

# **Alteration of Growth of Cultured Neurons by the Conscious Intent of an Energy Healer**

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Running Title: Conscious Intent Alters Neurite Growth

## ABSTRACT

Cultured neurons are very sensitive to extrinsic factors in the environment including physical parameters and molecular components. We show that conscious intent (at least that of a practicing energy healer) must also be included as an environmental determinant of neurite growth. An energy healer was asked to either increase or decrease the growth of cultured chick embryo ciliary ganglion cells. He did this in 3 independent experiments by modifying the “energy” in tissue culture medium prior to its addition to the cultures as well as by treating the cultures through the door of the incubator. Several parameters of neuronal survival, neuronal differentiation, neuronal shape and rate of neurite outgrowth were measured for every cell in the culture dishes. The results demonstrate that the healer’s conscious intent had little or no effect on neuronal survival and shape but strongly influenced neuronal differentiation and rate of neurite growth. These responses of cultured neurons to “energy” treatment are similar to those produced by changing the concentration of the trophic factor GDNF. The experiments were controlled so as to eliminate the possibility of investigator fraud or bias as well as to decrease other possible causes of variation in neuronal growth among the culture dishes. The application of a well-characterized *in vitro* system to demonstrate the significant interactions of an energy healer with a biological system has the advantage of not needing to consider the role of placebo effects and has the potential to lead to the identification of possible molecular sites of action.

## INTRODUCTION

There is considerable controversy as to the role of “conscious intent” in the functioning of the nervous system. This term implies the ability of conscious thought to initiate activity in neuronal networks leading to a change in behavior. This concept is supported by the fact that we all have the subjective experience that we control voluntary but not reflex movements. In spite of this, most neuroscientists take the counterintuitive approach that intentionality is a byproduct of or the same thing as neuronal activity and has no independent role in the initiation of behavior. However, a causative role for volition need not contradict materialist neuroscience, which would consider intention to be something, as yet unknown, that alters the activity in neuronal networks. Because of the vast interconnectivity of the brain a change of activity in one part of a network could alter the structure and activity at synapses a considerable distance away.

Even more difficult to explain is the possibility that through conscious intent one person can alter the nervous system, behavior and state of health of others without involvement of the known senses, without direct physical contact and often over great distances. This is supported by the vast literature on extrasensory perception (reviewed in Alcock et al., 2003) and by the demonstration that changes in activity in the brain of one person can produce changes in that of another who is a considerable distance away and is shielded so as to prevent any sensory interactions (Tart, 1963; Duane and Behrendt, 1965; Grinberg-Zylerbaum et al., 1994; Sabell et al., 2001; Wackermann et al., 2003; Radin, 2004; Standish et al., 2004). In addition, certain individuals, many considered energy healers, believe that their conscious intent has the power to influence

others. Although they may invoke many different explanations of how they do this, they typically have a conscious image of an energy field that they are able to manipulate through their conscious intent. One of us (MSS) is such an individual. His clinical experiences have been described (Southwood, 1995). We would like to hypothesize that such interactions initiated by conscious intent alter the recipient's nervous system. Although there is evidence that plants (Grad, 1963, 1964; Haid and Hupikar, 2001; Creath and Schwartz, 2004) and isolated non-neuronal cells (reviewed in Jonas and Crawford, 2003) are affected by such healers we propose that the most important function that is being altered is the ability of the cells to communicate with one another, a characteristic of the nervous system essential for its function.

Clinical studies have indicated that the conscious intent of the healer may change the subject in such a way that symptoms are alleviated, diseases are cured or treatment time in the hospital is shortened. However, these results are open to the criticism that they reflect placebo, psychosomatic effects. One way to control for these effects is to perform the experiments in an appropriately blinded manner. Another way is to examine whether energy healers can alter a cellular system *in vitro*. We have used the latter approach and selected cultured neurons as the target in order to more closely approximate the situation in which the conscious intent of one person can alter the nervous system of another. MSS was asked to try to enhance or reduce the growth of separate cultures of chick embryo ciliary ganglion neurons. He was successful in these efforts and altered the growth of these neurons in a manner similar to that observed by changing the concentration of a target-derived trophic factor essential for growth of these neurons.

## MATERIALS AND METHODS

### *Neuronal cell culture*

The ciliary ganglion is part of the parasympathetic nervous system and at the cellular level is relatively homogenous, containing only 2 types of neurons. The ciliary neurons innervate striated muscles in the iris and ciliary body of the eye where they cause the pupil to constrict and the lens to become more convex for near vision. The choroid neurons innervate smooth muscles in the blood vessels that supply the retina and the back of the eye. This preparation has often been used by neuroscientists interested in examining synapse formation, axon growth, signaling by neurotrophic factors and transport of endocytic vesicles (reviewed in Bernstein, 2003). We have had extensive experience with this experimental system and the methods used to culture neurons from the ciliary ganglia were identical to those previously described (Denburg et al., 2005).

In outline the procedure consisted of removal of the ganglia from the embryos, dissociation of the ganglia into single cells, removal of debris and non-neuronal cells from the cell suspension and plating of the neurons on to an adhesive surface upon which they will extend neurites. More specifically, the ciliary ganglia were removed from 7-8 day old (stage 31-33) chick embryos. At this stage of development many of the neurons have not yet started to extend axons and there are relatively fewer non-neuronal cells present. The ganglia were kept in a Hank's buffered salt solution (HBSS), pH 7.4 containing NaCl (137 mM), KCl (5mM),  $\text{KH}_2\text{PO}_4$  (0.3 mM),  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$  (0.3 mM), HEPES (10mM), glucose (5.5 mM), streptomycin (50U/ml) and penicillin (50U/ml) until 6-12 of them were dissected. In order to dissociate the ganglia into separated neurons they were treated for 15 minutes at 37° C with trypsin (1mg/ml). The reaction was

stopped by washing the ganglia 4 times with culture medium composed of Minimum Eagle's Medium and 10% fetal bovine serum. Trituration released the cells from the ganglia. In order to remove debris and non-neuronal cells this cell suspension was preplated on a plastic substrate (Falcon 1008 Petri dish) to which the neurons did not adhere tightly. After 30 minutes in the incubator at 37° C, 5% CO<sub>2</sub>, the neurons were selectively resuspended in the medium by gently shaking the dish. During this preparation process any axons or dendrites that had extended from the cells were shorn off. The spherical neurons were suspended in a liquid medium that contained all the nutrients required to keep them alive and growth factors that enabled them to grow long cytoplasmic extensions. It is not possible to definitively identify these processes as either dendrites or axons. Therefore the more neutral term neurites will be used. The volume was adjusted to contain cells from 1.5 ganglia/ml and between 200-500 cells/dish were plated on to an adhesive surface. This substrate consisted of carefully washed glass cover slips to which sequentially poly-L-lysine (0.25 mg/ml in borate buffer pH 8.4) and laminin (0.01 mg/ml in HBSS) had been adsorbed. After allowing the neurons to adhere to the dish for 30 minutes in the incubator a large volume (3 ml) of medium containing a glial cell line-derived neurotrophic factor (GDNF) that is essential for neurite outgrowth was added. A suboptimal concentration of GDNF (3 ng/ml) was used in order to facilitate the detection of any change in cell growth due to treatment by MSS. After 4 hours in the incubator at 37° C, 5% CO<sub>2</sub> growth was stopped by fixation with 4% paraformaldehyde in 0.1 M phosphate buffer, pH7.4 at 4° C. This treatment killed the neurons and made them rigid in order to preserve their structure during the measurement process.

### *Energy treatment of the cultured neurons*

All experiments involving the energy treatment of cultured neurons were performed in a laboratory situation, an environment often found hostile by healers. It is possible that excellent healers could feel uncomfortable in the laboratory environment and not be able to produce results on the simplified experimental systems. This was not the case with MSS. A close supportive relationship was developed with the other investigators and MSS had total confidence in his ability to perform the requested tasks.

