A Randomized Trial Investigating Effect of Intentionally Treated Water on Growth of *Arabidopsis thaliana* Seeds with Cryptochrome Mutations

ABSTRACT

Objective: A previous experiment suggested that consumption of intentionally treated tea influenced mood under double-blind conditions, as compared to untreated tea. To investigate this effect objectively, we studied whether *Arabidopsis thaliana* seeds hydrated with intentionally treated vs. untreated water would show differences in hypocotyl length, anthocyanin, and chlorophyll.

Design: Three Buddhist monks were asked to focus their intention on commercially bottled water with the goal of improved growth; water from the same source served as an untreated control. Seeds with three variations of cryptochrome (CRY) – a photosensitive flavoprotein – were used. They included the wild type *Arabidopsis* plant (Columbia-4), a gain-of-function mutation (*His-CRY2*), and a loss-of function mutation (*cry1/2*), where "gain" and "loss" refer to enhanced or reduced CRY sensitivity to blue light. The seeds were randomly hydrated with treated or untreated water under blinded conditions, then placed in random positions in an incubator and exposed either to blue light or blue plus far-red light. The germination process was repeated three times in each experiment, each using new seeds, and then the entire experiment was repeated four times.

Results: The results, evaluated blind with respect to the condition, showed a significant decrease in hypocotyl length in the *His-CRY2* seedlings (treated mean 1.31 ± 0.01 mm, untreated mean 1.43 ± 0.01 mm), a significant increase in anthocyanin with all three forms of *cry*, particularly *His-CRY2* (treated mean 17.0 ± 0.31 mg, untreated mean 14.5 ± 0.31 mg), and a significant increase in chlorophyll in *His-CRY2* (treated mean 247.6 ± 5.63 mg, untreated mean 230.6 ± 5.63 mg). These outcomes, especially in *His-CRY2*, were in alignment with the monks' intentions because a decrease in hypocotyl and increase in anthocyanin and chlorophyll are associated with enhanced photomorphogenic growth. These experiments suggest that *His-CRY2* may be viewed as a "detector" of intention. We propose a speculative model for this effect based on the purported quantum properties of cryptochrome.

Key words: Cryptochrome, intention, mind-matter interaction

INTRODUCTION

Why does mother's homemade chicken soup often seem to be more satisfying than the same soup from a tin can? Besides differences in food quality and freshness, one possibility is that the homemade meal contains an extra ingredient: loving intentions. Most cultures have maintained the belief that intentions can be imprinted into food or beverage, as commonly observed through the act of blessing water, wine, or bread. Such rituals are often performed in religious contexts, but the secular practice of toasting or offering special salutes with food or drink is practically universal.

To explore the role of intention in consumables, in two previous double-blind placebo-controlled experiments we tested whether intentionally treated chocolate in one case [1], and oolong tea in a second case [2], would enhance participants' mood. Experienced Buddhists monks in the United States and a visiting Mongolian shaman were invited to provide the intentional treatments in the chocolate study, and Buddhist monks in Taiwan provided it in the tea study. Both experiments showed statistically significant changes in participants' mood under double-blinded conditions using validated mood-reporting questionnaires [3].

To explore this effect in more detail without having to rely on subjective measures, and to explore a tentative explanatory model, in the present study we investigated the effect of intentionally imprinted water on the growth of seeds. We used water as a target of intention partially to follow up on our previous study investigating tea, but also because other experiments have reported that intentionally treated vs. untreated control water showed differences in ice crystal formation [4,5], infrared spectroscopy [6], pH [7,8], as well as enhanced germination and growth of barley, rye, lettuce and other plants [9-12].

For the plant we chose *Arabidopsis thaliana*, a small flowering weed in the mustard family with the popular name "mouse ear cress." This is one of the most-studied plants as well as a model eukaryotic system, thus relevant to all of biology [13]. Its genome was first completely sequenced in 2000 and shortly afterwards it was discovered that a majority of genes suspected or known to play a role in human disease had orthologs in *Arabidopsis,* making it a key resource in the study of the genetics of both plant and human health.

Arabidopsis grows quickly in the laboratory and it contains a photosensitive flavoprotein called cryptochrome (CRY) [14]. Three different CRYs act either as photoreceptors or as transcription regulators, called CRY 1, 2 and 3. These proteins play key roles in photomorphogenesis, circadian clocks, flowering time, seed germination, etc [15]. Shiah [16] proposed that the CRY might be a possible "transducer" of intention because it is present in all living systems and it is known to have quantum biological properties thought, among other things, to account for an exquisite sensitivity to magnetic fields as observed in bird magnetonavigation [17,18].

This suspected quantum biological property made *Arabidopsis* an ideal system to use for exploring a speculative model for how intention might affect physical systems.

