 1 qPM for 3 m 1 yr ago
M	70+	I	10	T	no burning pain	Aspirin 81 mg Advil PRN
M	50+	I	10	T	numbness & tingling, mild burning pain	Gabapentin for 1 m 1.5 yr ago Lyrica BID for 2 w 1 yr ago Metanx BID
F	60+	D	20+	T	numbness and tingling, no burning pain	Gabapentin 600 mg TID for multiple years until 4-6 w ago Metformin 150 mg TID
M	50+	I	5-6	T	numbness and tingling, no burning pain	Gabapentin 5-7 pills daily until 3 y ago Sudafed PRN
F	70+	I	9	T	-	Gabapentin 300 mg PRN, "took one last week," 600 mg TID for 1 w in 2006 Lyrica for several m. in 2006 Levothyroxin 137 mcg qd
F	60+	I	15	C	burning only in feet	Topamax 50 mg qd Sumatriptan PRN 100 mg for foot pain Naproxen 100 mg BID Celexa 40 mg qd Flexeril 10 mg qd Diphenhydramine (allergy) 25-75 mg qd Valium 10 mg qd Ambien 10 mg qd

F	60+	I	20	C	numbness, tingling, and burning in both feet	Oxycodone 300 mg q 4h for 3 d 2 m ago. Advil, ibuprofen PRN Synthroid Metformin 250 mg BID Progesterone 100 mg BID
F	60+	R S D	11	T	burning in upper forearms	Neurontin trial 7 y ago Excedrin PRN migraines Topazepam PRN sleep Atenolol 25 mg qd
M	60+	S	20+	C	lumbar pain L5-S1 sciatica bilateral into feet pain mostly on lateral sides of legs	
M	50+	I	3	C	pain in toes and balls of feet	
F	60+	C	4	T	numbness & tingling in both feet	
M	50+	D	10+	T	no burning pain reported	Gabapentin TID Oct 2014-Feb 2015 Lyrica 3-4 y ago Insulin (short and long lasting) Metformin Drugs for acid reflux, high cholesterol, and high blood pressure
M	60+	D	3	C	numbness & tingling	Milxicam PRN pain Tylenol 650 mg PRN pain Peroxetine Metformin Glimeperide Losartan Claritin D 24-Hr Multivitamin

[1]Peripheral Neuropathy Diagnosis – self-reported: I, idiopathic; D, diabetic; C, Chemotherapy-induced; S, spinal injury; RSD, reflex sympathetic dystrophy; [2]Treatment group: T, TENS; C, Calmare. Summary: Average female age was 59.1 (35-71) years and average male age was 59.4 (51-70). 55.6% of the subjects reported an idiopathic type of peripheral neuropathy, 22.2% reported diabetic, and 22.2% of the subjects were distributed amongst chemotherapy-induced, spinal injury, RSD, and undefined diagnoses. Pain duration ranged from 2 to over 20 years. Although the intent was to gather subjects with burning pain in the extremities and with limited anti-seizure pain med exposure, many of the subjects reported no pain, just numbness and tingling upon arrival for the study.

Table S3. Ten-treatment study subject characteristics

Sex	Age	Dx[1]	Yrs of Pain	Rx[2]	Verbal Pain Description	Drugs
M	60+	I	10	C	Dull ache in muscles, tender pressure points, stabbing pain, burning in feet	
M	30+	D	17	T	Feet keep getting tired, discomfort walking on objects.	
F	60+	I	15	C	Burning	
M	30+	C	2	C	Feet started to feel pain and have increased ever since	
M	50+	D	13	T	Shooting and deep, in feet and then in thigh. A little bit in hands.	Gabapentin (not in 2 weeks)
M	70+	I	12	C	Pins and needles on surface of toes and bottom of feet	
M	60+	I	20	T	Keeps him awake	Tramadol (not in 2 weeks)
M	60+	I	12	C	Pins and needles, numbness and tingling on feet	
F	50+	H	13	C	Begins in hands and spreads	Lyrica
M	60+	H	10	T	Begins in feet	
F	60+	I	4	T	Pain in both feet and legs	
F	30+	D	5	T	Stinging, hot and cold simultaneously	Gabapentin (not in 2 weeks)
F	60+	D	5	C	Stinging, burning pain in toes and feet.	
F	50+	D	3	T	Numb, sharp pain on toes. Cold, like bugs crawling	Gabapentin (not in 2 weeks)
M	60+	I	3	T	Numb, sharp pain in toes	
M		I	15	C	Burning Pain on feet, shins, and hand	
F	50+	I	2	T	Feet and ankles	Lyrica
F	30+	C*	8	C	Left side all the way through arm and back	Tramadol
F	60+	I	-	T	Burning pain on tops and bottoms of feet	Gabapentin
F	40+	S	24	C	Sharp, burning pain in left foot	