MSS was shown a dish that already had been cultured for 4 hours and that contained cells with various degrees of growth so that he could have a picture in his mind of what the neurons look like. In the experiments he was asked to use his energy treatments to increase the growth in some dishes and to decrease that in others. Two different methods were used to apply “energy” to the cultured neurons. The first was used in the preliminary experiment. MSS was given sealed (screw top) plastic test tubes containing the nutrient medium in which the cells were to be grown. One was to receive "positive" or "healing" energy and another "negative" or "harmful" energy. Each of these two test tubes were held in MSS's hands for 10 minutes during which he used his conscious intent to alter the “energy” content of the contained medium. A third test tube was not treated and served as the control. After treatment the test tubes were placed in the incubator in order to return to 37° C. Throughout MSS's treatment of the media he was accompanied by one of the investigators (PRB). Only MSS and PRB knew the contents of each of the numbered test tubes. The other investigators (RWH and JLD) who used the medium from each test tube to prepare neuronal cultures in 3 dishes were

blind as to the treatment of the media. All 9 dishes used in this preliminary experiment were placed on the same shelf in the same incubator at 37° C and 5% CO<sub>2</sub>.

The second method of energy treatment was used in the 2 larger scale experiments. MSS wanted to directly treat the neurons. Thirty culture dishes were used in each experiment. Ten dishes, already containing the plated neurons and nutrient medium, were placed in each of 3 different incubators all set to the same temperature (37° C) and CO<sub>2</sub> content (5%). The outer doors of the incubators were opened and for 10 minutes MSS projected energy through an inner glass door that was kept closed throughout the process. This method reduced any fluctuations in temperature and pH during the energy treatment. MSS did not make physical contact with any of the cultures and was again always accompanied by PRB. One set of dishes received the "positive" energy and the other the "negative" energy. A third set of 10 untreated dishes was treated identically but received no projected energy and served as the control. Each set of 10 dishes remained in the incubator in which it had received treatment for 4 hours, after which the cells were fixed with paraformaldehyde. At this time the other investigators did not know which dishes had received treatment but were aware of the dishes that were grouped together in the same incubator. In all experiments, after fixation of the cultures each dish was randomly renumbered by either PRB or RWH so that the person doing the analysis of the neurons (JLD) was blind to the treatment received by each dish and to which incubator it had been in.

#### *Data collection in the preliminary energy treatment experiment*

The preliminary experiment was designed in order to allow a relatively rapid analysis of the cultured cells in order to determine whether MSS's treatment could

produce any observable change in the survival, differentiation or growth of the neurons. At the completion of the experiment the neurons were fixed with paraformaldehyde and kept in the refrigerator until they were microscopically examined. The following parameters were measured by JLD and RWN in a blind manner, not knowing what treatment the examined dish had received:

1. The total number of living neurons on each dish was counted. Any dead cells present could be distinguished by their opaqueness under phase microscopy. This parameter gives a measure of the ability of the energy treatment to alter the survival of the cells

2. The number of neurons in each dish that extended a neurite was counted. A cytoplasmic extension from the cell body was considered a neurite if its length was at least as long as the radius of the cell body (Fig. 1A). These data were used to calculate the average number of cells with neurites/dish, the percent of cells with neurites per dish and the total number of cells with neurites. These parameters give a measure of the ability of the energy treatment to alter the state of differentiation of the cells. When removed from the embryo the ciliary ganglia contained differentiated neurons that had already extended axons and undifferentiated cells which will extend axons at a later time. Immediately after establishing the cultures these cells cannot be distinguished since the axons have been torn off the neurons during the dissociation procedure. Therefore, after the 4-hour culture period those cells with neurites were either already differentiated into neurons prior to dissociation of the ganglia or they became differentiated during the time in culture.

3. The number of primary neurites extending from the cell body of each neuron was counted (Fig. 1B). These data were used to calculate the average number of neurites/cell/dish, the total number of cells with  $\geq 3$  neurites/cell, the total number of cells with  $< 3$  neurites/cell and the percent of cells with  $\geq 3$  neurites/cell. These parameters give a measure of the ability of the energy treatment to alter one of the basic characteristics of the shape of the neurons. Neurons are usually characterized as being unipolar, bipolar or multipolar depending on the number of primary neurites they possess.

4. The total number of branches extending from each cell (Fig. 1C) was measured. This is equivalent to the number of growth cones per cell, which was actually measured. The growth cone is the motile structure at the end of a growing neurite. These data were used to calculate the average number of growth cones/cell/dish, the total number of cells with  $\geq 4$  growth cones/cell, the total number of cells with  $< 4$  growth cones/cell and the percent of cells with  $\geq 4$  growth cones/cell. These parameters give a measure of the ability of the energy treatment to alter a second major characteristic of the shape of the neuron.

5. Neurons with neurites of various lengths were observed (Fig. 1D). The longest neurite for each neuron in all 3 dishes was identified. Its length was approximated by comparing the neurite to the diameter of the cell body. If the length of the neurite was  $> 3$  cell diameters it was assigned a value of 3. If it was greater than 2 and less than 3 cell diameters it was assigned a value of 2. If the length was greater than 0.5 and less than 2 it was assigned a value of 1. These assignments of length were made by visual inspection in order to make the measurement process more rapid. These data were used to calculate the average length of the longest neurite/cell/dish, the total number of cells with longest

neurites = 3 units in length, the total number of cells with longest neurite < 3 units of length and the per cent of cells with longest neurite = 3 units of length. These parameters give a measure of the ability of energy treatment to alter the rate of neurite elongation.

*Data collection in the large-scale energy treatment experiments*

The large-scale experiments were designed to have a greater number of culture dishes in order to facilitate the statistical analysis of the data. Each experiment used 30 culture dishes with groups of 10 dishes receiving the same treatment.

The dishes were examined microscopically so that highly magnified digital photographs of all of the neurons on the dish were obtained. Images were acquired using Openlab V 3 software to regulate a Micromax CCD camera from Princeton Instruments and a Nikon Diaphot 300 microscope with a 10x PLANAPO Phase objective. The software controlled moveable stage insured that all regions of the culture dish were photographed. The images are then analyzed using Image J software available from the National Institutes of Health. The previously described parameters assessing the survival, differentiation and basic shape of the neurons were measured in a manner identical to that in the preliminary experiment. However, the length of each neurite could now be accurately measured by tracing it with the mouse-controlled cursor and having the software calculate the number of pixels the cursor had moved. Similar measurements of a standard micrometer enabled the conversion of this length into  $\mu\text{m}$ . Since the length of every neurite of every neuron in each dish was measured the following parameters could be determined:

1. The average length of all the neurites/dish,
2. The average length of the longest neurite/cell/dish,

3. The average total length of all neurites and branches extending from each neuron/dish.

These parameters were used to more quantitatively assess the effects of energy treatment on the extent of growth of the neurites during the 4 hour culture period and were measured by JLD in a blind manner without knowing for each dish what treatment it had received nor which group of 10 dishes it had come from (what incubator it was in). The precision of these measurements was estimated by repeating the evaluation of all the parameters in a single dish. The two measurements of each of the 3 length parameters differed from each other 1.0, 1.0, and 2.7% respectively. The other parameters assessing survival, differentiation and the basic shape of the neurons differed from each other by 0-4% in the replicate measurements.

*Reduction of variation among the experimental parameters*

Some recent well-controlled studies (Yount et al., 2004; Taft et al., 2004, 2005) failed to detect any effect of “healing energy”. These experiments, like our own, have depended on an assay in which some property in each of the individual cells has been measured. They have been impeded by the relatively large variation in molecular and cellular parameters observed in apparently identical cells. Individual cells are highly complex systems containing networks of genes regulating their own expression and networks of physically interacting proteins. Even in cultures cloned from a single cell there is significant variation among the daughter cells arising from molecular stochastic events (reviewed in Kaern et al., 2005) and differential interactions with microheterogeneities in the environment. Since we are measuring the neurite growth in all of the cultured neurons it was imperative for us to take this inherent variation of the

system into consideration when designing and performing our experiments. A major effort was made to reduce the variation in the neurite growth properties of the cultured neurons.