MATERIALS AND METHODS

Intentional treatment procedure and blinding procedures

The water used to hydrate the *Arabidopsis* was purchased from Vedan, a commercial water bottling plant in Taiwan. A total of 24 bottles were used and randomly assigned by the first author into two groups using a truly random number generator available at www.random.org (Randomness and Integrity Services Ltd., Dublin, Ireland). If a number retrieved from this service was odd, a bottle was labeled A, otherwise B.

The intentional treatment was provided by Master Lu Cheng, a respected monk and Director of the Bliss Wisdom Buddhist Foundation in Taiwan, along with two other senior monks from the same Foundation. All three are accomplished meditators with experience in maintaining prolonged concentration. The explicit intention they were asked to provide was as follows: "The *Arabidopsis* that absorbs this water will manifest optimal growth; in particular it will have increased nutrition, energy, vigor and well-being." The monks mentally directed these intentions toward the bottles of water in an isolated room at the Foundation for 20 minutes. To avoid inadvertently influencing the control water, which was located in the same room as the treated water, an additional, closing intention was added to the monks' instructions: "This enhancement is only for this batch of water," referring to the treated bottles.

During this procedure only a research assistant and the three monks were present, and none of those individuals were involved in any other aspect of the experiment. None of the authors of this study were aware of the blinding assignments at this stage of the experiment.

After the intentional treatment process, the first author sent the prepared coded bottles to the third author, who handled all of the watering, seed germination, and measurement procedures. When all measurements were completed, and after the fourth author analyzed the blinded data, the first author contacted this research assistant to learn the blinding code (A = untreated, B = treated).

Seed preparation and general design

Three types of *Arabidopsis thaliana* were employed: (1) the wild type known as Columbia (Col-4), (2) a "loss-of-function" CRY mutation known as *cry1/2*, where "loss" indicates the seed was less sensitive to blue light than Col-4, and (3) a gain-of-function mutation called *His-CRY2*, which has enhanced sensitivity to blue light. All of the seeds were surface sterilized with 20% bleach (containing 0.5% Tween-20), planted in a greenhouse, and then the plants were allow to grow to maturity to provide a sufficient number of additional seeds for the experiment. In two experiments additional variations of CRY were also grown. Results of those tests will be discussed in other publications.

Seeds harvested for the experiment were again surface sterilized with 20% bleach (containing 0.5% Tween-20) and 30 to 40 seeds were subsequently sown by the third author on germination medium (GM) agar plates containing 0.3% sucrose. She then randomly distributed the two types of water among the separate GM agar plates. The plates were placed in a Conviron (Winnipeg, Canada) E2/7 temperature-controlled incubator chamber fitted with light emitting diode (LED) panels. They were grown under four conditions: continuous blue light at 5 μmol/m^{2}s (cBL), 5 μmol/m^{2}s (cBL) plus 5 μmol/m^{2}s far-red light (cFR), 10 μmol/m^{2}s (cBL), or 10 μmol/m^{2}s cBL plus5 μmol/m^{2}s far-red light (cFR). The seeds were allowed to grow for 3 days. The protocol is illustrated in Fig 1.

