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In vitro test of external Qigong

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Abstract

Background: Practitioners of the alternative medical practice 'external Qigong' generally claim the ability to emit or direct "healing energy" to treat patients. We investigated the ability of experienced Qigong practitioners to enhance the healthy growth of cultured human cells in a series of studies, each following a rigorously designed protocol with randomization, blinding and controls for variability.

Methods: Qigong practitioners directed healing intentionality toward normal brain cell cultures in a basic science laboratory. Qigong treatments were delivered for 20 minutes from a minimum distance of 10 centimeters. Cell proliferation was measured by a standard colony-forming efficiency (CFE) assay and a CFE ratio (CFE for treated samples/CFE for sham samples) was the dependent measure for each experiment.

Results: During a pilot study (8 experiments), a trend of increased cell proliferation in Qigong-treated samples (CFE Qigong/sham ratios > 1.0) was observed (P = 0.162). In a formal study (28 experiments), a similar trend was observed, with Qigong-treated samples showing on average more colony formation than sham samples (P = 0.036). In a replication study (60 experiments), no significant difference between Qigong-treated samples and sham samples was observed (P = 0.465).

Conclusion: We observed an apparent increase in the proliferation of cultured cells following external Qigong treatment by practitioners under strictly controlled conditions, but we did not observe this effect in a replication study. These results suggest the need for more controlled and thorough investigation of external Qigong before scientific validation is claimed.

Background

Healing practices that purportedly involve the mental or spiritual manipulation of some form of life energy associated with the body are popular forms of complementary and alternative medicine worldwide. The National Center for Complementary and Alternative Medicine currently supports research projects investigating such 'energy medicine' modalities [1]. Energy medicine practitioners

generally claim the ability to emit or direct "healing energy" to treat patients. Whether the apparent efficacy of such energy medicine practices is due to processes internal or external to the patients is not clear, however. The human body has an interwoven network of central nervous system and endocrine system processes involved in modulation of the immune system in response to psychological states [2,3]. Self-healing processes may thus be induced by psychological states triggered by the drama of a healing ritual or by the charisma of a practitioner. Do energy medicine modalities exert their effect (if any) through the power of suggestion, then, or does it have a direct effect on biological systems? This question may be answered using samples in the laboratory as targets of a practitioner's treatment. Such *in vitro* models effectively eliminate from experiments the factor of psychological cueing.

A vast majority of the energy medicine studies published over the past 20 years have been evaluations of Qigong. Qigong is a health-promoting meditative practice originating from traditional Chinese medicine. Like herbal remedies and acupuncture, its theoretical basis involves manipulation of the purported life energy, 'Qi'. According to traditional Chinese medicine principles, Qi can be regulated mentally as well as chemically (as with herbal remedies) or physically (as with acupuncture). A person's mind is believed to be capable of influencing Qi, which travels along the meridians in the body and promotes health. Most Qigong practice is self-meditation. Through daily meditation practices, sometimes involving simple movements or postures and breathing exercises, a person focuses his or her own intentionality on improving or maintaining health. Some individuals believe that, after many years of practicing such 'internal Qigong', they can develop the ability to manipulate Qi outside the body. This is the basis of 'external Qigong'. Supporting the validity of such external applications of Qigong, a database of reports from Asia translated into English contains approximately 90 abstracts [4] presenting evidence that external Qigong can influence biological targets *in vitro*.

Many in the Chinese scientific community question the credibility of these studies, however, and argue that therapeutic effects following Qigong treatment, if any, must result from the power of suggestion. Some in China have reported on experimental evidence supporting this view and have proclaimed the "end of fairy-tale external Qigong" [5,6]. Chinese research on both sides of the controversy over the efficacy of external Qigong does not appear to follow accepted scientific standards however, creating doubt as to its validity. As one example, "[Publication bias] appears to be so much ingrained in Chinese publications on ... Qigong that the literature cannot be trusted" [7]. Poor design and lack of adequate controls also plague even the benchmark studies in the Qigong research field. Based on only four trials that lacked blinding, controls for experimental conditions, and statistical analysis, for example, a frequently referenced report of using bacteria as an *in vitro* target [8] concludes that external Qigong can stimulate or inhibit the growth of *Escherichia coli*. Another general weakness in the field is the lack of replication of experiments. A recent critical review that assessed a sample of 58 *in vitro* studies of Qigong in the Asian literature found no independent replications on any single model [9].