Before it is possible to consider that the conscious intent of an energy healer can alter the growth of cultured neurons, it is necessary to identify and eliminate other factors in the experimental situation to which such growth is sensitive. Many such factors contribute to the relatively large variation that is observed at several levels in these experiments. First, there is variation in growth among all the neurons in a single dish. Second, there is variation in neuronal growth among the multiple dishes receiving the same treatment and placed in the same incubator. Third, there is variation in neuronal growth among dishes placed in different incubators. Fourth, there is variation in growth among neurons isolated on different days.

After 4 hours of culturing considerable variation is observed among the neurons in any single dish. Some can be observed that have either failed to extend a neurite or have done so with lengths varying from 5 – 185  $\mu\text{m}$ . Some of this variation may arise from the previously discussed heterogeneity in neuronal populations in the ciliary ganglia. However, of greater significance is variation in the state of differentiation of the neurons. In any single ganglion there are neurons that had already extended axons towards their target and others which have not yet acquired the developmental ability to do so. The former will be regenerating new axons in our cultures and may initiate rapid growth a short time after plating. The latter will have to continue differentiating before they are able to extend neurites and the 4 hours in culture may not be sufficient time to enable them to do so. They will comprise the large number of neurons with no neurites.

In between these extremes will be neurons that are able to differentiate further in culture and initiate neurite growth for the first time. There was no way that we could control this type of variation. In addition to stage of differentiation, neurite outgrowth is very sensitive to an adhesive interaction with laminin molecules adsorbed to the bottom of the dish. These are very large proteins that are normal components of the extracellular matrix. Microheterogeneity in the distribution of laminin on the surface may produce regions of the dish that differentially support neurite outgrowth. Considerable effort was made to reduce this by carefully selecting (Gold Seal) and washing (ethanol, water, no soap) the glass coverslips prior to the adsorption of poly-L-lysine and laminin. The same batch of laminin was used in all experiments.

In the preliminary experiment differences are observed in the neuronal growth among the 9 dishes treated in different ways and cultured on the same shelf of a single incubator. Before saying that the differences are the result of the energy treatments, it is necessary to consider the possibility that this variation is the result of the preparation of the cultures or to the position of the dishes in the incubator. This is particularly relevant because in the large-scale experiments when 10 plates are treated in an identical manner and placed on the same shelf in the same incubator, variation among the plates is observed in the measured means of several neuronal growth parameters. The coefficients of variation were between 2.2 and 20% and depended on the parameter measured. It is not likely that the previously described heterogeneity in the neuronal populations and their developmental state contributed to this variation because the same cell suspension was used as the source of plated cells in all dishes. The cell suspension was gently shaken before each aliquot was removed and added to the dishes. Similarly,

microheterogeneity in the distribution of laminin on the surfaces of the dishes was controlled as well as possible by careful attention to the precision of the washing and preparation of the coated coverslips. Additional causes of variation in neuronal growth among the 9 or 10 dishes may arise from fluctuations in the physical environment within a single incubator. All incubators were adjusted so that the digital read-out of the temperature was 37.0° C. During the 4-hour culture period this reading fluctuated by  $\pm$  0.2° C. The accuracy of the temperature reading was confirmed with a standardized thermometer that recorded the same temperature at all positions on the one shelf/incubator upon which the culture dishes were placed. All incubators were also adjusted to a digital read-out of 5% CO<sub>2</sub> and did not fluctuate in the course of the experiments. This serves to regulate the pH of the nutrient medium. Phenol red was added to the medium to serve as a pH indicator. There were no differences in the color of the media in dishes placed at various positions on the one shelf used in each incubator. The level of water in a dish at the bottom of the incubator was maintained in order to control the humidity. It is expected that the metal casing of the incubators and the water jackets used to reduce temperature fluctuations also served to shield the contents from electromagnetic fields. Every effort was made to maintain a constant and homogeneous physical environment within each incubator. In the preliminary experiment involving a total of 9 dishes, all were placed on one shelf in the same incubator. Another way to control for the possible lack of homogeneity within the incubator is to randomly select a dish from all those used in one experiment and to randomly position it in the incubator (Yount et al., 2004). This procedure was not followed in our large scale experiments because MSS wanted to treat all the dishes at the same time after they were placed in the

incubator and we were concerned that fluctuations in temperature and pH could occur when dishes were removed from one incubator after treatment and placed in another.

Because of the design of the large-scale energy treatment experiments it was necessary to place the 30 culture dishes in 3 different incubators with each incubator containing ten dishes all treated in an identical manner. This created the possibility that any differences observed among the dishes treated in different ways could arise from variation resulting from the use of 3 different incubators in one experiment. No differences in the physical environments within the incubators could be detected (see above). Although very small temperature fluctuations occurred, the maximum transient difference in temperature between any 2 incubators was no greater than 0.4° C. Another cause of variation arises from the experimental protocol when applied to 30 dishes. All 10 culture dishes were removed from one incubator at a time and fixed with paraformaldehyde. In both large-scale experiments the dishes were fixed in the same incubator sequence (A, B then C). Although this was performed as rapidly as possible, by the time that the last 10 dishes from incubator C were fixed they had been cultured for 20 minutes longer than the first set of 10 dishes from incubator A. It was not considered that this would make much of a difference in light of the 4-hour culture period. In spite of these potential disadvantages of using 3 incubators it was felt that this was a better way of performing the large-scale experiments in light of MSS's desire to treat the neurons after they were already plated on their culture dishes. Our procedure eliminated the opening and closing of the inner incubator doors and the removal and replacement of treated cultures. This prevented the dishes from being exposed to any changes in temperature and pH which could have been significant if many dishes had to be repositioned in

different incubators. In addition, there are reports that the effects of energy treatment can linger in the environment for periods of time after the treatment has ended and can influence apparently untreated cells (Radin et al., 2004; Kiang et al., 2005). If this phenomenon occurred it would have degraded the specificity of MSS's treatment if dishes from different treatments had been placed in the same incubator.

In order to determine whether our protocol was producing variation in dishes among the different incubators, a control experiment was performed in which neurons were plated on to 30 dishes and 10 were placed in each of the 3 incubators (A, B, C). The results are summarized in Table 4. When the averages of the several neuronal growth parameters measured in dishes in incubator A were compared to those in incubator B differences between 0.4 and 6.9% were observed depending on the parameter. None of these were statistically significant. This is important to note because in both of the large-scale energy treatment experiments it was the culture dishes in these 2 incubators that were always treated with either healing or harming energy. It would be expected that the differences between these two sets of dishes would be greater than any comparisons with the control, untreated dishes that were always placed in incubator C. Therefore, for the comparisons of expected enhanced growth cultures with expected reduced growth cultures incubator or protocol effects contribute little to any variation that may be observed between dishes in the 2 incubators. However, some statistically significant differences are observed between neuronal growth parameters measured in dishes in incubator C when compared to those in A or B. The directions of these differences for A vs C (26.5% greater number of cells with neurites, 15% increase in the per cent of cells with neurites, 11% increase in the average length of neurites, 10% increase in the average

length of the longest neurite/cell) indicate that these were caused by our experimental protocol which resulted in the cells in incubator C having up to 20 minutes extra time to grow and differentiate. The added time for neuronal differentiation would allow some of the cells which had not previously grown neurites to now do so. Similarly, the added time will allow those neurons which have already started to grow to continue doing so and thus produce longer neurites. If there is a lag period between the time the neurons are plated and the time they differentiate and acquire the ability to grow neurites, then the actual growth period is less than 4 hours. The extra 20 minutes in incubator C may then represent a larger percent of the actual growth period than was expected. Therefore, it is considered that the experimental protocol is responsible for an apparent effect involving incubator C. Since in the large-scale experiments this incubator always contained the untreated control cultures it was considered necessary to correct the values of the 4 sensitive neuronal growth parameters measured in these dishes in order to make them comparable to those calculated from cultures in incubators A and B. This correction was made by dividing the observed number of cells with neurites by 1.27, the percent cells with neurites by 1.15, the average length of all the neurites by 1.11 and the average length of the longest neurite/cell by 1.10.