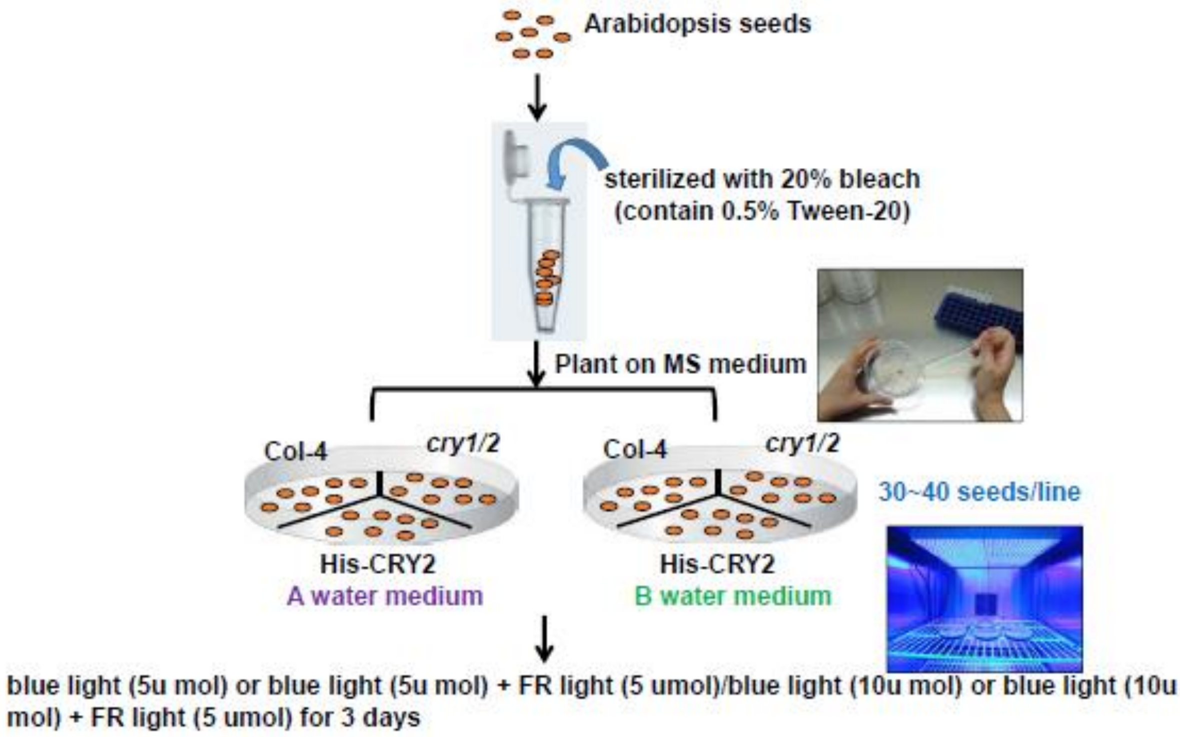

Figure 1. Protocol for growing Arabidopsis seeds.

This experiment thus included 3 types of *Arabidopsis* seeds × 25 or 15 seeds (25 for hypocotyl length; 15 for chlorophyll and anthocyanin measurements) per experiment × 2 types of water (treated or untreated) × 3 repetitions × 2 illumination conditions (cBL or cBL plus cFR). All plates, growth medium, pipettes, and seedling grinding materials were only used once in each replication to avoid the possibility of cross-study contamination.

In each experiment three analyses were conducted on the resulting seed growth: chlorophyll (green flavonoid pigments), anthocyanin (red-orange to blue-violet flavonoid pigments), and hypocotyl length (the stem of a germinating seedling). Increased levels of chlorophyll and anthocyanin are known to be beneficial for health, and a shorter hypocotyl length is associated with improved growth. Thus the intentional hypothesis predicted that seeds grown in treated water would show increased levels of chlorophyll and anthocyanin, and a shorter hypocotyl length (illustrated in Fig 2).

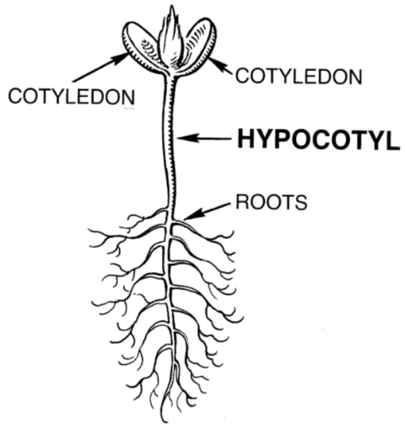

Figure 2. Anatomy of a seedling.

Chlorophyll extraction and quantification

Each plate was sown with 30 to 40 seeds. For analysis of chlorophyll only 15 of the most robustly germinated seeds were analyzed. That group of seeds was crushed together to form enough material to analyze for chlorophyll content (see Fig 3). One experiment consisted of 15 germinated seedlings (combined to form a single measure of chlorophyll) × 3 types of *Arabidopsis* variations × a lighting condition (cBL or cBL and cFR) × 2 water conditions (treated or untreated) = 180 seedlings. This process was repeated three times in each experiment, thus a total of 180 × 3 = 540 seedlings were used. The entire experiment was repeated four times, thus a total of 2,160 seedlings were involved in the measurement of chlorophyll.

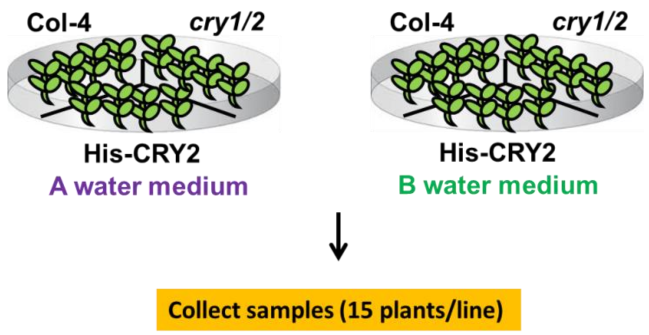

Figure 3. Protocol for analyzing chlorophyll in one experiment.

Each group of 15 harvested seedlings were extracted in 200 µl dimethylformamide (DMF) for 16 hours at 4℃ and vigorously mixed in darkness. 50 µl of extracts from this mixture were added into 450 µl of pure alcohol, mixed, and then 200 µl of the resulting liquid was used for spectrophotometric analysis. Absorbance at 750, 664, and 647 nm was measured with a Tecan Infinite 200 PRO (Tecan Group, Ltd, Switzerland) spectrometer distributed by pipette into a 96-well plate. Total chlorophyll content was determined according to the standard extinction coefficient as calculated by Porra et al. [19].

