Data Supplement for Telch et al., Post-Session Administration of USP Methylene Blue Facilitates the Retention of Pathological Fear Extinction and Contextual Memory in Phobic Adults.

S1. Supplementary Figures and Tables

FIGURE S1. Subject Flow

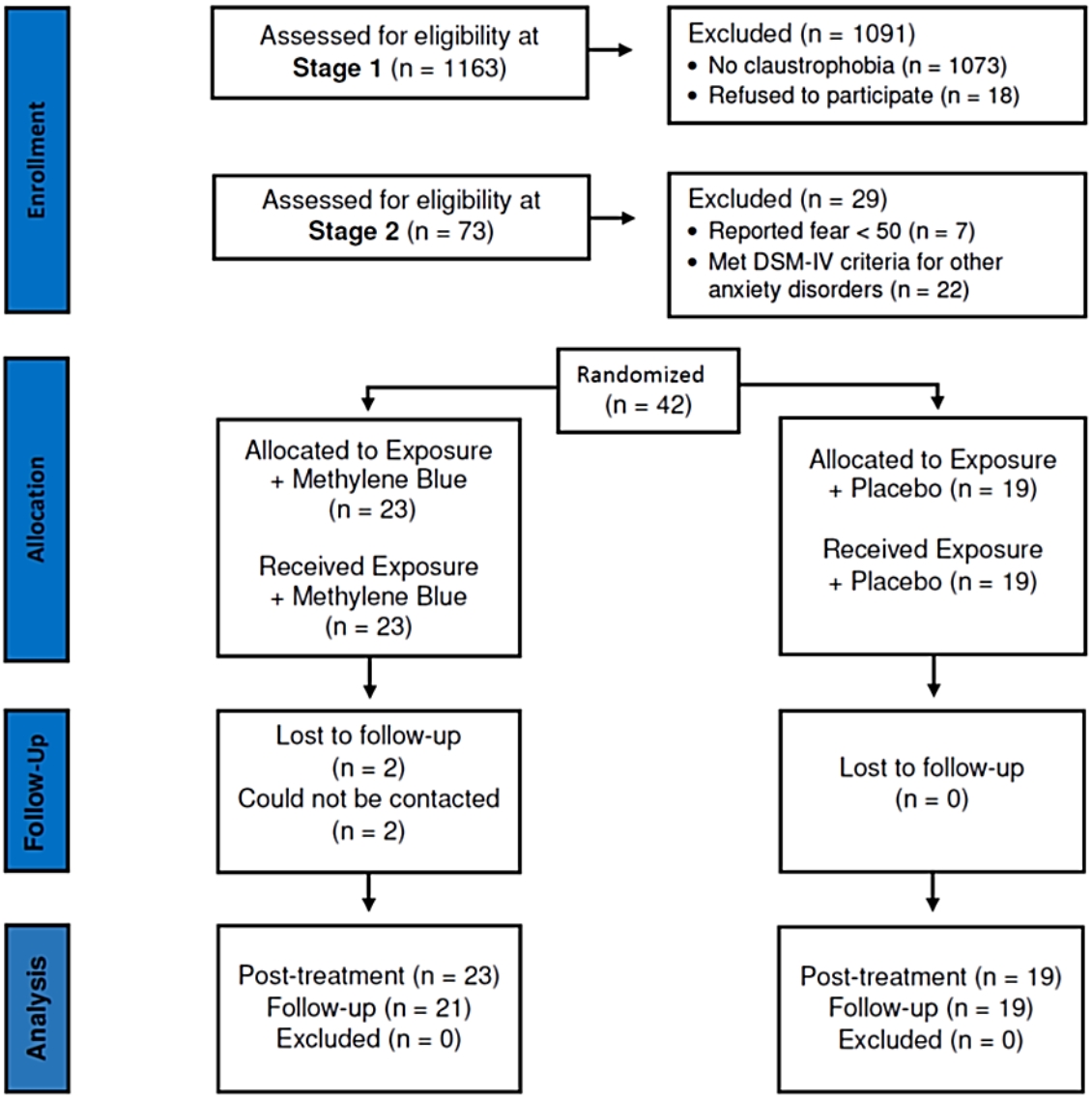

TABLE S1. Clinician and Self-Report Measures by Assessment Time Point [a]

Measure	Pre-training	Post-training	1-Month Follow-Up
Structured Clinical Interview for DSM-IV Axis I Disorders	✓		
Credibility and Expectancy Questionnaire	✓		
Claustrophobia Questionnaire	✓		✓
Claustrophobia Concerns Questionnaire	✓	✓	✓
Behavioral Approach Tasks 1 & 2	✓	✓	✓
Episodic Contextual Memory Test		✓	✓

[a] The Claustrophobia Questionnaire was administered in both the training and generalization contexts. Behavioral Approach Task 1 = training context. Behavioral Approach Task 2 = generalization context.