It was also observed that the values of the neuronal growth parameters varied among experiments performed on different days more than they did within any of the single treatment experiments. This is probably the result of slight variation in the age of the embryos used in each of the experiments which could result in differences in the percent of neurons that had differentiated axons prior to isolation. Also, slight differences in the enzyme treatment dissociating the neurons might produce variation in

the rate at which the cell recover when plated. These types of variation prevented us from pooling results obtained from experiments performed on different days.

## RESULTS

The preliminary experiment was designed to relatively rapidly determine whether energy treatment by MSS could alter some properties of cultured neurons and alter them in an intended direction. It consisted of 9 dishes containing a total of 6205 cultured neurons from chick embryo ciliary ganglia (Table 1). Three dishes received media treated with positive or healing energy and it would be expected that, in comparison to the 3 non-treated control dishes, these enhanced cultures would contain more neurons, a higher fraction of them would extend a greater number of neurites and that these branches would be of greater length. This assumes that cell survival, differentiation, shape and rate of neurite elongation are all sensitive to energy treatment. Conversely, it would be expected that the 3 dishes receiving media treated with negative or harmful energy would show changes in the opposite direction in comparison to the untreated controls. Similarly, the differences in the growth parameters observed between the enhanced and the reduced cultures should be greater than those observed when either one of these is compared to the untreated control cultures. The results as summarized in Tables 2 and 3 indicate that for most of the parameters these relationships were observed. The average values for the parameters in each of the 3 dishes were analyzed using a 2-tailed unpaired Student's t-test that accommodated unequal variance between the 2 samples. Most of the results were found to not be statistically significant ( $p > 0.05$ ). This was the case in some instances where even 30 - 50% differences between the means were observed. The lack of significance resulted from the small number of dishes used. However, even with these limitations, significant differences ( $p < 0.05$ ) in the parameters assessing rate of neurite elongation (length of the longest neurite/cell/dish, number of neurons with neurite length

= 3/dish, the percent of neurons with a neurite length of 3/dish) were detected when comparing enhanced cultures that received positive energy with the reduced cultures that received negative energy (Table 2). Some of the parameters were amenable to Chi-square analysis because every cell in all of the dishes was measured. The total number of cells with and without neurites in the treated cultures could be compared with that expected based on the values observed in the control untreated cultures. Highly significant results were obtained (Table 3) indicating that the treated cells were a different population from the control cells in regards to this parameter. Similarly, these parameters in the enhanced and the reduced cultures could be compared to each other using as expected values the proportions of cells in the appropriate rows and columns of the 2 x 2 contingency table. These results indicated an even more significant difference between the 2 populations of treated neurons. When a similar analysis was performed on the parameters assessing the basic shape of the cells (the total number of cells with either < 3 or  $\geq 3$  primary neurites; the total number of cells with either < 4 or  $\geq 4$  growth cones) no significant effect of treatment was observed. In contrast, the parameter assessing rate of neurite elongation (the total number of neurons with a neurite of length either < 3 or = 3) showed that energy treatment created very significantly different populations of neurons ( $p < 5 \text{ E-}8$ ). The preliminary experiment indicated that neuron survival and the basic shape of the neurons did not appear to be much affected by energy treatment while neuronal differentiation and the rate of neurite elongation were highly sensitive. In addition, the direction of the changes in the treated cultures was identical to that predicted from the treatment consciously intended by MSS. The results were sufficiently encouraging so that two larger experiments were performed.

Each of the two large-scale experiments contained 30 culture dishes and 7,979 and 14,753 cells respectively (Table 1). For reasons described in the Materials and Methods section these experiments were best performed by placing all ten dishes receiving the same treatment in the same incubator. In experiment 1 the enhanced, positive energy treated cultures were placed in incubator A, the reduced, negative energy treated cultures in incubator B and the control, untreated cultures in incubator C. To partially control for possible incubator effects the locations of the enhanced and reduced cultures were reversed in the second experiment while the control cultures remained in incubator C. In order to assess any effects due to placement of dishes in particular incubators or due to differences in the handling of the sets of dishes as a result of the experimental protocol a control experiment was performed in which untreated dishes were placed in all 3 incubators. The results (Table 4) were discussed in Materials and Methods and indicated the necessity of applying a correction factor to 4 of the parameters in the control dishes. This correction has been applied to the data in both of the large-scale experiments (Tables 5 and 6). An additional difference between these experiments and the preliminary one is the actual measurement of neurite length by direct tracing with a mouse-controlled cursor enabling the direct calculation of 3 different neurite growth parameters (average neurite length/dish, length of the longest neurite/cell/dish, total neurite length/cell/dish).

The results of experiment 1 are summarized in Table 5. The only parameter in which the enhanced, positive energy treated cultures significantly differed from the controls, and in the predicted direction, was the percent of cells with neurites. The reduced cultures treated with negative energy were very significantly different from the

controls in the parameter assessing neuronal differentiation (percent of cells with neurites) and rate of neurite elongation (average neurite length/dish, length of the longest neurite/cell/dish, total neurite length/cell/dish). The percent differences between these dishes varied from 25-32% and the p values of the t-tests were between  $10^{-4}$  and  $10^{-8}$ . Again, 2-tailed Student's t-tests accommodating unequal variance between the 2 samples were used. However, these differences were in the opposite direction from that expected. More cells extended neurites and these neurites were considerably longer even though the cultures had been treated with negative energy. These parameters were also significantly different when the enhanced cultures were compared to the reduced ones and again the direction was opposite from that expected.

The results from experiment 2 are summarized in Table 6. In this case it was the enhanced, positive energy treated cultures that were most different from the controls and in the expected direction. The three parameters assessing the rate of neurite elongation were all significantly different from those of the control cultures as was the parameter assessing neuronal differentiation, the percent of cells with neurites/dish. In comparing the reduced, negative energy treated cultures with controls none of the parameters were significantly different. In contrast, a comparison of enhanced with reduced cultures showed significant differences in all the parameters except for the 2 that assess basic cell shape.

In both of the large-scale experiments, measurements were made of the length of every neurite for every neuron in all of the dishes. These values did not have a normal distribution because many of the neurons were at different stages of development in spite of being the same chronological age. It was therefore possible to analyze these

measurements using the non-parametric Mann-Whitney method. It was found that in every case in which energy treatment altered a neurite length parameter in a significant manner as determined by the Student's t-test, similar or larger significant differences were confirmed by the Mann-Whitney test.