Our report presents the results of a collaborative effort between basic scientists and clinicians working with external Qigong practitioners in the United States and China to develop a rigorous *in vitro* protocol for evaluating potential direct effects of external Qigong. We chose a cell culture model known to be sensitive to conventional energy treatments (e.g., ionizing radiation) and sought an experimental design that would be easily reproducible in independent laboratories. Our experimental hypothesis was that experienced external Qigong practitioners could enhance the healthy growth of human cells under stressful culture conditions.

Methods

Overall study design

We conducted three independent studies of Qigong efficacy in two different geographic locations. All three studies incorporated rigorous experimental design, including (1) blinded observation and analysis of results, (2) randomized assignment to experimental groups, and (3) a nontreated control group (sham) to compare with each treatment group. In addition, we included an equal number of experiments comparing sham treatment to sham treatment (systematic negative controls) throughout the duration of the study to continually assess potential variability within the model system. All three studies used a well-established assay to measure cell proliferation in culture conditions in which a majority of the cells do not survive. The protocol was approved by the Institutional Review Board of the California Pacific Medical Center.

The first study was a pilot to evaluate the work of three Qigong practitioners who were in the San Francisco Bay Area at the time of the study. The setting was a basic science laboratory, but the room used for the experimental treatments was a nearby office. A scientist who was blinded as to the eventual status of the experiment (i.e., treatment by Qigong practitioner or sham treatment) brought randomly selected samples of cells to the treatment room. Ten minutes later, another scientist escorted a Qigong practitioner to the treatment room. The practitioners delivered Qigong treatment to the samples for 20

minutes. For each experiment, there was also a sham control session in which the cell cultures were handled identically except that no Qigong practitioner entered the treatment room. In this pilot study, a random number generator was used to decide which treatment, Qigong or sham, would be applied first. One of the practitioners performed two experiments; each of the other two performed a single experiment. Pilot experiments evaluating tumor cell killing or growth inhibition after Qigong treatment did not produce results that justified further investigation but experiments with normal cells did.

We designed the formal study to have 80% power to detect an apparent increased proliferation effect observed in cultures of normal human brain cells following Qigong treatment suggested in the pilot study. Power analysis of the results of the pilot study suggested that at least 14 Qigong/sham experiments would be needed for the formal study. This study was conducted at a basic science laboratory in Beijing, China, where more practitioners were readily available that we could verify were professionals of Traditional Chinese Medicine working within a Qigong department or division in a hospital. Three researchers traveled there to conduct the experiments and worked with nine practitioners from four traditional Chinese medicine institutions. Again, the room used for experimental treatment was a nearby office. The protocol was identical to that of the pilot study except that the sham always preceded the Qigong treatment. This variation in the protocol was at the request of the practitioners. Precedent in the Chinese literature indicates the possibility that residual Qi generated during a Qigong treatment session might linger in a treatment room and contaminate a subsequent sham treatment in the same room. To avoid such a "linger effect," the practitioners requested at the outset of the formal study that the sham treatments always precede the Qigong treatments. Nine practitioners in this study conducted a total of 14 experiments; five practitioners conducted two experiments each, and the remaining four conducted single experiments.