[1]Peripheral Neuropathy Diagnosis – self-reported: I, idiopathic; D, diabetic; C, Chemotherapy-induced; C*, Chronic Regional Pain Syndrome; S, spinal injury; H, Hereditary Sensory Neuropathy; RSD, reflex sympathetic dystrophy; [2]Treatment

group: T, TENS; C, Calmare. Summary: The average female age was 54.7 (36-68) years and male age 58.5 (37-67) years. 50% of the subjects reported an idiopathic diagnosis, 25% reported diabetic, and the other 25% were distributed amongst chemotherapy-induced, spinal injury, CRPS, and hereditary diagnoses. Most subjects had dealt with chronic pain for over 5 years, and the majority over 10 years. In this ten-treatment study, more care was taken than in the preliminary study in pre-screening the subjects for pain; also for medication use, although detail was only gathered on anti-seizure or opioid analgesic medications. 60% reported never having used anti-seizure or opioid analgesic medication. Twenty percent had terminated use of gabapentin or tramadol two weeks prior to the study and the remaining 20% terminated Lyrica, gabapentin or tramadol use 0-2 days prior to the study.
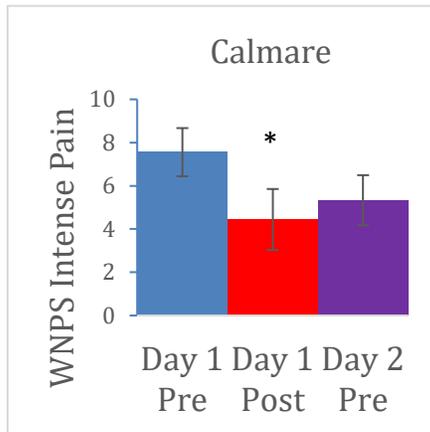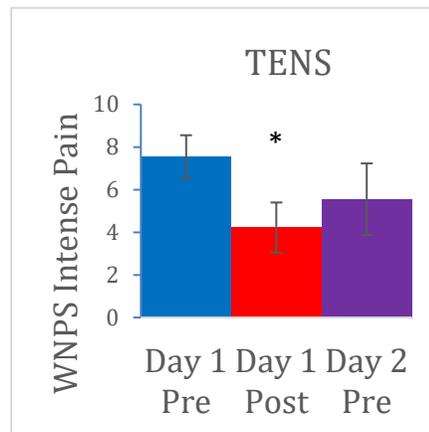
