Published in final edited form as:

Am J Psychiatry. 2014 October ; 171(10): 1091–1098. doi:10.1176/appi.ajp.2014.13101407.

Post-Session Administration of USP Methylene Blue Facilitates the Retention of Pathological Fear Extinction and Contextual Memory in Phobic Adults

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Abstract

Objective—Preclinical studies have shown that low-dose USP methylene blue increases mitochondrial cytochrome oxidase activity in the brain and improves memory retention after learning tasks, including fear extinction. We report on the first controlled experiment to examine the memory-enhancing effects of post-training methylene blue administration on retention of fear extinction and contextual memory following fear extinction training.

Method—Adults (N = 42) displaying marked claustrophobic fear were randomized to double-blind administration of 260 mg of methylene blue versus placebo immediately following six five-minute extinction trials to an enclosed chamber. Retesting occurred one month later to assess fear renewal as indexed by peak fear during exposure to a non-trained enclosed chamber with the prediction that methylene blue's effects would vary as a function of fear reduction achieved during extinction training. Incidental contextual memory was assessed 1 and 30 days after training to assess the cognitive enhancing effects of methylene blue independent of its effects on fear attenuation.

Results—Consistent with predictions, participants displaying low end fear at post-training showed significantly less fear at follow-up if they received methylene blue post-training relative to placebo. In contrast, participants displaying moderate to high levels of post-training fear tended to fare worse at follow-up relative to placebo. Methylene blue's enhancement of contextual memory was unrelated to initial or post-training claustrophobic fear.

Conclusions—Methylene blue enhances memory and the retention of fear extinction when administered after a successful exposure session, but may have a deleterious effect on extinction when administered after an unsuccessful exposure session.

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The authors report no financial relationships with commercial interests.

Keywords

CBT; Cognitive Behavioral Therapy; Exposure therapy; Fear extinction; Methylene Blue; Claustrophobia; Contextual Memory

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Introduction

Over the past four decades, research on exposure therapy has shifted from demonstrating therapeutic efficacy to the study of change mechanisms and augmentation strategies to improve short-term efficacy and reduce return of fear (1) (2). Significant advances in cognitive and behavioral neuroscience have led to a better understanding of fear extinction and have ushered in a new era of “translational” research focusing on the integration of findings from these basic science disciplines to improve extinction-based therapies for anxiety disorders (3, 4).

A recent development in exposure therapy research is the use of memory enhancing pharmacological agents to boost fear extinction during exposure therapy. The most widely studied of these is D-cycloserine, an N-methyl-D-aspartate receptor co-agonist. Based on early preclinical data demonstrating that D-cycloserine enhances fear extinction in rats (5), a series of randomized clinical trials have examined the clinical efficacy of exposure therapy with D-cycloserine augmentation across a range of anxiety disorders [see (6) for a review]. Overall, findings have been mixed with some showing strong augmentation effects (7-10), others showing weak (11-13) or negligible effects (14-16) and one study showing detrimental effects (17).

Another promising pharmacological agent that has received significant support in preclinical studies is methylene blue. Available in every emergency room as an antidote against metabolic poisons, methylene blue has been used safely for well over a hundred years (18). Methylene blue is a diaminophenothiazine drug that at low doses (0.5 - 4 mg/kg) has neurometabolic-enhancing properties (19). Preclinical research with rodents has shown that at low doses, methylene blue is a metabolic and cognitive enhancer that improves brain oxygen consumption, brain glucose uptake, cerebral blood flow, fMRI responses and memory consolidation by induction of cytochrome oxidase, the respiratory enzyme found within nerve cells (19-21). By enhancing cytochrome oxidase activity, methylene blue increases oxygen consumption and amount of ATP available in neurons during memory consolidation. Although methylene blue has the potential to enter any nerve cell, it preferentially accumulates in neurons with higher energy demand, such as those involved in memory consolidation after extinction training (22). Hence, by acting as a mitochondrial electron cyler and antioxidant, low-dose methylene blue increases cellular energy production and support enhanced memory consolidation in key brain regions associated with memory processing (19).

Post-training administration of low methylene blue doses in rodents has been shown to improve memory retention in a variety of tasks, including inhibitory avoidance (23, 24), spatial memory (24-27), discrimination learning (28), and most relevant to the present study, retention of conditioned fear extinction (22, 29). Gonzalez-Lima and colleagues (22, 29)

found that memory retention of extinction after Pavlovian fear conditioning could be improved with the administration of 4 mg/kg methylene blue post-extinction. Moreover, the rate of cytochrome c oxidation in brain homogenates of these animals showed a 38% increase in absolute brain metabolic activity relative to controls administered saline, whereas cytochrome oxidase histochemistry revealed the largest effect on the prefrontal cortex (22), a brain region clearly implicated in fear extinction (30-32).