A third study was conducted the following year in Beijing to assess the reproducibility of the findings of the formal study. The protocol was identical to that of the formal study, except that the order of treatments (Qigong or sham) was randomized to eliminate any potential confounding of treatment and time. This time there were 30 experiments conducted at two laboratories and involving eight practitioners; three conducted three experiments, four conducted four experiments, and one conducted five experiments. Two of the eight practitioners had participated in the previous Beijing study. A unique feature of this study was the addition of a temperature probe inside each treatment box to measure temperature (to 1/100°C in 30-second intervals). This was added to address the question of whether the proximity of the Qigong practitioner to the cells could be influencing subsequent proliferation through the mechanism of increasing temperature.

Outcome measure and statistics

We assessed cell proliferation by quantifying the colony-forming efficiency (CFE) of normal brain cells growing in culture plates. The assessment of CFE measures a cell's ability to duplicate itself again and again, forming a colony, under various treatment conditions. The methodology, first developed in the 1950s [10], was considered by the 1970s to be the gold-standard assay in studies of *in vitro* sensitivity to therapeutic agents [11]. It remains a mainstay in the measurement of cell response *in vitro* [12]. In our studies, we counted colonies of 50 or more cells manually using a low-power stereoscope, and we documented images of each plate using a digital imaging system.

For each experiment, 100 cells were placed into each of 12 culture plates. The dependent variable was the ratio of the CFEs between cell cultures in two treatment boxes (six plates in box A and six plates in box B) for a given experiment. Variation within each experiment was measured and differences between experiments tested using Bartlett's test.

To find the mean, the CFEs for the six plates in each box were averaged following standard and previously published methods [13,14]. This method reduces the variability of the CFE outcome measure, thus increasing the power to detect potential effects of treatment. The ratio of the averages (A/B) where then computed, giving a single data point for each experiment. Because averaging ratios can result in a skewed distribution, the statistics are presented as Log values. Unlike the mean Qigong/sham ratios, the Log values reflect whether a value is above or below chance expectation (CE), with negative values below CE and positive values above CE.

Cell culture

We used human astrocytes as the target of healing intentionality in these studies after evaluating these normal brain cells in parallel pilot experiments with glioma tumor cells, a common type of brain cancer. The glioma cells appeared to be less responsive to Qigong treatment. Based on a comparison of four experiments comparing Qigong treatment to sham treatment and four experiments comparing sham treatment to sham treatment (systematic negative controls), we found that the mean Qigong/sham CFE ratio for glioma cells was 1.047 (sd = 0.153) and the mean sham/sham ratio was 0.986 (sd = 0.107). Using a t-test to compare conditions yields $t(6) = 0.651$, which is an effect size only in the 0.02 – 0.25 range.

Given the small effect size, we determined not to pursue this model. Parallel experiments with astrocytes showed a greater effect size (0.30 – 0.35) with the mean CFE ratio being 1.042 (sd = 0.046) for Qigong/sham samples and 0.972 (sd = 0.129). Comparing treatment conditions for astrocytes yields $t(6) = 1.016$, $P = 0.349$.

The astrocyte cell culture model is used widely and is responsive to conventional therapies utilizing emitted energy (i.e., ionizing radiation). The model can demonstrate both positive and negative responses (cell proliferation and cell death), depending on the treatment. Astrocytes were isolated by established methods [15] and were confirmed to be astrocytic by uniform staining with an anti-GFAP antibody (Beohringer Mannhiem). Cells were grown in a DME H-21-based medium with 10 ng/ml EGF, 10 nM hydrocortisone, 10 ng/ml biotin, ITS supplement, transferrin 50 μg/ml, biotin 10 ng/ml (all from Sigma), and 0.2% bovine pituitary extract (Clonetics). This was mixed with DME/10% fetal calf serum at a 10:2 ratio. Cells were passaged at confluence using trypsinization and were expanded to a large population size, aliquoted, and frozen viably for long-term storage. A fresh aliquot was thawed at the start of each experimental trial to ensure uniformity in the genetic profile of the target cells throughout the project.

Conditioning of the plastic surface of the culture plates with organic material is necessary to promote proliferation of experimental cells. We provided minimal conditioning (a small number of lethally irradiated "feeder" cells) so that as few as 1% of the cells survived and were able to form colonies under control conditions. Thus, the majority of the normal brain cells were not expected to survive the stress of the culture conditions.