TABLE S2. Pre-treatment, Post-treatment, and 1-Month Follow-Up Measures [a]

	Methylene Blue + Exposure			Placebo + Exposure		
Pre-treatment	**n**	**M**	**SD**	**n**	**M**	**SD**
Credibility and Expectancy Questionnaire	23	5.65	1.53	19	5.29	0.96
Claustrophobia Questionnaire – Total Score	23	70.91	10.64	19	68.42	12.88
Claustrophobia Questionnaire – Suffocation Subscale	23	31.91	5.46	19	31.79	7.66
Claustrophobia Questionnaire – Restriction Subscale	23	39.00	6.88	19	36.63	7.37
Claustrophobia Concerns Questionnaire – Training	23	78.26	16.71	19	79.47	12.71
Claustrophobia Concerns Questionnaire – Generalization	23	72.50	20.31	19	71.45	14.89
Peak Fear: Behavioral Approach Task – Training	23	77.39	17.38	19	68.95	16.96
Peak Fear: Behavioral Approach Task – Generalization	23	66.09	16.72	19	63.68	15.35
Post-treatment	**n**	**M**	**SD**	**n**	**M**	**SD**
Claustrophobia Concerns Questionnaire – Training	23	32.83	32.03	19	16.84	19.79
Claustrophobia Concerns Questionnaire – Generalization	23	47.93	29.49	19	25.26	21.57
Peak Fear: Behavioral Approach Task – Training	23	23.91	27.59	19	16.84	23.58
Peak Fear: Behavioral Approach Task – Generalization	23	35.22	31.60	19	25.26	22.70
1-Month Follow-up	**n**	**M**	**SD**	**n**	**M**	**SD**
Claustrophobia Questionnaire – Total Score	21	59.43	14.20	19	60.63	13.30
Claustrophobia Questionnaire – Suffocation Subscale	21	28.22	6.46	19	29.16	7.23
Claustrophobia Questionnaire – Restriction Subscale	21	31.22	8.41	19	31.47	6.92
Claustrophobia Concerns Questionnaire – Training	21	35.00	27.21	19	20.26	21.78
Claustrophobia Concerns Questionnaire – Generalization	21	31.55	25.97	19	23.68	22.92
Peak Fear: Behavioral Approach Task – Training	21	27.39	23.01	19	19.47	20.94
Peak Fear: Behavioral Approach Task – Generalization	21	24.35	18.79	19	17.37	20.51

[a] Total score on the Claustrophobic Concerns Questionnaire (55) and peak fear expression during the Behavioral Approach Tasks are reported for both the training and generalization contexts. Total and subscale scores (i.e., Suffocation and Restriction) are reported for the Claustrophobia Questionnaire (54). The Claustrophobia Questionnaire was not administered at post-treatment.

S2. Participant Exclusion Criteria

1. Lifetime history of bipolar disorder, schizophrenia, psychosis, delusional disorders, obsessive-compulsive disorder, post-traumatic stress disorder, or current diagnosis of a substance use disorder or another anxiety disorder (other than specific phobia) as determined by the Structured Clinical Interview for DSM-IV Axis-I Disorders (SCID-I-IV)
2. Current use of psychotropic medication
3. Current treatment for claustrophobia
4. Known hypersensitivity to methylene blue
5. Uncontrolled hypertension manifested by systolic blood pressure >170 or diastolic blood
6. pressure >100 mm Hg
7. History of supraventricular arrhythmia with an uncontrolled ventricular response (mean heart rate >100 bpm at rest) or history of spontaneous or induced sustained ventricular tachycardia (heart rate >100 bpm for >30 sec), or history of congestive heart failure;
8. Current suicidality; (h) history of severe renal impairment
9. Glucose-6-phosphate dehydrogenase deficiency
10. Methemoglobinemia
11. Body weight over 250 lbs.
12. Current pregnancy or breast feeding.

S3. Additional Information on the Behavioral Approach Tests

Two behavioral approach tests were performed at each of the three assessment points (pre-extinction training, post-extinction training, and one-month follow-up). These tests were procedurally identical but used different stimuli (claustrophobia chambers), both of which were located in a darkened room in our laboratory. The two chambers were wooden boxes measuring 41 cm. x 76 cm. x 180 cm. The fear extinction training chamber and the generalization assessment chamber were intentionally designed to differ from one another on several sensory dimensions in order to enhance the distinctiveness of the two assessment contexts (i.e., training vs. generalization).