Based on these preclinical studies, we sought to test whether post-extinction methylene blue administration enhances the retention of pathological fear attenuation in phobic individuals receiving extinction training. Given the recent findings suggesting that the facilitative effects of other cognitive enhancers such as D-cycloserine (33) and yohimbine (34) may depend on the post-training clinical status of the patient, we hypothesized that methylene blue would *enhance* fear extinction retention at follow-up for participants who achieved low levels of fear at post-training. In contrast, we predicted that methylene blue may be less effective than placebo at follow-up for participants who continued to display higher levels of fear at post-training.

A second aim of the experiment was to address a significant limitation of all previous studies of cognitive enhancers and exposure therapy; namely, the lack of indexing cognitive enhancement independent of differences in fear responding. Toward this aim, we administered an episodic contextual memory test, with incidental encoding and free recall, 1 and 30 days after completing extinction training. We predicted that those administered methylene blue would display enhanced memory performance relative to those receiving placebo, irrespective of clinical status at post-training.

Method

Participants

Participants ($n = 42$) reporting marked claustrophobic fear (peak fear > 50 on a 100 - point scale) while performing two consecutive behavioral approach tests and meeting DSM-IV criteria for claustrophobia (excluding the requirement of functional impairment- criterion E) were recruited from a large participant pool of undergraduates ($n = 1163$) through a two-stage screening process. Detailed information on participant exclusion criteria are provided in S1 of the online data supplement.

Randomized participants ranged in age from 18 to 36 ($M = 19.3$) and were predominantly female (82%), White (76.19%) and non-Hispanic (71.43). See Table 1 for a more detailed description of the sample characteristics.

Study Design and Procedures

Potential study participants completed a brief online assessment of claustrophobia (Stage 1). Those reporting marked fear of enclosed spaces were invited to the laboratory for a face-to-face screening visit (Stage 2), which also served as their formal pretreatment assessment. This consisted of: (a) the Structured Clinical Interview for DSM-IV (SCID-IV); (b) completion of self-report patient-rating scales (see Measures); and (c) behavioral approach tests involving exposure to two distinct claustrophobic chambers (see Measures). Eligible

participants were stratified on gender and pretreatment claustrophobia severity and randomized in double-blind fashion to one of two treatment arms: (a) extinction training + post-session methylene blue vs. (b) extinction training + post-session placebo. All extinction training (see below) was completed in one session and was identical in the two treatment arms. Participants provided ratings of peak fear and end fear after each of the six 5-min. exposure trials. Outcome assessments identical to the pretreatment assessment were obtained immediately after extinction training and at a one-month follow-up visit (see Figure S1 in the online data supplement for a graphical depiction of the experimental design and subject flow). All study procedures were approved by the Institutional Review Board at the University of Texas at Austin.

Extinction Training Paradigm

The extinction training paradigm employed in the present study has been used in several published basic studies evaluating the mechanisms of change in exposure therapy (35-38). In brief, the paradigm consists of the following elements: (a) brief education about the nature of claustrophobia; (b) presentation of a therapy rationale emphasizing the fear-reducing effects of direct confrontation with the feared target; (c) six 5-min *in vivo* exposure trials in which the participant enters a tightly enclosed wooden chamber and remains inside in a supine position for a duration of 5 min.; and (d) completion of self-report rating scales after each exposure trial.

Medication

USP-grade methylene blue powder purchased from ScienceLab (Houston, TX) was put into gelatin capsules, which were identical in appearance to the placebo capsules that contained food dye indigo carmine powder (FD&C Blue No. 2, ScienceLab, Houston, TX). Capsules were provided to each participant in a sealed numbered envelope prepared by an unblinded pharmacist at the University of Texas at Austin. All other study personnel were blind to the drug condition. The 260 mg methylene blue dose corresponds to the 4 mg/kg dose shown to be effective in previously published preclinical studies of object recognition memory and fear extinction (22, 24, 29). Methylene blue and placebo were administered using a schedule divided into three 86.66 mg capsules. One capsule was administered immediately following the completion of extinction training. The participants were instructed to take the second capsule before going to bed that night (6-10 hours later), and to take the last capsule after waking up (another 6-10 hours later). The divided dosing schedule was intended to reduce possible urinary tract irritation sometimes associated with methylene blue excretion through the urine. Participants were instructed to take the capsules with a large glass of water, in order to further minimize the chance of urinary tract irritation and to reduce the intensity of urine discoloration. Since the average half-life for urinary excretion of methylene blue is 6.6 hours (39), our administration procedure served to maintain methylene blue in the circulation throughout the entire memory consolidation period following extinction training.

Twenty-four hours after completing extinction training, participants were administered a phone interview to assess medication adherence and side effects.

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 (generalization context) served as the primary index of clinical efficacy. More detailed information on the behavioral approach tests are provided in S3 of the 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 behavioral approach tests. 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 (40), dyslexia (41), and Williams syndrome (42).