Randomization

After one day of incubation, each cell culture plate was randomly assigned to (1) one of the two treatment boxes labeled A or B (an opaque plastic box with lid closed tightly) and (2) a specific position within its assigned box. A computer program written specifically for this task (using ZBasic for Macintosh) produced equally likely assignment of plates to boxes and positions. Assignment was constrained to assure that each box contained six cell culture plates. This program produced a printed diagram of box A and box B, with plate positions labeled by identification codes. Just before experimental treatments were to be delivered, a researcher followed the printed diagram to prepare the two boxes and placed them in the incubator to allow the CO_2 concentration to equilibrate before the lid was closed. The boxes were then carried one after the other to a separate room to receive experimental treatments. We also assigned each cell culture plate randomly to a position in the incubator. A computer program written for the experiment (Microsoft Visual Basic 5.0) used a pseudo-random number generator to assign plates to positions on a grid marked on the incubator shelf. Each plate had equal likelihood of assignment to any incubator position. This allowed testing of whether placement in the incubator had any effect.

Qigong practitioners

One challenge in Qigong research is that there are numerous styles of Qigong practice and few practitioners have identical training. In our experience, practitioners often emphasize how different and unique their own style and ability is compared with that of others. They also emphasize how their ability may vary according to environmental factors, such as weather and stress. Because of such vast variance and the fact that there are no objective measures for the "amount" and "strength" of the Qi each practitioner emits each time (if that is indeed emitted), it is impossible to verifiably identify a group of practitioners who can give identical external Qigong treatment consistently. Since the goal of our study is to assess external Qigong treatment for patients, we looked for common denominators among the practitioners from a clinical standpoint. These are: 1) they claim that they can emit Qi externally for the purpose of treating physical illnesses, albeit with variations and through different styles, 2) they are professionals of Traditional Chinese Medicine working within a Qigong department or division in a hospital, 3) they have practiced such treatment regularly for at least five years, and 4) they have experience treating cancer patients. A total of 18 practitioners (with practice experience ranging from 5 to 17 years) participated in the three independent studies. We worked with practitioners at their convenience and were unable to balance the number of experimental sessions in which each participated. To avoid implicit endorsement, practitioners were compensated for their participation through honoraria and remain anonymous.

Qigong and sham treatments

External Qigong treatments by a Qigong practitioner typically involve "emission" of Qi with the intent to heal. In these studies, Qigong practitioners were instructed to try to direct emitted Qi toward cell cultures in an attempt to stimulate the growth of cell cultures inside an opaque plastic box. We described the cell culture system and the experimental hypothesis to the practitioners. For each experiment, one practitioner was escorted into a treatment room and observed to ensure that a minimum distance of 10 centimeters was maintained from the treatment box that had been placed on the laboratory bench. During a Qigong treatment, the practitioner performed a meditative practice similar to what would be done during a session with a patient. Some of the practitioners made gentle motions with their hands as if

collecting or channeling invisible streams of "healing energy" toward the cells. Others stood motionless, holding a specific posture. The duration of one treatment session was 20 minutes, a typical period of time for treating a patient. A sham treatment involved all the same physical manipulations of the cell culture plates as during an experimental treatment, except that nobody entered the treatment room (the same room used for both types of treatments) during the 20-minute period.

Systematic negative controls

To incorporate systematic negative controls, we changed randomly between experiments in which we compared Qigong treatment to sham treatment and experiments in which we compared sham treatment to another sham treatment. By allowing quantitative assessment of potential systematic errors associated with the methodology used, systematic negative controls tested the method's accuracy and reliability throughout the experimental series [16]. Questions about the potential influence of variations in physical parameters between treatment sessions could therefore be addressed.