Instructions and procedures for both behavioral approach tests were identical. Participants were shown the respective chamber and instructed to enter with the expectation that they should remain inside with the door locked until the experimenter unlocked the door and prompted them to exit. However, they were informed that they could exit the chamber at any time by knocking on the door, at which point, the experimenter would unlock the door to let them exit. Prior to entering the chamber, participants completed ratings of expected fear, perceived coping self-efficacy, and suffocation and entrapment concerns each on a 0 to 100 scale ranging from (not at all) to100 (extreme). Upon exiting, participants provided their peak fear rating using a similar scale (0= no fear; 50 = moderate fear; 100 = extreme fear or panic. Our primary outcome measure for determining the clinical efficacy of post-session methylene blue administration was participants' fear responding one month later when placed inside the non-trained claustrophobia chamber (i.e., generalization context). Because extinction training took place in different chamber, fear responding in the non-trained chamber represents a reasonable index of the generalization of extinction training to a new context.

S4. Measures

In Vivo Fear Responding to Behavioral Approach Tests. Two behavioral approach tests were performed at each of the three assessment points (pre-extinction training, post-extinction training, and one-month follow-up). These tests were procedurally identical but used different stimuli (claustrophobia chambers), both of which were located in a darkened room in our laboratory. As reported elsewhere, participants' fear responding one month later when placed inside the non-trained claustrophobia chamber (i.e., generalization context) served as the primary index of clinical efficacy. More detailed information on the behavioral approach tests are provided in S3 of this online data supplement.

Assessment of Fear Extinction. Every 5-min. during extinction training, participants rated their peak fear on the same 0 to 100 scale used during the BATs. Consistent with our previous research (33), fear ratings obtained at the conclusion of the final exposure trial served as the primary index of fear extinction, with lower ratings indicating greater within-session extinction learning success and higher ratings indicating less extinction learning success.

Assessment of Episodic Contextual Memory. Inside the extinction training chamber secured at each corner of the inner upper surface of the door were four 2 in. single-digit glow-in-the-dark numbers positioned in direct sight of the participant as they lay on their back inside the chamber. These numbers and their locations served as the target stimuli for our context memory test. Memory encoding of the numbers was incidental as no instructions were provided to participants to attend to the numbers, nor did the experimenter make reference to them during extinction training. One and 30 days after completing extinction training, participants were provided a sheet of paper with a proportionally equivalent outline of the chamber and were asked to recall and record the numbers in their correct locations. The number of correct responses, defined as the sum of correctly recalled numbers in their correct locations, served as the primary index of contextual memory. Similar tasks have been used to investigate contextual memory deficits in depression {Correa:2012ii}, dyslexia {Vicari:2005cc}, and Williams syndrome {Vicari:1999eo}.

Structured Clinical Interview for DSM-IV Axis I Disorders. The **Structured Clinical Interview for DSM-IV Axis I Disorders** is a widely used structured diagnostic interview for assessing the presence of Axis I disorders {First:1994vt}. This interview was conducted at pre-treatment by a trained post-doctoral clinician as the means of assessing whether subjects (a) met diagnosis for specific phobia of enclosed spaces (yes vs. no), and (b) met criteria for comorbid Axis I disorders (yes vs. no). Reports of this instruments psychometric properties indicate inter-rater reliabilities across diagnoses ranging from acceptable to very good {Lobbestael:2011kf} and comparable performance among experienced and neophyte assessors {Ventura:1998vy}. Test-retest reliability for the DSM-III-R version of this instrument range from fair to excellent {Williams:1992ws}.

Claustrophobia Questionnaire. Claustrophobia severity was measured using the Claustrophobia Questionnaire {Radomsky:2001tj} at pre-treatment and at the 1-month follow-up. Items are rated on a 0 (not at all anxious) to 4 (extremely anxious) Likert scale. In addition to a total score, this instrument yields two subscales, including (a) suffocation and (b) restriction fear. This measure has demonstrated good predictive and discriminant validity, as well as good internal consistency and test-retest reliability.

Claustrophobia Concerns Questionnaire. The Claustrophobia Concerns Questionnaire {Valentiner:1996ez} is an empirically-derived measure of danger appraisals associated with claustrophobic fear. This self-report measure was administered at pre-treatment, post-treatment, and at the 1-month follow-up. Items (e.g., “I might be trapped”) are rated on a Likert scale from 0 (no concern) to 100 (extreme concern). This measure has demonstrated high internal consistency and test-retest reliability.

S5. Assessment and Monitoring of Treatment Fidelity

Treatments were conducted by either an advanced clinical psychology graduate student or a post-doctoral neuroscience research fellow. As in our previous published claustrophobia experiments, those delivering the intervention followed a detailed step-by-step manual of each procedural element of this single session in vivo exposure protocol. Specific procedural steps for each of the six 5-min. exposure trials were identical. They included (a) Opening the door of the claustrophobia chamber and instructing subjects to look inside and then complete brief rating forms of their anticipated reactions prior to entering the chamber; (b) Assisting the subject into the chamber and instructing them to lay on their back with eyes facing up towards the chamber door; (c) Closing the door of the chamber and starting a 5-min. timer; (d) Opening the door and assisting them in exiting the chamber; (e) Providing subjects with instructions and paper forms for collecting post-trial peak fear ratings (i.e., Subjective Units of Distress). Given the straightforward nature of the exposure procedures, formal fidelity data were not collected.