The results of the preliminary and the 2 large-scale experiments can be combined by calculating for each of the parameters the fraction that changed in a statistically significant manner (Table 7). The parameters measuring a single neuronal property were also combined. The conclusions from the preliminary experiment are robustly confirmed in that neuron survival and the basic shape of the neurons did not appear to be much affected by energy treatment whereas neuronal differentiation and the rate of neurite elongation were more sensitive. A schematic representation showing the effects of the different energy treatments on the length of the neurites and the absence of changes in numbers of neurites and growth cones is shown in Figure IF. The effects of energy treatment are seen when comparing treated (enhanced or reduced) cultures with controls (untreated) as well as when comparing enhanced and reduced cultures to each other. It is these latter comparisons that are the most significant and they are the ones that are least affected by incubator or protocol effects. In every single experiment MSS was successful in altering neuronal differentiation and neurite elongation in the energy treated cultures. However, his ability to enhance or reduce the growth of the neurons in the intended direction was barely above chance. Some possible reasons for this are explored in the Discussion

It is possible that one can identify the molecular target of the energy treatment by attempting to find chemical agents that produce the same selective effects on the growth

parameters of the cultured neurons. One candidate for this is the growth factor GDNF, which was added to the culture medium at a suboptimal concentration of 3 ng/ml in all the energy treatment experiments. This possibility was examined by culturing the neurons in medium containing various concentrations of GDNF (0, 0.3, 1.0, 3.0, 10, 30 ng/ml). Five culture dishes were used at each concentration and the same growth parameters were measured as in the energy treatment experiments. The results are summarized in Figure 2. It was observed that the number of cells/dish (Fig. 2A), the number of primary neurites/cell (Fig. 2C), and the number of growth cones/cell (Fig. 2D) are relatively insensitive to changes in the concentration of GDNF. Their values do not change much around the value of 3 ng/ml used in the energy treatment experiments. In contrast, the percent of cells with neurites/dish (Fig. 2B), the average neurite length (Fig. 2E), the length of the longest neurite/cell (Fig. 2F) and the total length of neurites/cell (Fig. 2G) are clearly more sensitive to the concentration of GDNF. This demonstrates that the growth parameters assessing neuronal differentiation and neurite elongation are the most sensitive to changes in concentration of GDNF and are also the most sensitive to energy treatment. Thus it is likely that the interaction between this growth factor and its receptor or the signal transduction pathways within the neuron are targets for the energy treatment.

## DISCUSSION

The results presented in this communication clearly demonstrate that in all 3 separate energy treatment experiments, using 69 culture dishes and involving the detailed analysis of 28,937 cells the healer MSS was able to alter growth of cultured neurons. Although the changes in the treated cells were not always in the intended direction in comparison to untreated cells, the most significant effects were always seen when comparing cultures in which MSS intended to enhance growth with those in which he intended to reduce it. Needless to say these results will be met with great skepticism by neuroscientists and every effort was made to identify and eliminate other factors that could account for the results.

One of the first things often questioned is the competence of the investigators. Two of us have received funding from the National Institutes of Health for research on the development and regeneration of the nervous system (JLD) and on sensory and motor aspects of the nervous system (PRB). The techniques used in this paper have been the center of recently published work on the mechanisms of signaling within neurites of cultured developing ciliary ganglion neurons (Denburg et al., 2005). The opportunity to work with MSS enabled us to apply established techniques and experimental methods with which we had extensive experience.

Another issue to consider is the possibility of experimental fraud and investigator bias. The healer, MSS, was only in the laboratory for the short times needed for the energy treatments. During these times he was continuously monitored by PRB. His treatments consisted of holding a sealed test tube containing tissue culture medium or of

holding his hand near the closed glass door of the incubators. There was no opportunity for him to commit fraud. Certainly the other investigators had their own sets of conscious expectations and unconscious biases. The experiments were designed so that these did not contribute to the results. The neurons used in each experiment were pipetted from the same stock solution. The cultures were prepared by JLD and RWH either at a time prior to energy treatment in the incubator or by the addition of already treated tissue culture medium. In both cases they did not know what type of treatment was received by specific cultures. The cells were fixed (killed and made rigid) after 4 hours in culture and data collection and analysis was performed in a blind manner by JLD. There was no way that investigator bias could contribute to the changes in the treated cultures.

Another possibility is that the various sets of dishes were being altered by differential fluctuations in the physical environments within the incubators. This was controlled by using top of the line tissue culture incubators (Forma Scientific Model 3326 stacked, water jacketed, CO<sub>2</sub> incubators) that rigidly maintained a constant temperature, CO<sub>2</sub> level and humidity. In the preliminary experiment all dishes were on the same shelf in the same incubator. In the large-scale experiments it was felt to be better to keep the dishes in the same incubator after the energy treatments in order to prevent environmental fluctuations arising from removing the dishes and randomly placing them in other incubators. However, this created a protocol problem in that the last set of 10 culture dishes removed for fixation received up to 20 extra minutes of growth time. The control experiment in which all 3 sets of dishes were untreated, showed that this produced a significant difference in 4 of the growth parameters when the last set of 10 dishes fixed

were compared to the first set. There were no significant differences between the first and the second set of dishes that were fixed. Since these were always the treated samples, the large-scale control experiment indicates that any differences among the incubators contributed little to the changes seen in treated cultures. On the other hand, the control experiment showed that the untreated cultures that were always the last fixed required some corrections in the calculation of certain of their growth parameters. The necessary corrections have been described.

The possibility that the results are an artifact due to inappropriate use of statistics must also be considered. In all cases we attempted to use the most conservative statistical application. The mean values of each parameter were calculated for each dish. Then the means for all the dishes in a particular set were calculated and the significance of differences among sets of dishes was assessed by 2 tailed Student's t-tests accommodating unequal variance between the 2 samples. However, since every cell in all of the dishes was analyzed data was available for an alternative statistical test. The Chi-square test in the preliminary experiment (3 dishes/set) detected significant treatment effects that were not observed in a t-test (compare Table 2 and 3). In the large-scale experiments (10 dishes/set) the same type of Student's t-tests detected significant differences in the parameters assessing neuronal differentiation and rate of neurite growth. Some of these could be checked by the non-parametric Mann-Whitney test using the length measurements of all of the neurites for all of the neurons. In each case where a significant difference was detected by the Student's t-test it was also found using the Mann-Whitney test. Therefore, the statistically significant changes are not likely to have occurred by chance.

We are led to conclude that it was something that MSS did during his energy treatments that produced the observed changes in the cultured neurons. Several established energy systems (Qigong, Reiki, Johrei, Therapeutic Touch, Vortex Healing) have been studied in the laboratory. MSS does not follow any of these systems. When asked to describe what he does he says he can detect and intentionally alter the flow of energy in his and the patient's the body. In our experiments he said he added energy to the cultures or media in which he intended to enhance growth and removed it from those in which he intended to reduce growth. He was successful in changing the growth of the neurons in the intended direction in only 2 out of the 3 experiments. In the preliminary experiment where he treated the tissue culture medium in a test tube MSS reported no qualms about what he was being asked to do (harm some of the cells). However, in the first large-scale experiment where he was directly treating the living cells in the dishes he immediately expressed remorse when it was suggested by PRB that it would be all right if he actually killed the cells he was trying to make grow less well. He said, "I never intentionally harm living things with my treatments". Although he emotionally responded in this way, he nevertheless made a conscious effort to reduce the growth of the cells in incubator B. He had already treated the cells in incubator A for enhancement. MSS always treated first for enhancement and then for reduction of growth. It was this experiment (Table 5) where the most significant differences between treated and control cultures were observed, but they were not in the intended direction. It is possible that the quandary in which MSS found himself made him subconsciously send energy to those cultures for which he was, with great hesitation, trying to decrease growth with his conscious intent. MSS now believes this is what happened. The second large-scale

experiment, treated in the same manner, did not produce an emotional response from MSS (PRB said nothing about killing cells) and the changes were all in the intended direction, although reduction was weak and often absent relative to the untreated controls. The results summarized in Table 7 demonstrate that MSS's ability to alter cultured neurons in the intended direction is not robust. However, they do show that in all experiments he was able to alter the neurons.

Although these experiments do not provide us with a mechanism as to what MSS is actually doing during his energy treatments, some interesting observations can be made. The "energy" is able to penetrate the walls of the plastic test tubes and the glass doors of the incubators. The "energy" is able to be stored in the medium which in the preliminary experiments was applied to the cells 15-20 minutes after treatment. A short 10 minute treatment had effects on the neurons that lasted for the entire 4-hour culture period.