Data Analysis

Consistent with recommendations outlined by Kraemer et al. for testing putative moderators in clinical trials (43), we performed a multiple regression analysis in which peak fear at follow-up was predicted by drug condition (methylene blue vs. placebo), end fear (fear at the last exposure trial), and their interaction. To enhance confidence that end fear was responsible for this effect and not other third variables associated with end fear, we followed the suggestions of Steiner et al (44) and controlled for other relevant variables that may be related to both end fear and peak fear at follow-up. Those control variables were initial fear at the first exposure trial, post-exposure fear in the generalization context, and the presence (at baseline) of other Axis I disorders.

To test the hypothesis that methylene blue would enhance contextual memory at follow-up, we performed two additional regression analyses in which drug condition (methylene blue vs. placebo) was the predictor of participants' scores on our context memory index

(separately at post-test and at follow-up). For consistency with our analyses of peak claustrophobic fear at follow-up, the presence of another Axis 1 disorder was dichotomously coded and included as a covariate in the model.

Results

Effects of Methylene Blue on Fear Extinction

Mean fear was 73.0 (SD = 20.0, range: 20-100) at the first exposure and 23.5 (SD = 23.3; range: 0-90) at the last exposure (end fear). As hypothesized, there was a significant drug condition $\times$ end fear interaction, $b = -0.74$, $t(34) = 2.71$, $p = .011$, $d = .93$. We probed this interaction following procedures recommended by Aiken and West (45). This entailed two follow-up analyses, with end fear centered alternatively at low (end fear = 0; 1 SD below the mean) and high (end fear = 47; 1 SD above the mean) levels of fear. These analyses showed that for participants with low end fear (end fear=0), those given methylene blue had significantly lower levels of fear at the 1-month follow-up (peak fear=10.7) than those given placebo (peak fear = 29.8), $b = 19.2$, $t(34) = 2.21$, $p = .035$, $d = .76$ (see Figure 1). For those with higher levels of end fear, the opposite tended to be true: those given methylene blue had marginally higher levels of fear at the 1-month follow-up (peak fear = 33.0) than those given placebo (peak fear = 17.4), $b = -15.6$, $t(32) = 1.78$, $p = .084$, $d = .63$.

We used this same procedure to examine the model-based relation between end fear at the last exposure trial and peak fear at the 1-month follow-up for those given methylene blue versus those given placebo. For those given methylene blue, end fear at the last exposure trial significantly predicted peak fear at the 1-month follow-up, $b = 0.48$, $t(32) = 2.43$, $p = .021$, $d = .86$, with higher end fear associated with higher fear 1 month later. However, for those given placebo, there was no relationship between end fear and peak fear at the 1-month follow-up, $b = -.27$, $t(32) = 1.10$, $p = .281$, $d = .39$.

Effects of Methylene Blue on Contextual Memory

We also hypothesized that scores on our incidental contextual memory test given at the 1-day and 1-month follow-up assessments would reveal enhanced performance for those given methylene blue versus placebo. Although those given methylene blue did not have greater recall 1-day after completing extinction training ($p = .40$), they did demonstrate better free recall performance at the 1-month follow-up (recall score = 1.4) compared to those given placebo (recall score = 0.7), $b = 0.69$, $t(37) = 2.06$, $p = .047$, $d = .68$ (Figure 2).

We next attempted to verify that recall assessed by our incidental context memory recall index was not merely a result of lower fear among the methylene blue participants. We found that peak fear at post-treatment was unrelated to incidental recall at post-treatment or at follow-up, across drug conditions (p 's $> .26$), or within drug conditions (p 's $> .12$). Similarly, peak fear at follow-up was unrelated to free recall at follow-up, both across drug conditions (p 's $> .57$) and within each drug condition (p 's $> .23$). The reverse possibility also received no support - incidental recall at post-treatment was unrelated to peak fear at follow-up, both across ($p = .88$) and within (p 's $> .33$) drug conditions.

Furthermore, because incidental recall could be associated with overall memory functioning and hence could be related to emotional memory of end fear at the last exposure trial, we investigated whether memory recall moderates the effect of end fear on peak fear at follow-up (i.e., end fear could be highly related to peak fear at follow-up for those with greater recall, but less related to peak fear for those with poor recall). No evidence was found for this possibility (p 's $> .24$). Thus, it appears that methylene blue's memory facilitation effects at follow-up are independent of any differential changes in fear between methylene blue and placebo treated participants.

Side Effects and Adverse Reactions

Tables 2 gives the frequency and mean severity of side effects reported during the study. Minor side effects were reported in each drug condition. The three most commonly reported side effects in the methylene blue group included urine discoloration, increased frequency of urination, and dizziness; whereas the three most common side effects in the placebo group included urine discoloration, headaches and fecal discoloration. These side effects were generally mild and no serious adverse reactions were observed. No problems with medication adherence were reported.