Anthocyanin extraction and quantification

For anthocyanin measurements, 15 newly harvested seedlings were ground in liquid nitrogen, and total plant pigment was extracted overnight in 300 µl 1% HCl in methanol. After the addition of 200 µl H_2O, chlorophyll was separated from the anthocyanin by extraction with an equal volume of chloroform. The content of anthocyanin was then quantified by spectrophotometer [20].

Measurement of hypocotyl length

For hypocotyl length, 25 germinated seeds were analyzed in each of three replications per experiment. The germinated seedlings were placed horizontally on agar plates and a photo taken with a Canon digital camera. The hypocotyl length in the image was subsequently measured using the imaging software, ImageJ (Rasband, 1997-2016).

The experiment involved 25 seedlings × 3 types of *Arabidopsis* variations × 2 water conditions (treated or untreated) × 3 repetitions per experiment × 2 lighting conditions (cBL or cBL and cFR). Each of four experiments thus included 600 seedlings. This process was repeated three times in each experiment, for a total of 1,800 seedlings.

The previous successful studies involving chocolate [1] and tea [2] involved a total of 62 participants (Cohen's d = 0.45; p = .04, one-tailed) and 189 participants (Cohen's d = 0.65; p = .02, one-tailed) , so the present study was assumed to have sufficient statistical power to detect the hypothesized intentional effect.

RESULTS

Hypocotyl

Data from the four experiments were analyzed with a two-factor ANOVA using Statistica 8.0 (Tulsa, OK): 4 levels for *experiment* × 2 levels for *condition*. This analysis combined data from the different illumination sources. The effect of different light frequencies and intensity, as well as other CRY mutations, will be described in another publication. The comparison of primary interest was the main effect for *condition*. The results for hypocotyl length are shown in Fig 4 and 5. The means observed in each of the four experiments are shown in Fig 6.

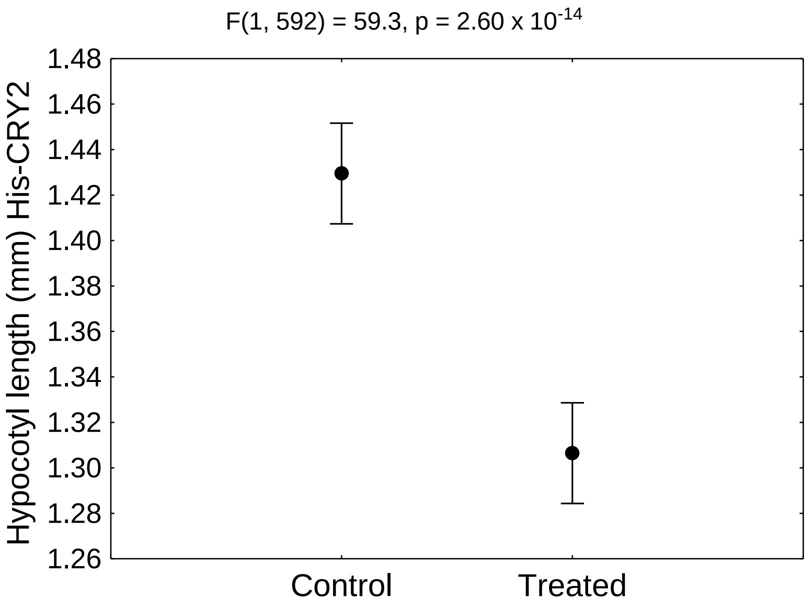

Figure 4. Grand mean hypocotyl length with 95% confidence intervals for *His-CRY2* seedlings in control and treated conditions, combined across all four experiments. (All error bars in graphs show 95% confidence intervals.)

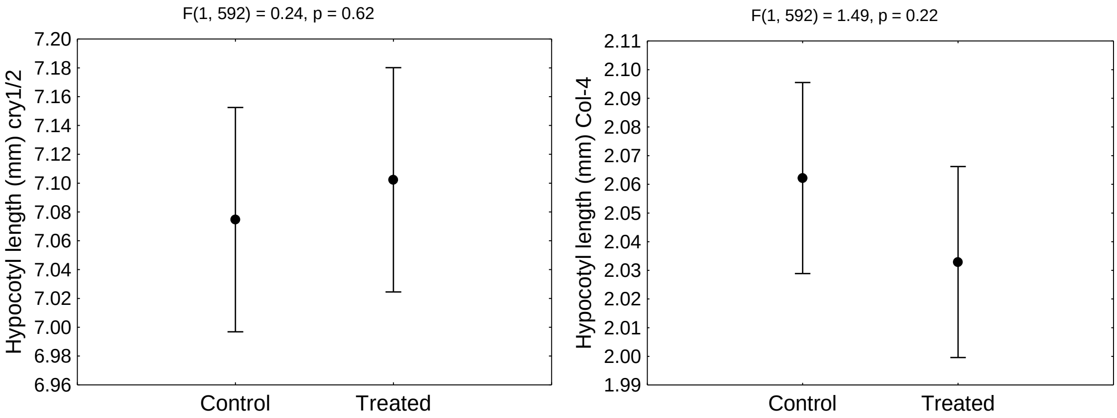

Figure 5. (Left) Mean hypocotyl length for *cry1/2*. (Right) Same for *Col-4*.

