ORIGINAL RESEARCH

Effects of Intentionally Treated Water on Growth of *Arabidopsis thaliana* Seeds with Cryptochrome Mutations

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Objective: A previous experiment suggested that consumption of intentionally treated tea influenced subjective mood under double-blind, controlled conditions. To investigate that effect objectively, again under double-blind, controlled conditions, 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 focused their intention on commercially bottled water with the goal of improving the growth of seeds; bottled water from the same source served as an untreated control. Seeds with the following three variations of cryptochrome (CRY) were used: the wild type *Arabidopsis* (Columbia-4), a gain-of-function mutation (*His-CRY2*), and a loss-of function mutation (*cry1/2*), where "gain" and "loss" refer to enhanced and reduced sensitivity to blue light, respectively. Seeds were hydrated with treated or untreated water under blinded conditions, and then placed in random positions in an incubator. The germination process was repeated three times in each experiment, each time using new seeds, and then the entire experiment was repeated four times.

Results: Data combined across the four experiments 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, $P < 10^{-13}$), 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, $P < 10^{-4}$), and a modest increase in chlorophyll in *His-CRY2* (treated mean 247.6 ± 5.63 mg, untreated mean 230.6 ± 5.63 mg, $P = .05$). These outcomes conformed to the monks' intentions because a decrease in hypocotyl length and increase in anthocyanin and chlorophyll are associated with enhanced photomorphogenic growth. These experiments suggest that the *His-CRY2* mutation of *Arabidopsis* may be an especially robust "detector" of intention.

Keywords: cryptochrome, intention, mind–matter interaction

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INTRODUCTION

Rituals involving the blessing of water, wine, or bread are common in religious contexts, as are the secular practices of toasting and saluting with food or drink. These customs are so familiar that beyond their purposes in prayer, celebration, and social bonding, other effects associated with the intentional aspects of these rituals may go unnoticed.

To explore the possibility that intention plays a role in what might called "ingesting rituals," in previous double-blind placebo-controlled experiments we tested whether intentionally treated chocolate in one case,[1] and oolong tea in a second case,[2] enhanced participants' subjective mood. Experienced Buddhist monks in the United States and a visiting Mongolian shaman were invited to provide the intentional treatments in the first study, and Buddhist monks in Taiwan provided it in the second study. Both experiments showed statistically significant changes in participants' mood using validated mood-reporting questionnaires,[3] suggesting that under double-bound conditions the act of intention alone affected subjective moods.

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.

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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 most of the 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 variations of CRY act either as photoreceptors or as transcription regulators; they are known as CRY 1, 2, and 3. These proteins play key roles in photomorphogenesis, circadian clocks, flowering time, seed germination, etc.[15] Shiah[16] proposed that CRY might be a possible "transducer" of intention because it is present in all living systems and it is suspected to have quantum biological properties.[17,18] Among other things, these quantum effects are thought to account for the exquisite sensitivity to magnetic fields in living organisms, as observed for example in bird magnetonavigation.[17,18] In light of experiments indicating a relationship between consciousness and the behavior of photons in double-slit optical systems,[19,20] the potential quantum biological properties of *Arabidopsis* made it an interesting system for exploring intentional effects.

MATERIALS AND METHODS

Intentional Treatment Procedure and Blinding Procedures

The water used to hydrate the *Arabidopsis* was from Vedan, a commercial water bottling plant in Taiwan. A case of 24 bottles was purchased by a Research Assistant (RA), and that water was used for all of the experiments described here. The RA brought the bottles to the Bliss and Wisdom Buddhist Foundation (BWBF) in Taiwan, where the first author randomly assigned and labeled the bottles 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 RA then took the labeled bottles to a separate room in the BWBF, where the intentional treatment was provided by Master Lu Cheng, a respected monk and Director of the BWBF, along with two other senior monks from the same Foundation. All three monks 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 this intention toward one set of bottles of water (they collectively selected the B group) for 20 minutes. To avoid inadvertently influencing the bottles of control water (the A group), which were 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 (B group).

During this procedure only the RA who purchased the bottles and the three monks were present, and none of those individuals were involved in any other aspect of the experiment. At this stage of the experiment, none of the authors of this study were aware of the conditions assigned to bottles in group A or B.

After the intentional treatment, RA noted the assignment of the conditions on a piece of paper, sealed it in an envelope, then collected all 24 bottles and brought them to the first author. He in turn brought them to the third author, who handled all of the watering, seed germination, and measurement procedures. After all measurements were completed and analyzed by the fourth author, the first author contacted RA to learn the conditions assigned to the bottles.