Discussion

Based on a fundamental assumption that the outcome of administering a memory enhancing agent in combination with psychotherapy depends on what is learned during therapy, we hypothesized that methylene blue would promote the retention of fear extinction at follow-up relative to placebo for participants achieving marked fear attenuation during extinction training. In contrast, we hypothesized that for those showing minimal or no fear attenuation during training, methylene blue would show a less favorable outcome at follow-up relative to placebo, due to the potential strengthening of threat associations.

As predicted, end fear at post-training significantly moderated the effects of methylene blue on claustrophobic fear in the non-trained, generalization context at follow-up. Specifically, participants displaying low end fear at post-training showed significantly less fear at follow-up if they received methylene blue post-training. The opposite pattern emerged for those displaying high levels of post-training fear; that is, participants receiving methylene blue tended to fare worse at follow-up relative to those who received placebo. These findings are consistent with preclinical studies in which methylene blue given after fear extinction improves the retention of extinguished fear memories (22) and with more recent clinical studies using other cognitive enhancers such as D-cycloserine (33), glucocorticoids (46) and yohimbine (34). Although the implications for those achieving average post-training reduction in fear are less clear, the data underscore the importance of considering individual differences in patients' response to exposure therapy as an important factor in deciding *when* to use cognitive-enhancing agents in combination with psychotherapy.

Consistent with previous preclinical studies in rodents (19, 22-24, 27-29), our findings provide the first demonstration that low-dose methylene blue administration improves memory retention in humans. To determine whether the enhanced context memory performance observed in participants receiving methylene blue was a consequence of lower

fear levels or differences on other clinical status variables (e.g., presence of comorbid Axis I pathology), we controlled for these variables when testing the effects of drug condition on context memory performance. Results showed that the increased memory retention among methylene blue-treated participants was unrelated to individual differences in participants' fear responding or history of other Axis I disorders and thus supports the direct memory enhancing effects of methylene blue. There is no literature to indicate that methylene blue's efficacy may be affected by age or physical activity or other factors affecting neurobiological oxygen uptake. In contrast, methylene blue's neurobiological efficacy has been demonstrated in both normoxia and hypoxia conditions *in vivo* (21).

We also considered the possibility that methylene blue's enhancement of contextual memory could have detrimental effects on later fear responding by enhancing the context dependency of fear extinction learning. For example, if the contextual associative links formed in the extinction context are especially strong, methylene blue augmentation could serve to circumscribe the inhibition of fear to select extinguished contexts (47, 48). However, our results suggest just the opposite occurs. Methylene blue appears to promote generalization of the learning that occurs during extinction training, for better or for worse, depending on the degree of in-session fear attenuation.

We assessed methylene blue's effects on contextual memory independent of participants' fear responding. As mentioned, prior investigations of cognitive enhancing agents in combination with exposure-based treatments have inferred cognitive enhancement based on greater symptom improvement among those receiving the cognitive enhancer relative to those receiving placebo. However, if the goal for using a cognitive enhancer with exposure therapy is to facilitate memory-related neuroplasticity so as to facilitate neural adaptations brought about by the new learning occurring during exposure therapy (49), is it not reasonable to expect enhanced memory for context relevant information unrelated to the patient's emotional responding? If so, incorporating contextual memory tests, which have special relevance for fear extinction learning, provides an independent corroboration that memory-related neuroplasticity has indeed been enhanced.

Our findings should be interpreted in light of the neuropharmacological mechanisms governing methylene blue's metabolic enhancement effects (19). Methylene blue enhances brain energy metabolism in two ways. The first mechanism is global; Methylene blue produces a global increase in brain cytochrome oxidase activity above baseline levels, which leads to enhanced capacity for oxidative energy production (22). This global action has been confirmed by increased baseline glucose uptake and cerebral blood flow using PET and fMRI (20). However, this global effect is widespread and non-specific. The second mechanism shows regional activational specificity. That is, methylene blue selectively potentiates cytochrome oxidase activity and evoked fMRI responses in brain regions activated by a specific task or stimulus (21, 22). Relative to the global effects of methylene blue, this activational effect is more pronounced and is specific to the neural networks demanding more energy utilization. In the case of fear-related neural networks, for example, memory for fear expression in humans activates a network that includes the amygdala and the dorsal anterior cingulate cortex, whereas memory for fear extinction activates a different network involving the ventromedial prefrontal cortex and hippocampal formation (31,32).

Therefore, among participants with high fear expression at the end of training, methylene blue would be expected to facilitate the *fear-expression network*; whereas in participants displaying marked fear extinction, methylene blue would be expected to facilitate the *fear-extinction network*. Thus administering methylene blue at the conclusion of exposure therapy may thus lead to opposite psychological outcomes depending on whether fear-expression or fear-extinction neural networks are potentiated. In contrast, since all participants were exposed to the same claustrophobic chamber, they may show the same contextual memory-enhancing effect from methylene blue irrespective of their level of fear activation due to methylene blue's more global brain action to metabolically enhance their context memory, which is widely distributed in terms of neural networks (47, 50, 51).