It is important to consider why we were able to demonstrate effects of energy treatment on cultured neurons in well-controlled experiments while several other recent efforts (Yount et al., 2004; Taft et al., 2004, 2005) were not able to show that Qigong and Johrei practitioners could alter cells in tissue culture. We do not think that MSS is a better healer than those used in other studies that yielded negative results. Nor does this demonstrate that one system of healing is better than another. It is not possible to extrapolate from how energy healers perform in laboratory studies on *in vitro* systems to how they will do when treating patients. One can be an excellent healer as a result of one's personal interactions with patients and yet not be able to work on cultured cells in a laboratory situation. Performance in the laboratory may depend on creating a non-

hostile environment and using an energy healer who appreciates the significance of the appropriate methodology for scientific validation. We were fortunate to have both in our experiments. In addition, the selection of cultured neurons as a target system may have been fortuitous. The main function of these cells is to relay information by generating signals in vast networks. Within all of us conscious intent can alter activity of neuronal networks. We propose that the conscious intent of energy healers is actually altering the nervous systems of their patients, even though this may not be their intended target. According to this hypothesis, those parts of the body and their cells that are most sensitive to energy treatment are those most directly involved in communication and regulation. Cancer cells or transformed cell lines are sometimes used in energy treatment experiments. We would expect that these cells would be relatively less sensitive to energy treatment as a result of their having lost the ability to respond to signals regulating cell division. Similarly, at the molecular level we would expect increased sensitivity to energy treatment in those processes involved in cell-cell communication and regulation. This is supported by our observation that the pattern of changes in neuronal growth parameters produced by energy treatment was similar to that produced by changes in the concentration of the trophic factor GDNF. Such proteins are produced and secreted by target cells and they function as a signal to developing neurons that regulates cell survival, differentiation and neurite elongation. GDNF has been shown to be the *in vivo* trophic factor for the chick ciliary ganglion neurons (Hashino et al., 2001). Under the culture conditions of our experiments (4 hours *in vitro*) nearly all the plated cells survived and the cell count was not much affected by energy treatment or by changes in the concentration of GDNF. If we had allowed the cultures to incubate for much longer

periods of time the dependence of cell survival on GDNF also would have been observed and we predict that sensitivity to energy treatment would be detected too. The basic shape of the neurons, as assessed by the number of primary neurites/cell and the number of growth cones/cell, is generally considered to be sensitive to both extrinsic (environmental) and intrinsic properties of the cell (reviewed in Goldberg, 2004). Under our culture conditions in media containing 3 ng/ml of GDNF these parameters did not appear sensitive to energy treatment or to changes in concentration of GDNF around this basal level. However, neuronal differentiation as assessed by the ability to extend a neurite (percent of cells with neurites) and rate of neurite elongation as assessed by the 3 length parameters were sensitive to both energy treatment and changes in the concentration of GDNF. It should be noted that in each individual energy treatment experiment all cultures were prepared from the same batch of medium with the same concentration of GDNF. The conscious intent of the healer, MSS, was presumably not altering the concentration of GDNF but he may have been activating the receptor or the signal transduction pathway within the neuron. Consistent with these ideas are the observations of others that have shown energy treatment to increase the concentration of  $\text{Ca}^{2+}$  inside cancer cells (Kiang et al., 2002, 2005) and the activity of the enzyme phosphatidylinositol 3-kinase (Yan et al., 2004). These molecules are very important in the intracellular transduction pathways for cell signals. Similarly, the expression of the gene encoding the signal molecule insulin growth factor-I is increased by energy treatment (Yan et al., 2004).

When considering the medical implications of these experiments the first thing that comes to mind is to use such energy treatments to enhance axon regeneration after

spinal cord injury. Although the increase in neurite length was small (4-10  $\mu\text{m}$ ) it should be noted that this represented a 13-20 % increase after only 4 hours in culture and 10 minutes of prior energy treatment. In addition, short extensions of axons and dendrites might be sufficient to strengthen existing connections and to generate new synapses between neighboring neurons. The observation that energy treatments increase neuronal differentiation may have great significance in that it might be possible to use them to increase the likelihood that stem cell technology will be successful in the treatment of several degenerative neurological disorders.

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**TABLE 1. SUMMARY OF EXPERIMENTS PERFORMED TO ALTER GROWTH OF NEURONS BY ENERGY TREATMENT**

<b>Expt</b>	<b>Treated to ENHANCE Growth</b>		<b>Treated to REDUCE Growth</b>		<b>Untreated CONTROL</b>	
	<b>No. Dishes</b>	<b>No. Cells Analyzed</b>	<b>No. Dishes</b>	<b>No. Cells Analyzed</b>	<b>No. Dishes</b>	<b>No. Cells Analyzed</b>
<b>Preliminary</b>	3	2,155	3	1,959	3	2,091
<b>Large-scale 1</b>	10	2,633	10	2,700	10	2,646
<b>Large-scale 2</b>	10	5,178	10	4,598	10	4,977

**TABLE 2 Summary of Results of Preliminary Experiment Analyzed with Student's T-test**

	<b>CONTROL</b>	<b>ENHANCED</b>	<b>% Difference Between ENHANCED and CONTROL</b>	<b>T-test (p value)</b>	<b>REDUCED</b>	<b>%Difference Between REDUCED and CONTROL</b>	<b>T-test (p value)</b>	<b>% Difference Between ENHANCED and REDUCED</b>	<b>T-test (p value)</b>
1. Number of surviving cells/dish	697 ± 50	718 ± 56	3	0.649	653 ± 67	-6	0.420	10	0.268
2. Number of cells with neurites/dish	495 ± 36	559 ± 83	13	0.313	436 ± 52	-12	0.196	28	0.109
3. Per cent cells with neurites/dish	71 ± 2	78 ± 6	10	0.182	67 ± 4	-6	0.162	16	0.064
4. Number of 1° neurites/cell/dish	2.51 ± 0.08	2.47 ± 0.06	-2	0.604	2.47 ± 0.05	-2	0.593	0	1.0
5. Number of growth cones/cell/dish	3.79 ± 0.08	3.76 ± 0.19	-1	0.796	3.69 ± 0.02	-3	0.135	2	0.587
6. Length of longest neurite/cell/dish (arbitrary unit)	2.13 ± 0.11	2.25 ± 0.06	6	0.195	1.98 ± 0.02	-8	0.154	14	<b>0.012*</b>
7. Number of cells with neurite length = 3/dish (µm)	153 ± 58	231 ± 42	51	0.140	103 ± 161	-33	0.275	124	<b>0.023*</b>
8. Per cent cells with neurite of length=3	31 ± 10	41 ± 4	32	0.200	24 ± 4	-23	0.374	71	<b>0.004*</b>

\* indicates results that are significantly different (p < 0.05).