Seed Preparation and General Design

Three types of *A. 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*, where "gain" indicates enhanced sensitivity to blue light. All seeds were surface sterilized with 20% bleach (containing 0.5% Tween-20), planted in a greenhouse, and then the plants were allowed to grow to maturity to provide a sufficient number of additional seeds for the experiment. In two experiments, other 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 and 30–40 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 the following four lighting conditions: continuous blue light (cbl) at 5 $\mu mol/m^2s$, 5 $\mu mol/m^2s$ cbl plus 5 $\mu mol/m^2s$ continuous far-red light (cfrl), 10 $\mu mol/m^2s$ cBL, and 10 $\mu mol/m^2s$ cBL plus 5 $\mu mol/m^2s$ cfrl. The seeds were allowed to grow for three days. The protocol is illustrated in Figure 1.

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

In each experiment three analyses were conducted: amount of chlorophyll (green flavonoid pigments) and 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

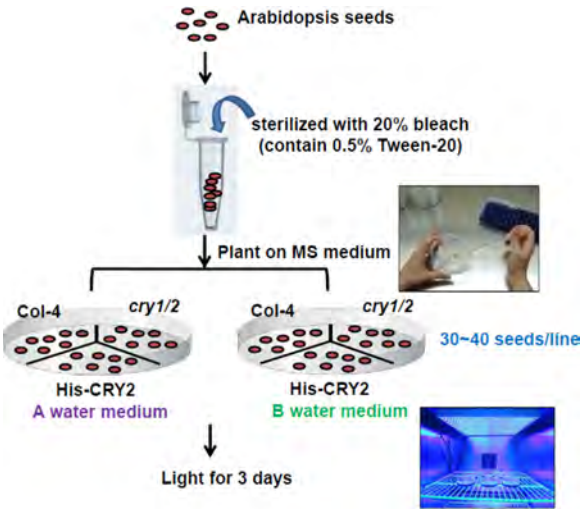

Figure 1. Protocol for growing *Arabidopsis* seeds.

health, and a shorter hypocotyl length is associated with improved seed 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 (as illustrated in Figure 2).

Chlorophyll Extraction and Quantification

Each plate was sown with 30–40 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 (Figure 3). One experiment consisted of 15 newly harvested seedlings germinated seedlings (combined to form a single measure of chlorophyll) × 3 types of *Arabidopsis* variations × a lighting condition (cbl or cbl plus cfrl) × 2 water conditions (treated or untreated) = 180 seedlings. This process was repeated three times in each experiment, thus a total of 180 × 3 = 540 newly harvested seedlings were used. This procedure was repeated four times, thus a total of 2160 seedlings were involved in the measurement of chlorophyll.

Each group of 15 harvested seedlings were extracted in 200 µl dimethylformamide (DMF) for 16 hours at 4°C and vigorously mixed in darkness. A 50 µl of extracts from the combined mixture were added to 450 µl of pure alcohol, mixed, and then 200 µl of the resulting liquid was used for

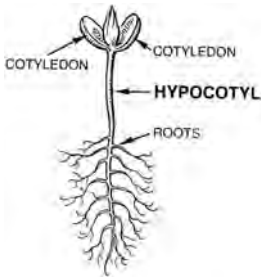

Figure 2. Anatomy of a seedling.

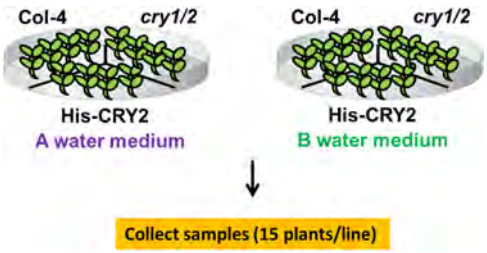

Figure 3. Protocol for analyzing chlorophyll in one experiment.

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, and total chlorophyll content was determined according to the standard extinction coefficient calculated by Porra et al.[21]

Anthocyanin Extraction and Quantification

For anthocyanin measurements, 15 newly harvested seedlings were ground together in liquid nitrogen, and total plant pigment from the combined mixture 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.[22]

Measurement of Hypocotyl Length

For hypocotyl length, 25 newly germinated seeds were analyzed in each of the three replications per experiment. The germinated seedlings were each placed horizontally on agar plates and a photo taken with a Canon digital camera. Hypocotyl length in each image was then measured using 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 plus cfrl). Each of four experiments thus included 600 seedlings. This process was repeated three times in each experiment, for a total of 1800 seedlings.

Because this study was a new design, we did not have effect size estimates to plan the sample size in advance. But given the promising results of the 2 previous studies that used subjective mood as measures, we anticipated that the present study would have sufficient statistical power to test the hypotheses of interest.