Study Limitations

First, our sample was relatively small and largely comprised of young, female university students thus replication with a larger, more diverse sample is warranted. Second, conclusions as to whether methylene blue enhances exposure therapy for other anxiety disorders awaits further investigation. Third, single-item fear ratings (i.e., SUDS) are a ubiquitous measure of fear responding in the phobia treatment literature; however, additional indices of fear, including physiological measurements, would have strengthened conclusions drawn from the present findings. Fourth, extinction training was delivered in a single session. Although single session extinction-based treatments have been shown to be efficacious (53), it remains unclear as to whether methylene blue enhances exposure therapy when delivered in a multi-session treatment format. Fifth, a 4 mg/kg post-training dosing strategy was selected because it has been shown to be the most reliable in rats for enhancing post-training fear extinction memory retention (22, 29) as well as long-term behavioral habituation and object memory recognition (24). Future studies are needed to determine the optimal methylene blue dosing strategies for enhancing exposure therapy. Finally, given the small sample size, it is unclear how effectively the control variables corrected the potential differences between groups and between participants with varying levels of end fear.

Clinical Implications

Our findings have several implications for clinical practice. First, because methylene blue appears to strengthen the memory for fear extinction learning (or lack thereof) that occurs during exposure therapy, administration of methylene blue at the beginning of a therapy session should be avoided, as clinicians cannot predict whether a session will be successful. Second, our findings suggest that post-session methylene blue administration should be done judiciously, after careful consideration of the patient's level of fear attenuation achieved in-session. Based on our findings, patients who continue to show moderate to high levels of fear at the conclusion of an exposure therapy session may have their fear inadvertently strengthened by methylene blue administration, thus leading to a less favorable therapeutic outcome. Finally, our findings highlight the need for research on the development of empirically based decision rules for administering methylene blue and other cognitive enhancers in the context of exposure therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Anushka Pai and Alicia Herbison for their assistance with this study. This work was supported in part by NIH grant R01 MH076847 to Dr. Gonzalez-Lima.

References

1. Neudeck, P.; Wittchen, HU. Exposure Therapy: Rethinking the Model - Refining the Method. New York, NY: Springer; 2012.
2. Telch, MJ.; Cobb, AR.; Lancaster, CL. Exposure Therapy for Anxiety Disorders: Procedural Variations, Clinical Efficacy, and Change Mechanisms. In: Emmelkamp, PM.; Ehring, T., editors. International handbook of anxiety disorders. Aimes, IA: Wiley-Blackwell; 2013. in press
3. McNally RJ. Mechanisms of exposure therapy: how neuroscience can improve psychological treatments for anxiety disorders. Clin Psychol Rev. 2007; 27:750–759. [PubMed: 17292521]
4. Craske MG, Kircanski K, Zelikowsky M, Mystkowski J, Chowdhury N, Baker A. Optimizing inhibitory learning during exposure therapy. Behav Res Ther. 2008; 46:5–27. [PubMed: 18005936]
5. Davis M, Ressler K, Rothbaum BO, Richardson R. Effects of d-cycloserine on extinction: translation from preclinical to clinical work. Biol Psychiatry. 2006; 60:369–375. [PubMed: 16919524]
6. Hofmann SG, Sawyer AT, Asnaani A. D-cycloserine as an augmentation strategy for cognitive behavioral therapy for anxiety disorders: an update. Curr Pharm Des. 2012
7. Ressler KJ, Rothbaum BO, Tannenbaum L, Anderson P, Graap K, Zimand E, Hodges L, Davis M. Cognitive enhancers as adjuncts to psychotherapy: use of D-cycloserine in phobic individuals to facilitate extinction of fear. Arch Gen Psych. 2004; 61:1136–1144.
8. Hofmann SG, Meuret AE, Smits JAJ, Simon NM, Pollack MH, Eisenmenger K, Shiekh M, Otto MW. Augmentation of exposure therapy with D-cycloserine for social anxiety disorder. Arch Gen Psych. 2006; 63:298–304.
9. Guastella AJ, Richardson R, Lovibond PF, Rapee RM, Gaston JE, Mitchell P, Dadds MR. A randomized controlled trial of D-cycloserine enhancement of exposure therapy for social anxiety disorder. Biol Psychiatry. 2008; 63:544–549. [PubMed: 18179785]
10. Otto MW, Tolin DF, Simon NM, Pearlson GD, Basden S, Meunier SA, Hofmann SG, Eisenmenger K, Krystal JH, Pollack MH. Efficacy of D-cycloserine for enhancing response to cognitive-behavior therapy for panic disorder. Biol Psychiatry. 2010; 67:365–370. [PubMed: 19811776]
11. Kushner MG, Kim SW, Donahue C, Thuras P, Adson D, Kotlyar M, McCabe J, Peterson J, Foa EB. D-cycloserine augmented exposure therapy for obsessive-compulsive disorder. Biol Psychiatry. 2007; 62:835–838. [PubMed: 17588545]
12. Wilhelm S, Buhlmann U, Tolin DF, Meunier SA, Pearlson GD, Reese HE, Cannistraro P, Jenike MA, Rauch SL. Augmentation of behavior therapy with D-cycloserine for obsessive-compulsive disorder. Am J Psychiatry. 2008; 165:335–341. [PubMed: 18245177]
13. Hofmann SG, Smits JAJ, Rosenfield D, Simon N, Otto MW, Meuret AE, Marques L, Fang A, Tart C, Pollack MH. D-cycloserine as an augmentation strategy with cognitive-behavioral therapy for social anxiety disorder. Am J Psychiatry. 2013; 170:751–758. [PubMed: 23599046]
14. Storch EA, Merlo LJ, Bengtson M, Murphy TK, Lewis MH, Yang MC, Jacob ML, Larson M, Hirsh A, Fernandez M, Geffken GR, Goodman WK. D-cycloserine does not enhance exposure-response prevention therapy in obsessive-compulsive disorder. Int Clin Psychopharmacol. 2007; 22:230–237. [PubMed: 17519647]
15. Guastella AJ, Dadds MR, Lovibond PF, Mitchell P, Richardson R. A randomized controlled trial of the effect of d-cycloserine on exposure therapy for spider fear. J Psychiatr Res. 2007; 41:466–471. [PubMed: 16828803]