**TABLE 3 Summary of Results of Preliminary Experiment Analyzed with  $\chi^2$  test**

	<b>CONTROL</b>	<b>ENHANCED</b>	<b>ENHANCED vs. CONTROL Chi Square Test (<math>\chi^2</math>,p value)</b>	<b>REDUCED</b>	<b>REDUCED Vs. CONTROL Chi Square Test (<math>\chi^2</math>,p value)</b>	<b>ENHANCED Vs. REDUCED Chi Square Test (<math>\chi^2</math>,p value)</b>
1. Total number of cells with neurites	1484	1677		1309		
Total number of cells without neurites	607	478		650		
Percent cells with neurites	71	78	<b>48.6,* &lt; 5 E-8</b>	67	<b>16.7,* &lt; 1 E-3</b>	<b>63,* &lt; 5 E-8</b>
2. Total number of cells with $\geq 3$ primary neurites	679	746		596		
Total number of cells with < 3 primary neurites	805	931		713		
Percent of cells with $\geq 3$ primary neurites	46	44	1.5, < 0.22	46	0, 1.0	0.35, < 0.554
3. Total number of cells with $\geq 4$ growth cones	793	895		699		
Total number of cells with < 4 growth cones	691	782		610		
Percent of cells with $\geq 4$ growth cones	53	53	0.09, < 0.764	53	0.15, < 0.699	0, 1.0
4. Total number of cells with neurite length = 3	459	695		309		
Total number of cells with neurite length < 3	1025	982		1000		
Percent of cells with neurite length = 3	31	41	<b>79,* &lt; 5 E-8</b>	24	<b>30,* &lt; 4.3 E-8</b>	<b>96.5,* &lt; 5 E-8</b>

\* indicates significant differences ( $p < 0.05$ )

**TABLE 4 Summary of Results of Control Experiment**

	<b>Incubator A</b>	<b>Incubator B</b>	<b>% Difference Between B and A</b>	<b>T-test (P value)</b>	<b>Incubator C</b>	<b>% Difference Between C and A</b>	<b>T-test (P value)</b>	<b>% Difference Between C and B</b>	<b>T-test (P value)</b>
1. Number of surviving cells/dish	174 ± 20	175 ± 20	0.6	0.956	191 ± 18	10	0.065	9	0.076
2. Number of cells with neurites/dish	67 ± 10	72 ± 12	8	0.349	85 ± 9	27	<b>7.9 E-4*</b>	18	<b>0.015*</b>
3. Per cent cells with neurites/dish	39 ± 6	41 ± 4	5	0.311	44 ± 3	15	<b>0.019*</b>	7	0.071
4. Number of 1° neurites/cell/dish	2.54 ± 0.17	2.53 ± 0.14	-0.4	0.805	2.50 ± 0.16	-2	0.540	-1	0.675
5. Number of growth cones/cell/dish	4.41 ± 0.36	4.29 ± 0.27	-3	0.383	4.31 ± 0.26	-2	0.511	-0.5	0.785
6. Average length of neurite/dish (µm)	21.7 ± 1.9	22.7 ± 0.9	5	0.173	24.1 ± 1.7	11	<b>8.7 E-3*</b>	6	<b>0.037*</b>
7. Length of longest neurite/cell/dish (µm)	31.5 ± 3.0	33.5 ± 1.6	6	0.08	34.7 ± 3.1	10	<b>0.033*</b>	4	0.325
8. Total neurite length/cell/dish (µm)	95.4 ± 11	96.9 ± 5.6	2	0.702	104.1 ± 12	9	0.106	7	0.109

\* indicates significant differences (p < 0.05).

**TABLE 5 Summary of Results of Experiment 1**

	<b>CONTROL</b>	<b>ENHANCED</b>	<b>% Difference Between ENHANCED and CONTROL</b>	<b>T-test (P value)</b>	<b>REDUCED</b>	<b>% Difference Between REDUCED and CONTROL</b>	<b>T-test (P value)</b>	<b>% Difference Between ENHANCED and REDUCED</b>	<b>T-test (P value)</b>
1. Number of surviving cells/dish	261 ± 47	263 ± 32	0.8	0.895	270 ± 22	3	0.587	-3	0.594
2. Number of cells with neurites/dish	140 ± 23	153 ± 22	9	0.194	185 ± 19	32	<b>1.3 E-4*</b>	-17	<b>2.8 E-3*</b>
3. Percent cells with neurites/dish	54 ± 3	58 ± 4	7	<b>0.01*</b>	69 ± 3	28	<b>5.0 E-9*</b>	-16	<b>4.0 E-6*</b>
4. Number of 1° neurites/cell/dish	2.32 ± 0.14	2.27 ± 0.1	-2	0.406	2.25 ± 0.1	-3	0.275	1	0.762
5. Number of growth cones/cell/dish	4.12 ± 0.23	4.07 ± 0.23	-1	0.676	4.03 ± 0.16	-2	0.360	1	0.658
6. Average neurite length/dish (µm)	28.1 ± 2.0	28.7 ± 1.7	2	0.494	35.8 ± 1.6	27	<b>2.1 E-8*</b>	-20	<b>1.5 E-8*</b>
7. Length of longest neurite/cell/dish (µm)	43.2 ± 2.8	43.7 ± 2.3	1	0.700	54.7 ± 2.1	27	<b>1.3 E-8*</b>	-20	<b>1.5 E-9*</b>
8. Total neurite length/cell/dish (µm)	115.4 ± 11	116.7 ± 9	1	0.766	144.5 ± 8.3	25	<b>4.1 E-6*</b>	-19	<b>1.2 E-6*</b>

\* indicates significant differences (p<0.05).

**TABLE 6 Summary of Results of Experiment 2**

	CONTROL	ENHANCED	% Difference Between ENHANCED and CONTROL	T-test (P value)	REDUCED	% Difference Between REDUCED and CONTROL	T-test (P value)	% Difference Between ENHANCED and REDUCED	T-test (P value)
1. Number of surviving cells/dish	491 ± 26	518 ± 38	5	0.086	460 ± 41	-6	0.061	13	<b>4.3 E-3*</b>
2. Number of cells with neurites/dish	197 ± 29	237 ± 28	20	<b>6.0 E-3*</b>	179 ± 31	-9	0.202	32	<b>4.3 E-4*</b>
3. Percent cells with neurites/dish	40 ± 6	46 ± 5	15	<b>0.029*</b>	39 ± 5	-3	0.635	18	<b>7.6 E-3*</b>
4. Number of 1° neurites/cell/dish	2.00 ± 0.12	2.08 ± 0.07	4	0.066	2.00 ± 0.17	0	0.867	4.0	0.209
5. Number of growth cones/cell/dish	3.55 ± 0.25	3.67 ± 0.26	3	0.326	3.45 ± 0.34	-3	0.447	6.0	0.127
6. Average neurite length/dish (µm)	19.2 ± 1.4	22.3 ± 1.4	16	<b>7.9 E-5*</b>	19.9 ± 2.3	4	0.393	12	<b>0.015*</b>
7. Length of longest neurite/cell/dish (µm)	28.4 ± 2.7	32.7 ± 2.8	15	<b>2.7 E-3*</b>	28.6 ± 3.9	1	0.936	14	<b>0.015*</b>
8. Total neurite length/cell/dish (µm)	69.4 ± 9.0	80.2 ± 7.8	16	<b>0.01*</b>	69.3 ± 13.9	0	0.985	16	<b>0.049*</b>

\* indicates significant differences (p<0.05).

**TABLE 7. SUMMARY OF STATISTICALLY SIGNIFICANT CHANGES IN CULTURED NEURONS AFTER ENERGY TREATMENT**

Cell Property	Combined TREATED vs. CONTROL		Combined ENHANCED vs. REDUCED	
	Fraction of parameters changed	Fraction of those that changed in the expected direction	Fraction of parameters changed	Fraction of those that changed in the expected direction
Neuronal survival	0/6	-	1/3	1/1
Neuronal differentiation	5/6	4/5	3/3	2/3
Basic cell shape	0/12	-	0/6	-
Neurite elongation	8/14	5/8	8/8	5/8

## FIGURE LEGENDS

**FIGURE 1.** The variation in several parameters of growth of chick embryo ciliary ganglion cells are shown schematically in A-D and can be compared with a typical field containing actual neurons (F). Neurons were assessed as to whether or not they extended neurites (A), how many primary neurites they did extend (B), how many branches were attached to each primary neurite (C) and the length of each neurite (D). Energy treatment had little or no effect on neuronal survival, the number of primary neurites/cell and the number of growth cones/cell. It resulted in a change in the percent of cells with neurites and in the length of these neurites (E).

**FIGURE 2.** The effects of changes in the amount of glial cell line-derived neurotrophic factor (GDNF) added to the medium on the parameters of growth of the cultured chick embryo ciliary ganglion cells. The number of surviving cells/dish (A), the number of primary neurites/cell (C) and the number of growth cones/cell (D) are relatively insensitive to the concentration of GDNF. In contrast, the percent of cells with neurites (B), the average neurite length/dish (E), the length of the longest neurite/cell (F) and the total neurite length/cell (G) are more sensitive to changes in the concentration of GDNF.