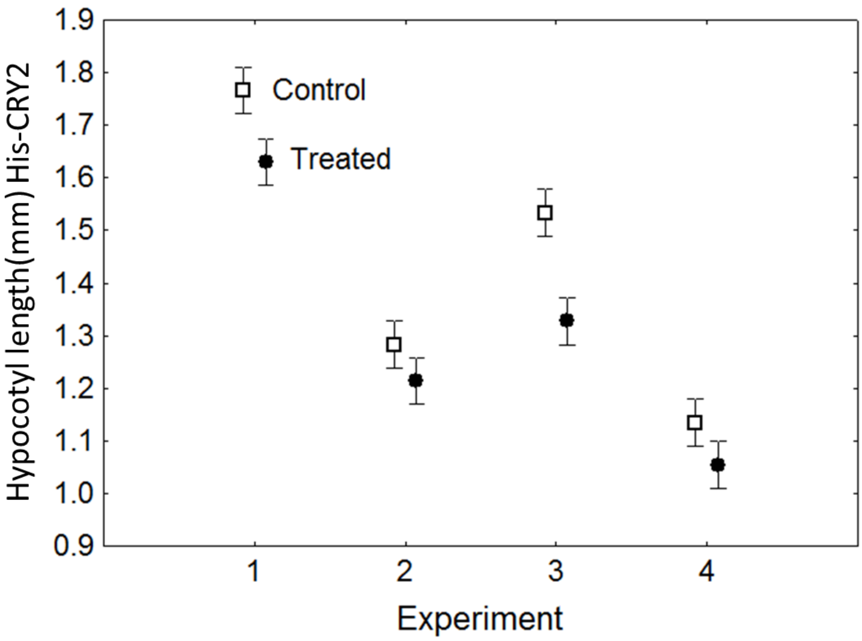

Figure 6. Mean hypocotyl length for *His-CRY2* seedlings in each of four experiments. The variation across experiments was due to different intensities and frequencies of light used in each study.

Anthocyanin

The combined main effects for anthocyanin are shown in Figs 7 and 8. There were a combined 15 × 3 × 4 = 180 seedlings in the control condition and 180 seedlings in the treated condition for each of the three *cry* types.

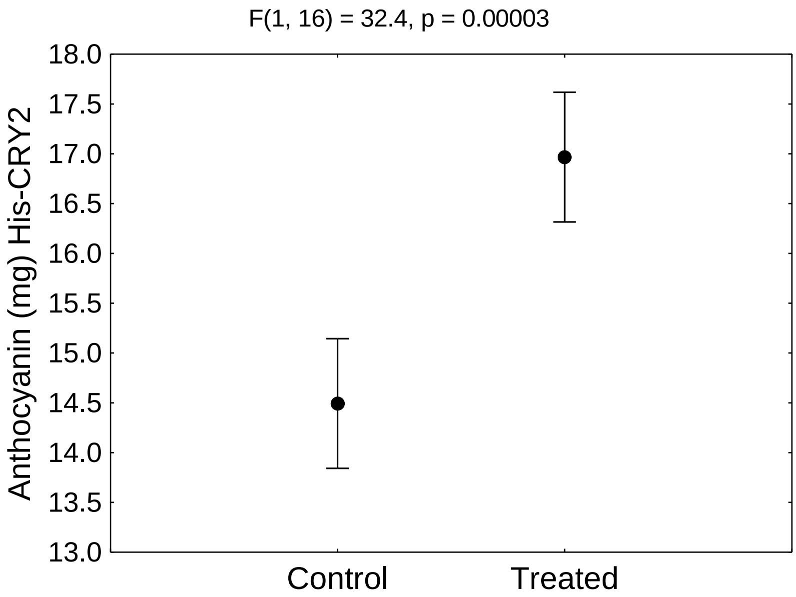

Figure 7. Grand mean anthocyanin content for *His-CRY2* seedlings.