16. Tart CD, Handelsman PR, DeBoer LB, Rosenfield D, Pollack MH, Hofmann SG, Powers MB, Otto MW, Smits JAJ. Augmentation of exposure therapy with post-session administration of D-cycloserine. *J Psychiatr Res.* 2013; 47:168–174. [PubMed: 23098672]
17. Litz BT, Salters-Pedneault K, Steenkamp MM, Hermos JA, Bryant RA, Otto MW, Hofmann SG. A randomized placebo-controlled trial of D-cycloserine and exposure therapy for posttraumatic stress disorder. *J Psychiatr Res.* 2012; 46:1184–1190. [PubMed: 22694905]
18. Wainwright M, Crossley KB. Methylene Blue--a therapeutic dye for all seasons? *J Chemother.* 2002; 14:431–443. [PubMed: 12462423]
19. Rojas JC, Bruchey AK, Gonzalez-Lima F. Neurometabolic mechanisms for memory enhancement and neuroprotection of methylene blue. *Prog Neurobiol.* 2012; 96:32–45. [PubMed: 22067440]
20. Lin AL, Poteet E, Du F, Gourav RC, Liu R, Wen Y, Bresnen A, Huang S, Fox PT, Yang SH, Duong TQ. Methylene blue as a cerebral metabolic and hemodynamic enhancer. *PLoS ONE.* 2012; 7:e46585. [PubMed: 23056355]
21. Huang S, Du F, Shih YYI, Shen Q, Gonzalez-Lima F, Duong TQ. Methylene blue potentiates stimulus-evoked fMRI responses and cerebral oxygen consumption during normoxia and hypoxia. *Neuroimage.* 2013; 72:237–242. [PubMed: 23357077]
22. Gonzalez-Lima F, Bruchey AK. Extinction memory improvement by the metabolic enhancer methylene blue. *Learn Mem.* 2004; 11:633–640. [PubMed: 15466319]
23. Martinez JL Jr, Jensen RA, Vasquez BJ, McGuinness T, McGaugh JL. Methylene blue alters retention of inhibitory avoidance responses. *Physiol Psychol.* 1978; 6:387–390.
24. Riha PD, Bruchey AK, Echevarria DJ, Gonzalez-Lima F. Memory facilitation by methylene blue: Dose-dependent effect on behavior and brain oxygen consumption. *Eur J Pharmacol.* 2005; 511:151–158. [PubMed: 15792783]
25. Medina DX, Caccamo A, Oddo S. Methylene blue reduces $\alpha\beta$ levels and rescues early cognitive deficit by increasing proteasome activity. *Brain Pathol.* 2011; 21:140–149. [PubMed: 20731659]
26. O'Leary JC, Li Q, Marinec P, Blair LJ, Congdon EE, Johnson AG, Jinwal UK, Koren J, Jones JR, Kraft C, Peters M, Abisambra JF, Duff KE, Weeber EJ, Gestwicki JE, Dickey CA. Phenothiazine-mediated rescue of cognition in tau transgenic mice requires neuroprotection and reduced soluble tau burden. *Mol Neurodegener.* 2010; 5:45. [PubMed: 21040568]
27. Callaway NL, Riha PD, Bruchey AK, Munshi Z, Gonzalez-Lima F. Methylene blue improves brain oxidative metabolism and memory retention in rats. *Pharmacol Biochem Beh.* 2004; 77:175–181.
28. Wrubel KM, Riha PD, Maldonado MA, McCollum D, Gonzalez-Lima F. The brain metabolic enhancer methylene blue improves discrimination learning in rats. *Pharmacol Biochem Beh.* 2007; 86:712–717.
29. Wrubel KM, Barrett D, Shumake J, Johnson SE, Gonzalez-Lima F. Methylene blue facilitates the extinction of fear in an animal model of susceptibility to learned helplessness. *Neurobiol Learn Mem.* 2007; 87:209–217. [PubMed: 17011803]
30. Barrett D, Shumake J, Jones D, Gonzalez-Lima F. Metabolic mapping of mouse brain activity after extinction of a conditioned emotional response. *J Neurosci.* 2003; 23:5740–5749. [PubMed: 12843278]
31. Quirk GJ, Garcia R, Gonzalez-Lima F. Prefrontal mechanisms in extinction of conditioned fear. *Biol Psychiatry.* 2006; 60:337–343. [PubMed: 16712801]
32. Milad MR, Quirk GJ. Fear extinction as a model for translational neuroscience: Ten years of progress. *Annu Rev Psychol.* 2012; 63:129–151. [PubMed: 22129456]
33. Smits JAJ, Rosenfield D, Otto MW, Powers MB, Hofmann SG, Telch MJ, Pollack MH, Tart CD. D-cycloserine enhancement of fear extinction is specific to successful exposure sessions: Evidence from the treatment of height phobia. *Biol Psychiatry.* 2013; 73:1054–1058. [PubMed: 23332511]
34. Smits JAJ, Rosenfield D, Otto MW, Davis ML, Julian K, Handelsman PR, Tuerk P, Shiekh M, Powers MB. Yohimbine enhancement of exposure therapy for social anxiety disorder: A randomized controlled trial. *Biol Psychiatry.* in press.
35. Powers MB, Smits JAJ, Whitley D, Bystritsky A, Telch MJ. The effect of attributional processes concerning medication taking on return of fear. *J Consult Clin Psych.* 2008; 76:478–490.