RESULTS

Hypocotyl

Data from the four experiments were analyzed with a two-factor analysis of variance (ANOVA) (4 levels of *experiment* × 2 levels of *condition*) using Statistica 8.0 (Tulsa, OK). This analysis combined data across the different illumination sources; the effect of different light frequencies and intensity, as well as other CRY mutations, will be described in future publications. The comparison of primary interest here was the main effect for *condition*; the *experiment* × *condition* interaction

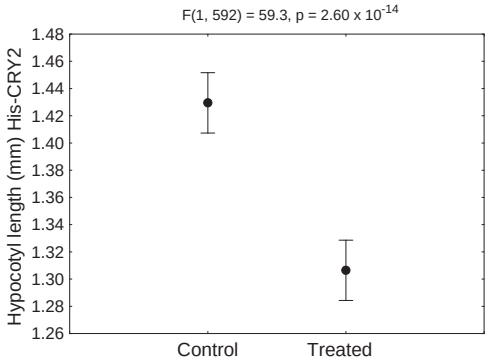

Figure 4. Grand mean hypocotyl length for *His-CRY2* seedlings in control and treated conditions, combined across all four experiments. All error bars in graphs show 95% confidence intervals.

is also shown. Figures 4 and 5 show mean values for hypocotyl length for the three types of seeds, Figure 6 shows means for *His-CRY2* across the four experiments, and the full ANOVA details are provided in Table 1.

Anthocyanin. The combined main effects for anthocyanin are shown in Figures 7 and 8; the ANOVA details are shown in Table 2. This test involved 15 seedlings × 3 repetitions × 4 experiments = 180 seedlings in the control condition and the same number in the treated condition for each of the three CRY types. Note that the anthocyanin measure was the mean value for 15 seeds combined, so the ANOVA consisted of 4 repeated experiments × 3 means/experiment × 2 conditions.

Chlorophyll

The combined main effects for chlorophyll content are shown in Figures 9 and 10 and the ANOVA details in Table 3. 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. And similarly to the anthocyanin measure, the chlorophyll measure was the mean value for 15 seeds combined, so the ANOVA consisted of 4 repeated experiments × 3 means/experiment × 2 conditions.

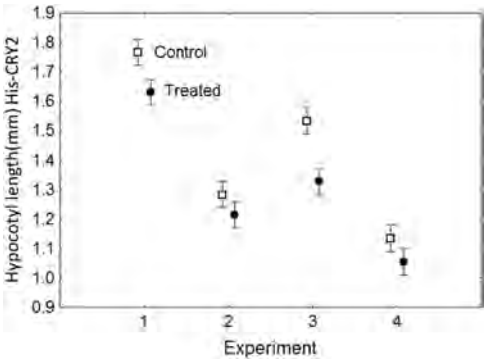

Figure 6. Mean hypocotyl length for *His-CRY2* seedlings in each of the four experiments. $F(3, 592) = 3.86$, $P = .009$.

DISCUSSION

The hypothesis that intentionally treated water would cause seeds to "manifest optimal growth" was confirmed, primarily in plants with the *His-CRY2* mutation. Similar outcomes were observed under double-blind conditions in each of four separate experiments, as shown in Figure 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.

Phenotypic responses examined in this study included hypocotyl length, chlorophyll content and anthocyanin accumulation because these phenotypes serve as markers of plant health and metabolic efficiency. Specifically, hypocotyl length is a measure of effectiveness of light signal transduction.[23] Chlorophyll is an essential component for converting light into chemical energy for cellular metabolism.[24] And anthocyanin has an antioxidant role against reactive oxygen species induced by abiotic stress,[25] and it also protects plants from intense light irradiation.[26]

Alternative Interpretations

Could one or more conventional effects have produced the observed outcomes? One possibility is that the investigator responsible for growing the seeds knew which source of water was treated vs. untreated. With such knowledge, she might have been unconsciously biased to place treated seeds in the

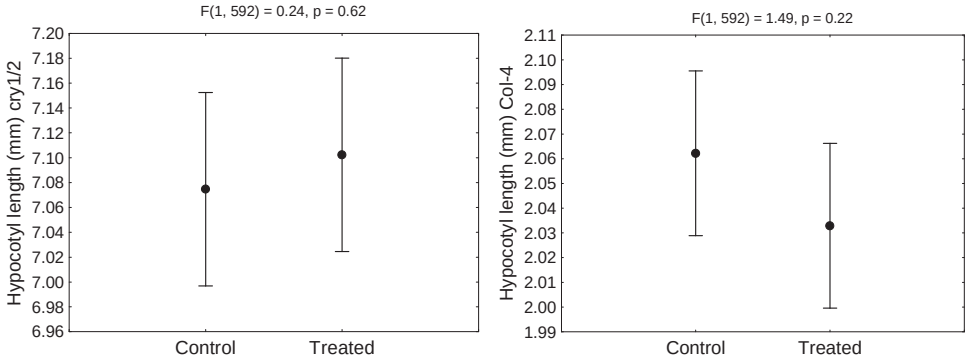

Figure 5. (Left) Mean hypocotyl length for *cry1/2 mutation*. (Right) Same for *Col-4*.