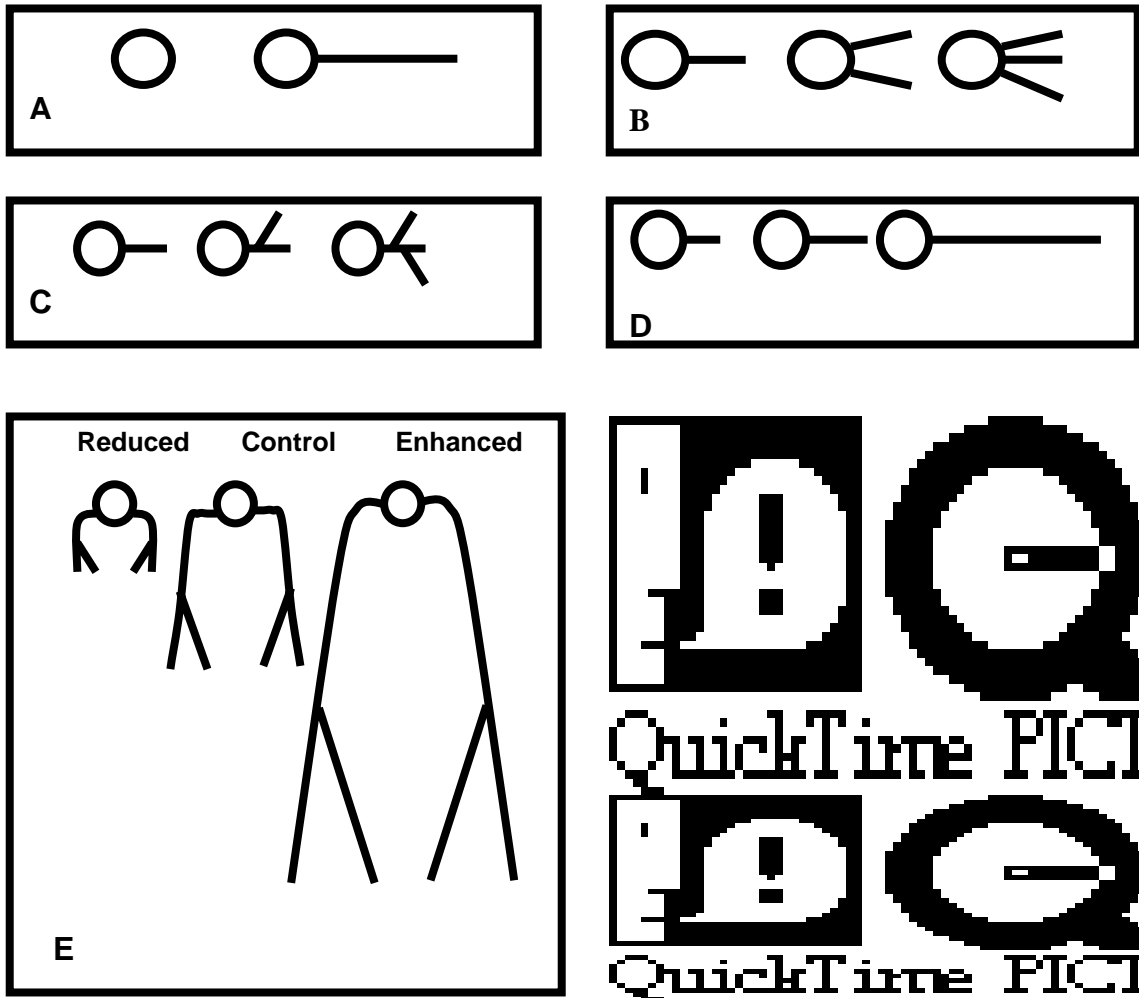
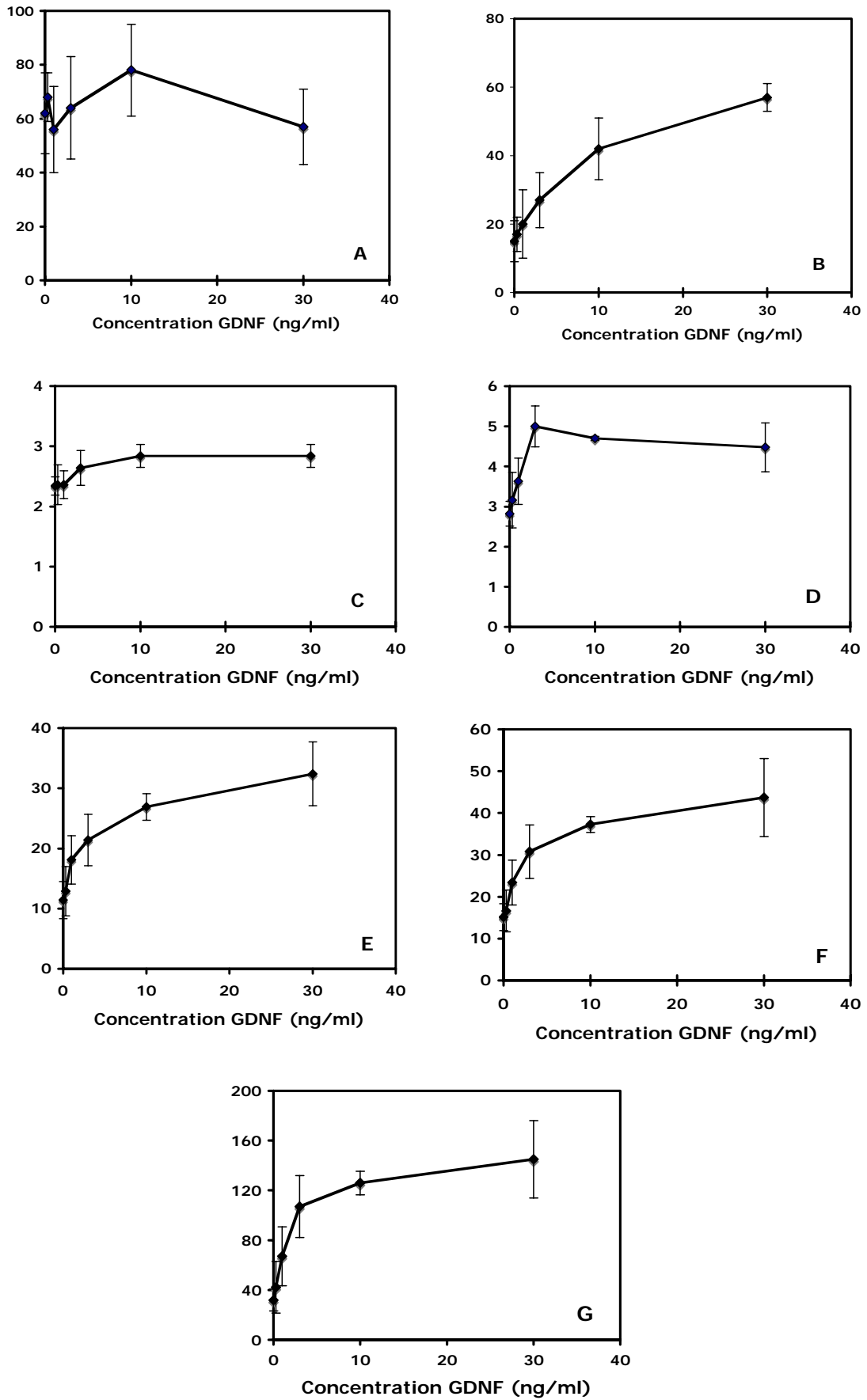


FIGURE 1



**FIGURE 2**