Blinding procedures

Experiments were conducted with blinding applied to each of the four scientists and the biostatistician involved, following previously reported methods [17]. Briefly, Scientist #1 handled the cell culture plates, labeled them with random identifying codes, and always placed them according to randomly assigned positions provided by Scientist #2. Scientist #1 would signal Scientist #3 via an electronic signaling system (a nonverbal signal) when samples were ready for treatment so that Scientist #3 could escort the practitioners in and out of the treatment room at the appropriate times. Thus, the scientist handling the cells knew which cells were inside treatment boxes A and B but did not know what treatments (i.e., Qigong or sham) were delivered. Likewise, the scientist aware of the treatment schedule did not know which cells were being treated at any given time (i.e., which cells were in the boxes). A fourth scientist was responsible for counting colonies in each sample using only the random identifying codes. For the studies conducted in Beijing, the cell culture plates were carried back to San Francisco after the colonies were fixed for counting. Each scientist sent blinding codes and data to an outside institute to be kept by an independent peer (a code keeper). A biostatistician, who was blinded to what treatment each group had received, conducted statistical analysis using only the code number of the culture plates.

Results

The results show a trend toward increased cell proliferation in the treated samples in the pilot study (Qigong/sham CFE ratio > 1.0), a statistically significant trend of increased proliferation following Qigong treatment in the formal study, and a nonsignificant, slight increase in proliferation following Qigong treatment in the replication study. We pooled the results from all three studies to form summary statistics, including an overall *t*-test for significance. The mean for the pooled Qigong/sham data was >1.0 but was not statistically significant. The pooled sham/sham data was also not significantly different than 1.0. Table 1 summarizes the results of the three independent studies and the original data are provided as supplementary material [see Additional file 1]. Figure 1 plots the CFE ratios for each experiment.

A striking feature of these three studies is the increase in variability as we went from the small pilot study in San Francisco to the formal study in Beijing and then to the larger replication study in Beijing. This is likely due to the sub-optimal cell culture conditions at the Beijing laboratories (two sites for the replication study). Each increase in variability is highly statistically significant in the Qigong/sham experiments and also in the sham/sham experiments ($P < 0.001$ for each pair-wise variance comparison).

Measuring the temperature inside the treatment boxes (replication study only) revealed that the temperature-to-CFE relationship in the replication study is rather complex. Overall, temperature and CFE seem to correlate strongly ($r[122] = 0.72$, $P < 0.0001$). Upon closer inspection, however, this appears to be an artifact of large changes in both CFE and temperature across the consecutive days of the experiment. The within-day correlations between temperature and CFE are modest, although relatively consistent (mean $r = 0.22$). More importantly, neither the CFE nor the temperature is significantly related to the CFE ratio (Qigong/sham), either overall or on any given day. No significant temperature differences were found between A and B sessions. The temperature is thus unlikely to have influenced the results of the main hypothesis of this study. While the astrocytes are sensitive to ambient temperature, there is no indication that treated or untreated samples fared differently. Similarly, analysis of the randomly assigned position of cell culture plates in the incubator for the formal study showed no relation between incubator position and colony counts ($P = 0.99$).

We were curious whether the failure to find significant results in the replication study was because the new practitioners were less efficacious than the practitioners from the first formal study. After we analyzed the replication study data, comparing results from experiments involving practitioners who had also participated in the earlier formal study in Beijing (n = 9, mean CFE = 1.10, SD = 0.68) with those from experiments involving the new practitioners (n = 21, mean CFE = 1.08, SD = 0.49), we determined that this is unlikely. We tested for a difference