S6. Additional Study Limitations

Given that this was a tightly controlled experiment, employing a sample likely not representative of true clinical populations, the real-world clinical significance of our findings are unclear. Furthermore, the use of a limited follow-up period does not allow strong inferences as to whether the observed effects are robust across time. However, our aim in this translational analog treatment experiment was to replicate in humans, findings observed in rodents demonstrating that methylene blue enhances fear extinction and spatial memory. To accomplish this aim, we chose a population displaying marked pathological fear in a circumscribed domain (i.e. claustrophobia), a well-established single session extinction training protocol, and widely used indices of fear attenuation that most closely resemble the fear extinction research in rodents (i.e., fear responding in the presence of a conditioned stimulus). Considering these aims and the translational nature of this investigation, we contend that the question of clinical significance, while an important one, is better addressed using a clinical population and a more traditional randomized controlled trial design.

Another noteworthy limitation of this study is that we did not assess exposure practice between the post-training and 1-month follow-up assessments. Indeed, in an effort to conduct a more stringent test of the efficacy of methylene blue as an exposure enhancement strategy, we did not encourage exposure practice outside the treatment context, nor did we assess whether subjects engaged in self-directed exposure. In our experience treating claustrophobic samples over the last 15 years, it is evident that few subjects engage in self-directed exposure practice outside of therapy sessions unless instructed to do so. However, differential practice effects, albeit unlikely, cannot be ruled out as an alternative explanation for our findings.

We also cannot rule out the possibility that demand characteristics may have been operating, given the different side effect profiles between those administered methylene blue versus placebo. However, given that the medication was administered following treatment, demand characteristics could not have influenced patients' initial gains during exposure therapy. Additionally, the inclusion of food dye to provide similar urine discoloration for the placebo group served to ensure subjects were not aware of what would have been the most readily obvious difference between treatments. However, it is possible that differences in side effect profiles between patients receiving methylene blue and placebo lead to differential expectations, which in turn may have affected subjects' fear responding at the one month follow-up assessment. The fact that there was no significant main effect of methylene blue over placebo makes this confound less likely, but does not rule it out completely. Unfortunately, we did not assess participants' or clinicians' attributions of medication assignment.

S7. Additional Analyses

Moderation of methylene blue's effects by the Claustrophobia Concerns Questionnaire. We conducted exploratory analyses to test the hypothesis that methylene blue might impact claustrophobia-related cognitions as indexed by the Claustrophobia Concerns Questionnaire. Our original hypothesis was that methylene blue's effects would be moderated by what was learned during the exposure treatment as indexed by participants' peak fear at the end of fear extinction training (a hypothesis guided by our recently reported moderator findings with D-cycloserine and yohimbine). Consequently, we would have predicted no main effect of methylene blue on claustrophobic cognitions. To test whether methylene blue had a main effect on claustrophobic concerns, or whether it interacted with end of session scores on this measure as it did with the index of end fear, we performed a multiple regression analysis analogous to the one performed to examine the moderating effects of end fear on methylene blue. In this analysis, claustrophobic concerns at follow-up was predicted by drug condition (methylene blue vs. placebo), claustrophobic concerns at the last exposure trial, and their interaction. To enhance confidence that final scores on this claustrophobia measure itself was responsible for any interactive effects with methylene blue (rather than some other third variables associated with claustrophobic concerns), we followed the suggestions of Steiner et al. (44) and controlled for other relevant variables that may be related to both (a) claustrophobic concerns at the end of training and (b) at follow-up. Those control variables were initial scores on the Claustrophobia Concerns Questionnaire at the first exposure trial, post-exposure scores on this measure in the generalization context, and the presence (at baseline) of other Axis I disorders. As expected, results showed no main effect for methylene blue on claustrophobic concerns. The interaction of methylene blue and claustrophobic concerns at the end of training showed results similar to those found for end fear, but did not attain traditional levels of statistical significance (2-tailed p=.127, 1-tailed p=.064). Participants low on claustrophobic concerns who received methylene blue scored 20 points *lower* on the Claustrophobia Concerns Questionnaire at follow-up than those who received placebo. In contrast, those scoring high on claustrophobic concerns at the end of extinction training who received methylene blue scored 17 points *higher* at follow-up than those receiving placebo.

Post-hoc power analyses indicated we had limited power (β = .66) to detect a medium effect size for this measure.