36. Powers MB, Smits JAJ, Telch MJ. Disentangling the effects of safety-behavior utilization and safety-behavior availability during exposure-based treatment: A placebo-controlled trial. *J Consult Clin Psych.* 2004; 72:448–454.
37. Sloan T, Telch MJ. The effects of safety-seeking behavior and guided threat reappraisal on fear reduction during exposure: an experimental investigation. *Behav Res Ther.* 2002; 40:235–251. [PubMed: 11863235]
38. Telch MJ, Valentiner DP, Ilai D, Petruzzi D, Hehmsoth M. The facilitative effects of heart-rate feedback in the emotional processing of claustrophobic fear. *Behav Res Ther.* 2000; 38:373–387. [PubMed: 10761281]
39. Peter C, Hongwan D, Küpfer A, Lauterburg BH. Pharmacokinetics and organ distribution of intravenous and oral methylene blue. *Eur J Clin Pharmacol.* 2000; 56:247–250. [PubMed: 10952480]
40. Corrêa MS, Balardin JB, Caldieraro MAK, Fleck MP, Argimon I, Luz C, Bromberg E. Contextual recognition memory deficits in major depression are suppressed by cognitive support at encoding. *Biol Psychol.* 2012; 89:293–299. [PubMed: 22115881]
41. Vicari S, Finzi A, Menghini D, Marotta L, Baldi S, Petrosini L. Do children with developmental dyslexia have an implicit learning deficit? *J Neurol Neurosurg Psychiatr.* 2005; 76:1392–1397. [PubMed: 16170083]
42. Vicari S, Bellucci S, Carlesimo GA. Visual and spatial long-term memory: differential pattern of impairments in Williams and Down syndromes. *Dev Med Child Neurol.* 1999; 47:305–311. [PubMed: 15892372]
43. Kraemer HC, Kiernan M, Essex M, Kupfer DJ. How and why criteria defining moderators and mediators differ between the Baron & Kenny and MacArthur approaches. *Health Psychol.* 2008; 27:101–108.
44. Steiner PM, Cook TD, Shadish WR, Clark MH. The importance of covariate selection in controlling for selection bias in observational studies. *Psychol Methods.* 2010; 15:250–267. [PubMed: 20822251]
45. Aiken, LS.; West, SG. Multiple regression: Testing and interpreting interactions. Thousand Oaks, CA: Sage Publications Inc; 1991.
46. Soravia LM. Glucocorticoids reduce phobic fear in humans. *Proceedings of the National Academy of Sciences.* 2006; 103:5585–5590.
47. Maren S, Phan KL, Liberzon I. The contextual brain: implications for fear conditioning, extinction and psychopathology. *Nat Rev Neurosci.* 2013:1–12. [PubMed: 23232605]
48. Orsini CA, Kim JH, Knapska E, Maren S. Hippocampal and prefrontal projections to the basal amygdala mediate contextual regulation of fear after extinction. *J Neurosci.* 2011; 31:17269–17277. [PubMed: 22114293]
49. Krystal JH. Neuroplasticity as a target for the pharmacotherapy of psychiatric disorders: new opportunities for synergy with psychotherapy. *BPS.* 2007; 62:833–834.
50. Eich E. Context, memory, and integrated item/context imagery. *J Exp Psych Learn.* 1985; 11:764.
51. Hintzman DL. Judgments of frequency and recognition memory in a multiple-trace memory model. *Psychol Rev.* 1988; 95:528.
52. Kamphuis JH, Telch MJ. Effects of distraction and guided threat reappraisal on fear reduction during exposure-based treatments for specific fears. *Behav Res Ther.* 2000; 38:1163–1181. [PubMed: 11104181]
53. Wolitzky-Taylor KB, Horowitz JD, Powers MB, Telch MJ. Psychological approaches in the treatment of specific phobias: A meta-analysis. *Clin Psychol Rev.* 2008; 28:1021–1037. [PubMed: 18410984]
54. Rachman PDS, Taylor PDS. Analyses of claustrophobia. *J Anxiety Disord.* 1993; 7:281–291.
55. Valentiner DP, Telch MJ, Ilai D, Hehmsoth MM. Claustrophobic fear behavior: a test of the expectancy model of fear. *Behav Res Ther.* 1993; 31:395–402. [PubMed: 8512539]