Table 1. Two-Factor ANOVA for Hypocotyl Length

	SS	df	MS	*F*	*P*
His-Cry2					
Intercept	1122	1	1122	29,354	$<10^{-6}$
Experiment	30.3	3	10.1	263	$<10^{-6}$
Condition	2.3	1	2.3	59.3	$<10^{-6}$
Expt × condition	0.4	3	0.1	3.9	.009
Error	22.6	592	0.04		
cry1/2					
Intercept	30,148	1	30,148	64,011	$<10^{-6}$
Experiment	4736	3	1578	3352	$<10^{-6}$
Condition	0.1	1	0.1	0.2	.62
Expt × condition	11.6	3	3.9	8.2	.00002
Error	278.8	592	0.5		
Col-4					
Intercept	2515	1	2515	29,132	$<10^{-6}$
Experiment	158	3	52.8	611	$<10^{-6}$
Condition	0.1	1	0.1	1.5	.22
Expt × condition	0.9	3	0.3	3.7	.01
Error	51.1	592	0.1		

ANOVA, analysis of variance; df, degrees of freedom; MS, mean square; SS, sum-of-squares.

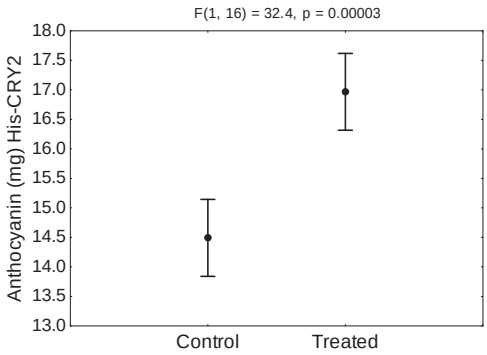

Figure 7. Grand mean anthocyanin content for *His-CRY2* seedlings.

incubator in favorable positions, or to select seedlings to measure when more seeds germinated than were required by the preplanned design. Such biases were avoided by keeping that individual (the third author) blind to the water condition throughout the experiments; this individual also did not know the research assistant or the monks who provided the intentional treatment.

A second source of bias could have been the analyst, who might have tried different analytical procedures to discover 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 double-check the analysis, a second statistician —also blind to the water conditions—was later asked to independently repeat the analysis, and the same results were

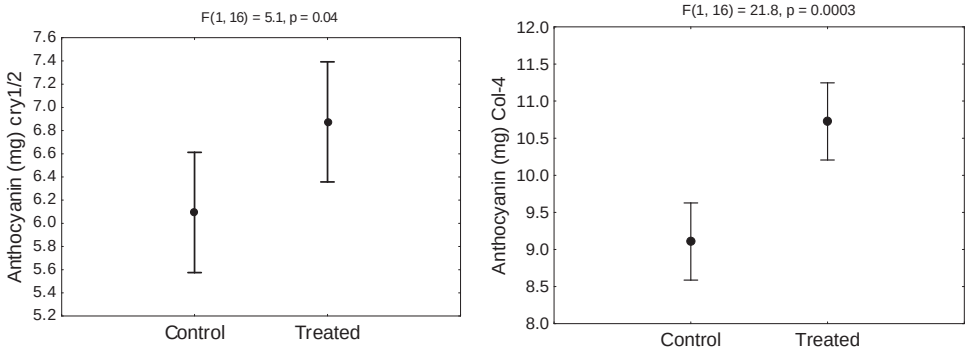

Figure 8. (Left) Mean anthocyanin content for *cry1/2*. (Right) Same for Col-4.