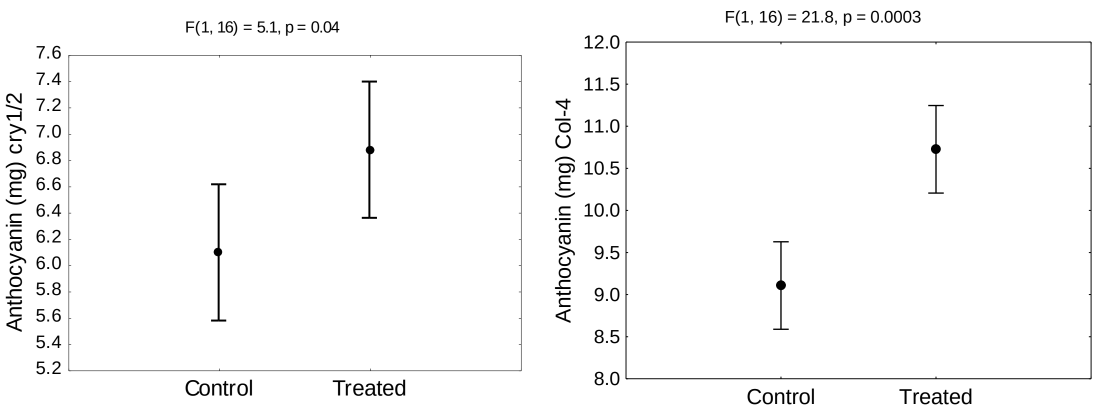

Figure 8. (Left) Mean anthocyanin content for *cry1/2*. (Right) Same for Col-4.

Chlorophyll

The combined main effects for chlorophyll content are shown in Figs 9 and 10. There were a combined 15 × 3 × 4 = 180 seedlings in the control condition and 180 in the treated condition for each of the three *cry* conditions.

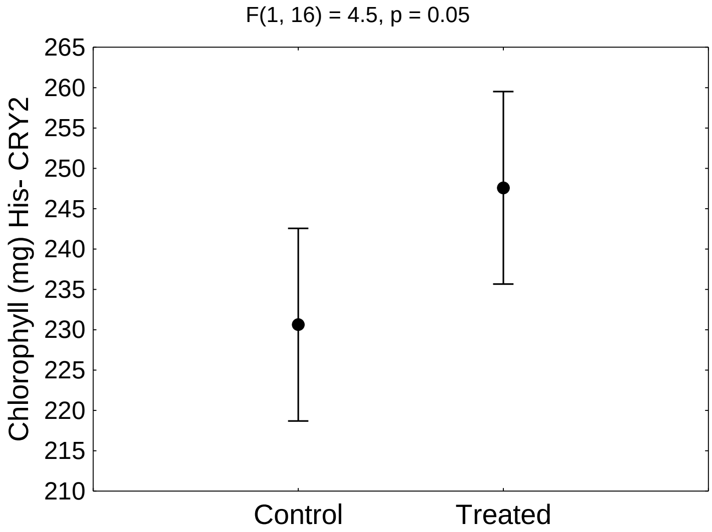

Figure 9. Grand mean chlorophyll content for *His-CRY2* seedlings.

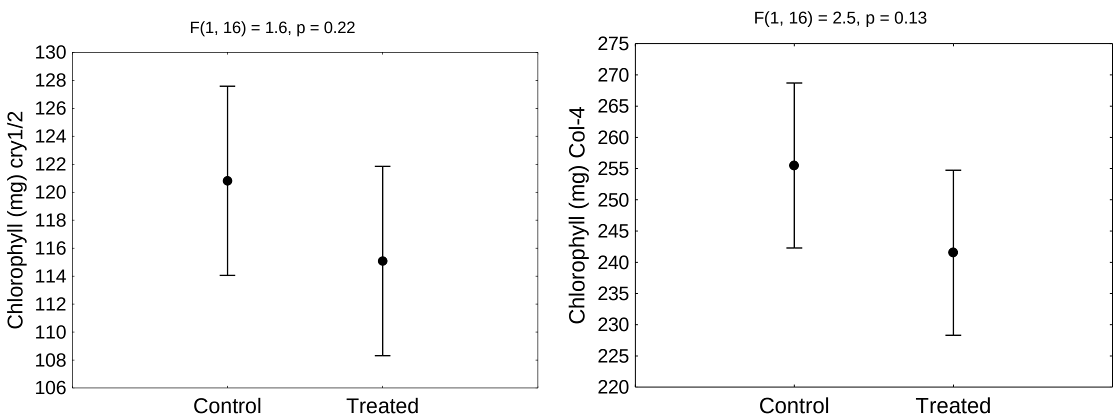

Figure 10. (Left) Mean chlorophyll content for *cry1/2*. (Right) Same for Col-4.

Discussion

The hypothesis that intentionally treated water would cause seeds to "manifest optimal growth" was confirmed, primarily in plants with the *His-CRY2* mutation. This outcome was repeatedly observed under double-blind conditions in each of four separate experiments, as shown in Fig 4. The grand mean difference in hypocotyl length with the *His-CRY2* mutation was associated with an effect size $\eta^2 = 0.63$, considered a "large" effect size and indicating that 63% of the measurement variance was attributable to the treatment.

Alternative Interpretations

In considering these results, it is important to consider conventional explanations that might have accounted for the observed outcomes. The most obvious source of bias would have been if the investigator responsible for handling the seeds knew which source of water had been treated vs. untreated. That knowledge might have biased the placement of the seeds in the incubator, selection of seedlings to be measured, or the measurements themselves. This possibility was prevented by keeping that person (the third author) blind to the water condition throughout the experiments.