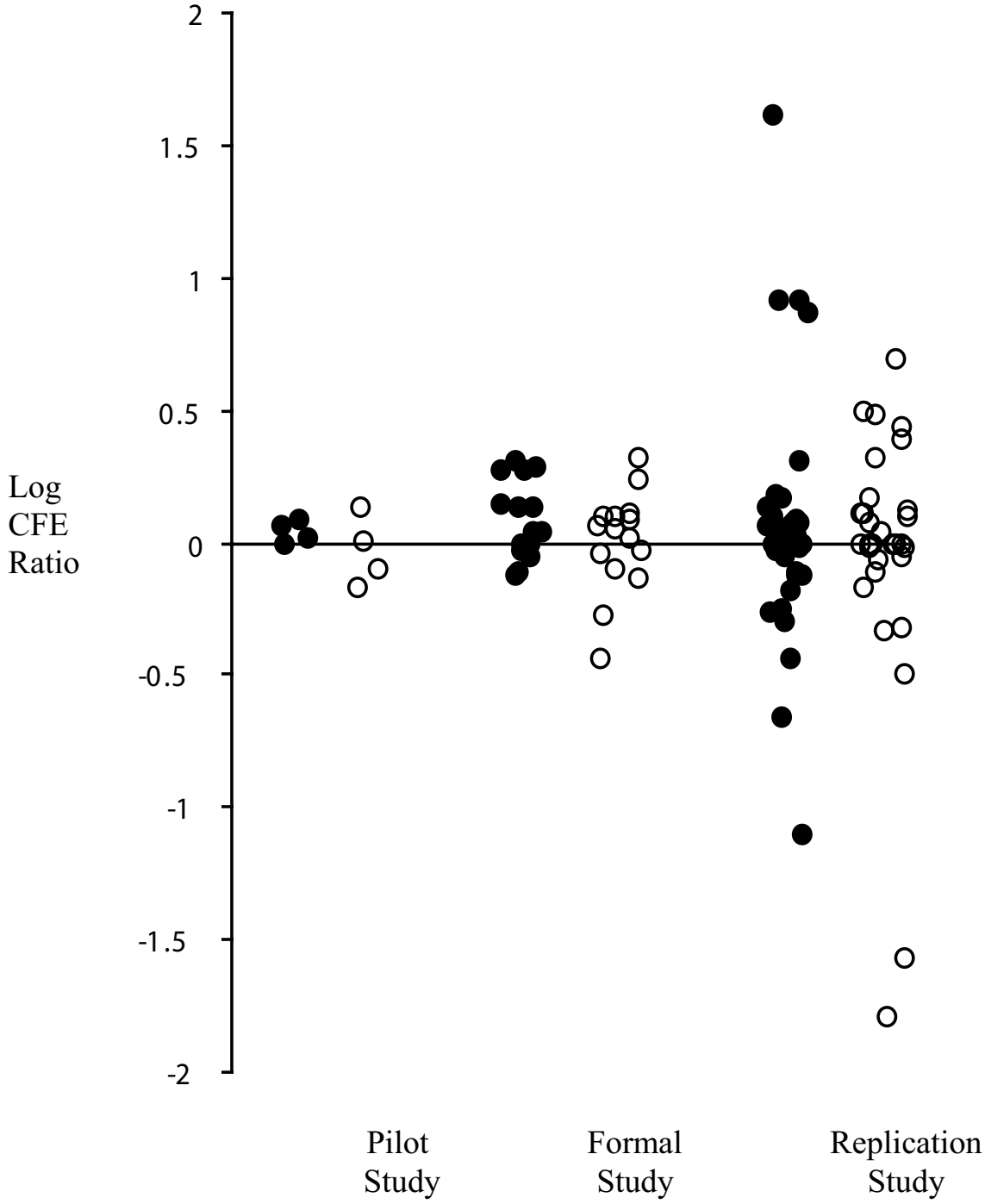

Figure 1
Colony forming efficiency ratios for three studies. Natural Log Qigong/sham ratios are graphed for all experiments in the pilot study (left), the first formal study (middle) and the replication study (right). Qigong/sham ratios are shown as *solid circles*; sham/sham ratios are shown as *open circles*.