Table 2. Two-Factor ANOVA for Anthocyanin Content

	SS	df	MS	*F*	*P*
His-Cry2					
Intercept	5938	1	5937	5245	$<10^{-6}$
Experiment	1300	3	433	382	$<10^{-6}$
Condition	36.7	1	36.7	32.4	.00003
Expt × condition	48.8	3	16.2	14.4	.00008
Error	18.1	16	1.1		
cry1/2					
Intercept	1009	1	1009	1406	$<10^{-6}$
Experiment	132	3	44.2	61.5	$<10^{-6}$
Condition	3.6	1	3.6	5.1	.04
Expt × condition	5.5	3	1.8	2.5	.09
Error	11.4	16	0.7		
Col-4					
Intercept	2360	1	2360	3272	$<10^{-6}$
Experiment	372	3	124	171	$<10^{-6}$
Condition	15.7	1	15.7	21.7	.0003
Expt × condition	7.8	3	2.6	3.6	.04
Error	11.5	16	0.7		

ANOVA, analysis of variance; df, degrees of freedom; MS, mean square; SS, sum-of-squares.

obtained. A straightforward two-factor ANOVA was used in both cases.

A third possibility is that the water in the bottles differed in some way and the random assignment into the two conditions systematically matched those potential differences. We considered this unlikely, but in hindsight we might have conducted chemical analyses of the water before it was used, or alternatively we could have filled the bottles from a single source. In the present experiments, all of the water from the 24 bottles was used to hydrate the seeds, so we could not test it after the fact. One or both of these protocol additions would be worthwhile enhancements to consider in conducting future studies of this type.

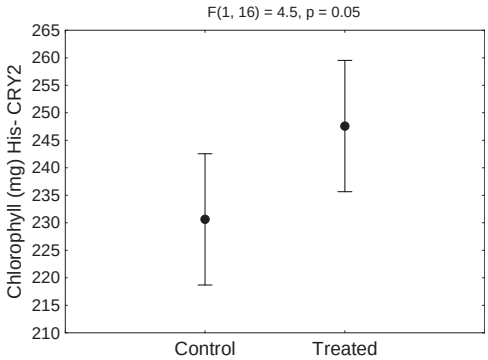

Figure 9. Grand mean chlorophyll content for *His-CRY2* seedlings.

Speculative Model

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

Compared to the small magnitude effects typically obtained in experiments studying intentional influences on physical systems, the present study suggests that elementary living systems with quantum-biological properties may be especially responsive targets.[16] If future studies continue to replicate the results of the present experiments, then quantum-inspired models may be useful guides in developing hypotheses for understanding and testing the role of intention in the physical world.

Acknowledgements

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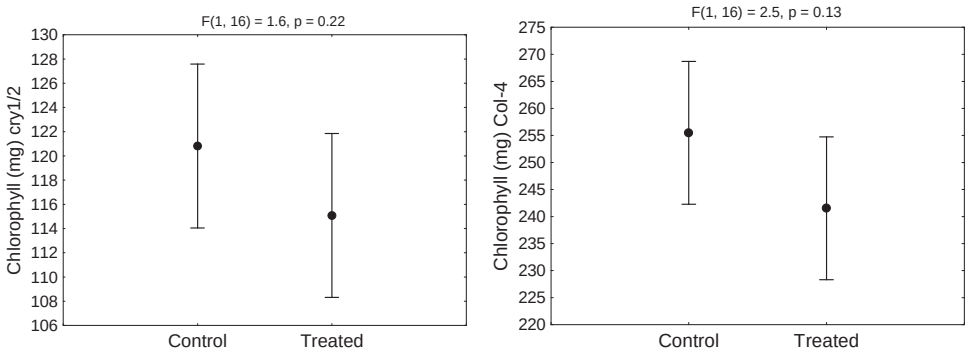

Figure 10. (Left) Mean chlorophyll content for *cry1/2*. (Right) Same for Col-4.

Table 3. Two-Factor ANOVA for Chlorophyll Content

	SS	df	MS	*F*	*P*
His-Cry2					
Intercept	1,372,158	1	1,372,158	3609	$<10^{-6}$
Experiment	7039	3	2346	6.2	.005
Condition	1726	1	1726	4.5	.05
Expt × condition	2240	3	747	1.9	.22
Error	6082	16	380		
cry1/2					
Intercept	3,33,889	1	3,33,889	2731	$<10^{-6}$
Experiment	23,389	3	7796	63	$<10^{-6}$
Condition	197	1	197	1.6	.22
Expt × condition	901	3	300	2.4	.10
Error	1955	16	233		
Col-4					
Intercept	1,482,140	1	1,482,140	3181	$<10^{-6}$
Experiment	12,997	3	4332	9.3	.0009
Condition	1169	1	1169	2.5	.13
Expt × condition	758	3	253	0.5	.66
Error	7455	16	466		

ANOVA, analysis of variance; df, degrees of freedom; MS, mean square; SS, sum-of-squares.

reagents, materials and analysis tools, and the last author analyzed the data.

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