A second source of bias could have been the analyst, who might have tried different analytical procedures to find one that was most favorable to the hypothesized outcome. This was prevented by keeping the analyst (the fourth author) blind to the water conditions until after all analyses were completed. To check the analysis, a second statistician – also blind to the water conditions – was later asked to independently examine the data; the same results were obtained. A straightforward two-factor ANOVA was used on the raw data in both cases.

Speculative Model

Given that the experimental protocol excluded mundane biases, the results appear to objectively confirm what was previously observed only in subjective terms [1,2]. This study thus justifies continued use of *Arabidopsis* as a model plant system, especially because of the finding that a particular cryptochrome mutation (*His-CRY2*) appears to be an unusually robust detector of intentional effects.

What is especially novel about the present results, as compared to previous proof-oriented experiments exploring intentional influences, is that it provides a potential mechanism that may account for these effects [16]. The model offered is clearly speculative and incomplete, but it may prove to be useful in designing a framework for understanding how these effects occur. It consists of five parts:

(1) John von Neumann's analysis of the quantum measurement problem (i.e. that quanta behave differently when observed than when unobserved) proposes a special role for consciousness in the act of quantum measurement [21]. This is sometimes discussed in terms of observation "collapsing" the quantum wavefunction [22]. Von Neumann's proposed effect has been observed most directly in experiments involving observed versus unobserved interference patterns in double-slit optical systems [23,24], but there is a large additional literature reporting studies using quantum-based random number generators (RNG) that offer further support for von Neumann's idea [25-27].

(2) The above experiments suggest that focused attention in effect "steers" the behavior of quanta in a manner resembling the quantum Zeno effect [28]. That is, the act of observation can either freeze the evolution of a quantum system or accelerate it, depending on the form of observation. This suggests that observational effects on physical systems are active influences. And that in turn implies that *intention* may play a role in the behavior of a quantum system.

(3) Water, like all physical systems, has quantum properties [29,30], and other experiments suggest that various properties of water may be influenced by the application of focused attention [4,5].

(4) Cryptochrome, found in all living plant and animal systems studied so far, is suspected to have quantum properties responsible, among other things, for its exquisite sensitivity to magnetic fields. E.g., some believe that magnetonavigation in birds may be due to radical pairs of electronics within cryptochrome [18,31,32].

(5) “Entanglement swapping” is a means by which quantum properties in one system (e.g., water) may be shared with another system (e.g., a plant) [33].

Connecting the dots among these speculations allows us to form a half-baked model whereby (a) the focused attention and intentions of the monks influenced a property of water, which (b) was swapped with the quantum properties of the cryptochrome, which (c) expressed in different ways according to the specific form of CRY, which in turn (d) manifested in various ways in the seedlings. Besides the main effect of enhanced photomorphogenic growth, additional support for this model was observed in the way that the various forms of CRY appeared to modulate the effect of intention in hypocotyl and chlorophyll measurements. That is, in the gain-of-function *His-CRY2* seeds a robust enhancement in hypocotyl was observed, but in the loss-of-function *cry1/2* seeds a small but *opposite* effect was observed. Likewise, *His-CRY2* seeds showed an enhancement of chlorophyll while *cry1/2* seeds showed a reduction.

Given the speculative nature of the underlying mechanisms, no conclusions may be drawn at this stage. However, if future replications continue to rigorously support the finding that intentional treatments influence seed growth, then we trust that we will eventually learn if a cryptochrome model is on the right or wrong track.

Acknowledgements

We thank Professor Chen-Tao Lin, Department of Molecular, Cell, and Developmental Biology of the University of California, Los Angeles, for providing the *His-CRY2* seeds. We also thank Lukas Lasota for independently double-checking the statistical analyses. This study was generously supported by the Bial Foundation (Fellowship #287/14). All authors conceived and designed the experiments and contributed to writing the paper. The first three authors performed the experiments, the third author contributed the reagents, materials and analysis tools, and the last author analyzed the data.