Figure S5. Average intense pain (WNPS) ratings for ten-treatment study before first treatment (after fMRI), after first treatment, and (before second treatment) next day for A) Calmare group *P<0.01 (treatment effect, Post Tx (N=10) vs Pre Tx (N=8), unpaired, one-tailed T test), B) TENS group (N=9) *P<0.01 (treatment effect, Post Tx vs Pre Tx, paired, one-tailed T test). Error bars are ±2 S.E.M.
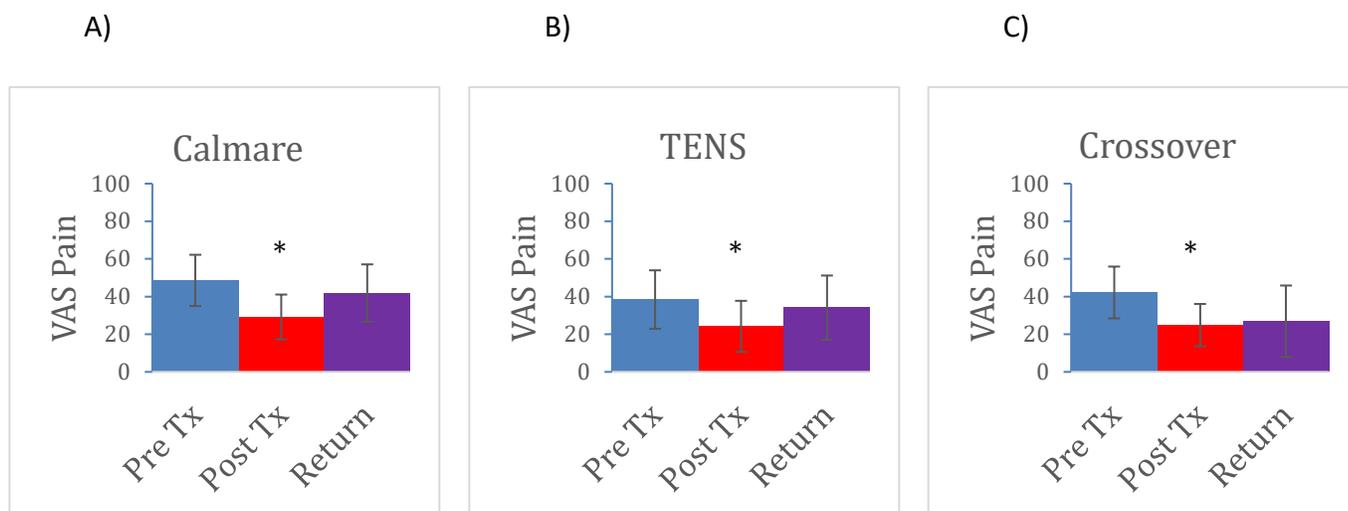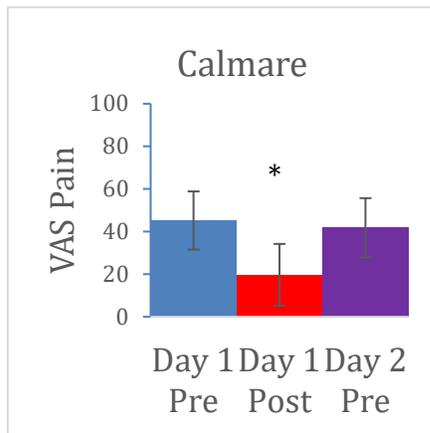
Figure S6. Average subject Visual Analog Scale (VAS) pain ratings in mm for single-treatment study before treatment, after treatment, and next day for A) Calmare group (N=9) *P<0.05 (treatment effect, Post Tx vs Pre Tx, paired, one-tailed T test), B) TENS group (N=9) *P<0.01 (treatment effect, Post Tx vs Pre Tx, paired, one-tailed T test), and C) Crossover group (N=8), *P<0.01 (treatment effect, Post Tx vs Pre Tx, paired, one-tailed T test). Error bars are ±2 S.E.M.
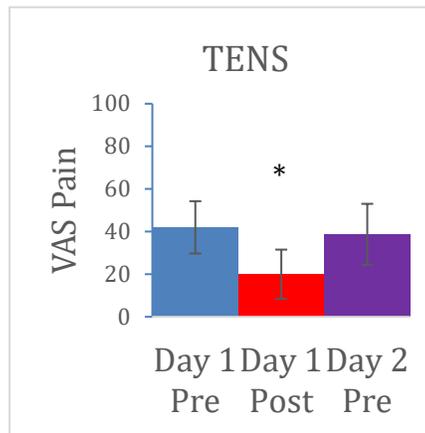
Figure S7. Average subject VAS pain ratings in mm for ten-treatment study before first treatment (after fMRI), after first treatment, and (before second treatment) next day for A) Calmare group *P<0.05 (treatment effect, Post Tx (N=10) vs Pre Tx (N=9), unpaired, one-tailed T test), B) TENS group *P<0.05 (treatment effect, Post Tx (N=9) vs Pre Tx (N=10), paired, one-tailed T test). Error bars are ±2 S.E.M.