Table 1: Summary of study results

Experiment	No. of Qigong Practitioners	No. of Exps.	Mean* CFE Ratio	St Dev*	*t*-statistic	2-sided p
Qigong/Sham						
Pilot study	3	4	1.041	0.046	1.845	0.162
Formal study	9	14	1.099	0.167	2.333	0.036
Replication	8	30	1.070	0.534	0.740	0.465
Pooled data	**18**	**48**	**1.076**	**0.440**	**1.238**	**0.222**
Sham/Sham						
Pilot study	0	4	0.966	0.126	-0.526	0.635
Formal study	0	14	1.037	0.155	0.905	0.382
Replication	0	30	0.955	0.489	-0.491	0.627
Pooled data	**0**	**48**	**0.979**	**0.412**	**-0.347**	**0.730**

*Mean and standard deviation based on logarithmic values.

between these two groups of practitioners based on a two-sided two-sample *t*-test and found no significant difference (P = 0.83). Another difference between the formal study and the replication study is that sham treatments always preceded Qigong treatments in the formal study, while the order of treatment was randomized in the replication study. This difference in protocols does not appear to account for the difference in outcomes of the two studies because there were no differences in the sham results, regardless of whether they preceded or followed Qigong treatment. Sham median counts were 6.6 (95% CI 1.6 to 11.5) in the formal study and 5.5 (95% CI 2.6 to 8.4) in the replication study when sham was first, and 6.8 in the replication study (95%CI 3.9 to 9.8) when sham was second. None of the pairwise differences are statistically significant based on a Wilcoxon rank sum test, which was used since there were outlier values.

Discussion

The systematic negative controls included in the protocol were helpful when considering the significance of an outlier in the data. One of the Qigong/sham experiments in the replication study yielded an exceptionally high CFE ratio (see Figure 1). If it were not for the information gained from the sham/sham experiments, this data point could easily be interpreted as an exceptional performance (i.e., stimulating cell proliferation) by a particular practitioner in that particular experiment. Evidence of intrinsic variability of the system of similar magnitude does not support this speculation, however. Two data points from sham/sham experiments were also outliers to a similar degree. Thus, the outlier in the Qigong/sham experiment falls within the range of variability associated with the experimental model. The variability of the model must yield to the laws of probability as long as there are sufficient numbers of experiments, careful randomization, and strict blinding to eliminate artifact and bias. In this series, the systematic negative controls provided an additional level of confidence in the validity of the results. Indeed, even three outliers in the data - all in the direction suggesting an influence of the Qigong treatment (one high Qi/sham CFE and two low sham/sham CFEs), did not sway the results into spurious significance.

Our report presents a protocol that can easily be replicated in independent laboratories to assess potential direct influences of energy medicine modalities. Following this protocol, we did not observe reproducible effects of external Qigong treatment on the colony-forming efficiency of normal astrocytes. Application of protocols of comparable rigor to other outcome measures (preferably with less variability) is necessary to address the question of the mechanisms of external Qigong. Likewise, exploration of other outcome measures that more closely approximate physiological conditions may also be warranted. If reproducible positive results are observed in other model systems, it will be important to include specific experimental conditions to control for the possibility that physical parameters associated with the proximity of a human body (practitioner) are sufficient to influence samples. Pheromones or other chemical signals, for example, might stimulate biological samples. The demonstration that single cells in mice respond to pheromones at concentrations below 10^{-11} molar [18] increases speculation along these lines. Examples of appropriate control conditions could include having a practitioner present without delivering treatment and having a nonpractitioner "confederate" present with or without mimicking the practitioner's behavior.

Competing interests

None declared.

Authors' contributions

GY and YQ conceived of the study and participated in its design, coordination and implementation. JS and MS par-

ticipated in the design of the study. DM performed the statistical analysis. KA prepared samples and MR performed colony counts. All authors read and approved the final manuscript.

Additional material

Additional File 1

Includes original data for all experiments: The supplementary table presents original data for all experiments in the three studies, including: 1) whether each experiment was comparing Qigong/sham or sham/sham, 2) the mean colony counts for each group of samples, 3) the ratio of these mean values and 4) the natural logarithm of these ratios.
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