REFERENCES

1. Radin D, Hayssen G, Walsh J. Effects of intentionally enhanced chocolate on mood. *Explore (NY).* 2007;3(5):485-492.
2. Shiah YJ, Radin D. Metaphysics of the tea ceremony: a randomized trial investigating the roles of intention and belief on mood while drinking tea. *Explore-J Sci. Heal.* 2013;9(6):355-360.
3. McNair DM, Loor M, Droppleman LF. *Profile of Mood States.* San Diego, California: Multi-Health Systems, Inc; 1992.
4. Radin D, Hayssen G, Emoto M, Kizu T. Double-blind test of the effects of distant intention on water crystal formation. *Explore-J Sci. Heal.* 2006;2(5):408-411.
5. Radin D, Lund N, Emoto M, Kizu T. Effects of distant intention on water crystal formation: A triple-blind replication. *Journal of Scientific Exploration.* 2009;22(4):481-493.
6. StephanA.Schwartz, J.DeMattei R, Jr. EGB, Spottiswoode SJP. Infrared spectra alternation in water proximate to the palms of therpeutic practitioners *Explore (NY).* 2015;11(2):143-115.
7. Tiller W, Dibble W, Shealy C, Nunley R. Toward general experimentation and discovery in conditioned laboratory spaces: part II. pH-change experience at four remote sites, 1 year later. *J Altern Complement Med.* 2004;10:306-310.
8. Dibble WE, Tiller WA. Electronic device-mediated pH changes in water. *Journal of Scientific Exploration.* 1999;13:155-176.
9. Grad B. A telekinetic effect on plant growth. *International Journal of Parapsychology.* 1963;5:117-133.
10. Saklani A. Follow-up studies of PK effects on plant growth. *Journal of the Society for Psychical Research.* 1992;58:258-265.
11. Munson RJ. The effects of PK on rye seeds. *J Parapsychol.* 1979;43:43.
12. Roney-Dougal S, Solfvin J. Field study of enhancement effect on lettuce seeds: their germination rate, growth and health. *Journal of the Society for Psychical Research.* 2004;66:129-142.
13. Jones AM, Chory J, Dangl JL, et al. The impact of Arabidopsis on human health: diversifying our portfolio. *Cell.* 2008;133(6):939-943.
14. Ahmad M, Cashmore AR. Seeing blue: the discovery of cryptochrome. *Plant*

Mol Biol. 1996;30(5):851-861.

15. Barinaga M. Clock photoreceptor shared by plants and animals. *Science.* 1998;282(5394):1628-1630.
16. Shiah Y-J. A possible mechanism for ESP at the initial perceptual stage. *J Parapsychol.* 2012;76(1):147-159.
17. Maeda K, Robinson AJ, Henbest KB, et al. Magnetically sensitive light-induced reactions in cryptochrome are consistent with its proposed role as a magnetoreceptor. *Proc Natl Acad Sci U S A.* 2012;109(13):4774-4779.
18. Maeda K, Henbest KB, Cintolesi F, et al. Chemical compass model of avian magnetoreception. *Nature.* 2008;453(7193):387-U338.
19. Porra RJ, Thompson WA, Kriedemann PE. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta (BBA) - Bioenergetics.* 1989;975(3):384-394.
20. Chory J. A genetic model for light-regulated seedling Arabidopsis. *Development.* 1992;115(1):337-354.
21. von Neumann J. *Mathematical Foundations of Quantum Mechanics.* Princeton, NJ: Princeton University Press; 1955.
22. Thaheld FH. Does consciousness really collapse the wave function? a possible objective biophysical resolution of the measurement problem. *Biosystems.* 2005;81(2):113-124.
23. Radin D, Michel L, Delorme A. Psychophysical modulation of fringe visibility in a distant double-slit optical system. *Phys Essays.* 2016;29(1):14-22.
24. Radin D, Michel L, Pierce A, Delorme A. Psychophysical interactions with a single-photon double-slit optical system. *Quantum Biosystems.* 2015;6(1):82-98.
25. Bosch H, Steinkamp F, Boller E. Examining psychokinesis: the interaction of human intention with random number generators - a meta-analysis. *Psychol Bull.* 2006;132(4):497.
26. Radin D, Nelson R, Dobyns Y, Houtkooper J. Reexamining psychokinesis: Commentary on the Bösch, Steinkamp and Boller meta-analysis. *Psychol Bull.* 2006:529.

27. Radin D, Nelson R. Evidence for consciousness-related anomalies in random physical systems. *Found Physics.* 1989;19:1499-1514.
28. Fischer MC, Gutierrez-Medina B, Raizen MG. Observation of the quantum zeno and anti-zeno effects in an unstable system. *Phys Rev Lett.* 2001;87(4):040402.
29. Jiang B, Song H, Yang M, Guo H. Quantum dynamics of water dissociative chemisorption on rigid Ni(111): an approximate nine-dimensional treatment. *J Chem Phys.* 2016;144(16):164706.
30. Cheng B, Behler J, Ceriotti M. Nuclear quantum effects in water at the triple point: using theory as a link between experiments. *J Phys Chem Lett.* 2016:2210-2215.
31. Gegear RJ, Foley LE, Casselman A, Reppert SM. Animal cryptochromes mediate magnetoreception by an unconventional photochemical mechanism. *Nature.* 2010;463(7282):804-U114.
32. Solov'yov IA, Domratcheva T, Schulten K. Separation of photo-induced radical pair in cryptochrome to a functionally critical distance. *Sci Rep.* 2014;4:3845.
33. Ma X-S, Zotter S, Kofler J, et al. Experimental delayed-choice entanglement swapping. *Nat Phys.* 2012;8(6):479-484.