Figure 1. Methylene Blue's Effects on Peak Fear at 1-Month Follow-Up Moderated by End Fear at the Last Extinction Trial^a

^a Effects of methylene blue on peak fear in the generalization context at the 1-month follow-up for participants with low (0), average (23.5) and high (47) end fear at the last extinction trial. For participants with low end fear, those given methylene blue had significantly lower levels of fear at follow-up than those given placebo ($p = .035$). For those with higher levels of end fear, those given methylene blue tended to show marginally significantly higher levels of fear at follow-up than those given placebo ($p = .084$). * = $p < .05$.

Figure 2. Methylene Blue's Effects on Contextual Memory Performance at Post-training and 1-Month Follow-Up^a

^a Effects of methylene blue on contextual memory performance at post-training and 1-month follow-up. Memory index scores depicted on the y-axis were derived by summing the total number of items for which participants correctly recalled both the number and its location. As shown, participants given methylene blue post-training demonstrated significantly better contextual memory performance at the 1-month follow-up ($p = .047$), but not at the post-treatment assessment ($p = .396$). * = $p < .05$.

Table 1
Baseline Characteristics ^a

	Methylene Blue+Extinction Training (n = 23)		Placebo+Extinction Training (n = 19)	
Variable	M	SD	M	SD
Claustrophobia Questionnaire	70.91	10.64	68.42	12.88
Claustrophobia Concerns Questionnaire – Training	78.26	16.71	79.47	12.71
Claustrophobia Concerns Questionnaire – Generalization	72.50	20.31	71.45	14.89
Peak Fear: Behavioral Approach Task – Training	77.39	17.38	68.95	16.96
Peak Fear: Behavioral Approach Task – Generalization	66.09	16.72	63.68	15.35
Age	19.04	1.46	19.58	3.66
	n	%	n	%
Gender				
Male	5	21.74	5	26.32
Female	18	78.26	14	73.68
Race				
White	17	73.91	15	78.95
African American	1	4.35	0	0.00
Asian	5	21.70	3	15.79
Native Hawaiian/Pacific Islander	0	0.00	1	5.26
Other	0	0.00	0	0.00
Hispanic or Latino Ethnicity	7	30.43	5	26.32

^aTotal 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) for the Claustrophobia Questionnaire (54) are reported in Table S2 of the online data supplement.

Table 2
Reported Side Effects for Each Drug Condition

Side Effect	Methylene Blue		Placebo	
	% Reporting Side Effect	Mean Severity(0-4)	% Reporting Side Effect	Mean Severity(0-4)
Urine discoloration	95.6	3.3	21.1	2.9
Fecal discoloration	4.3	3.0	15.8	3.0
Increased frequency of urination	21.7	2.6	10.5	3.0
Indigestion	13.0	2.0	5.3	2.0
Diarrhea	8.7	2.5	0.0	----
Vomiting	4.3	2.0	0.0	----
Headache	13.0	2.0	21.1	2.3
Heart racing	4.3	1.0	0.0	----
Stomach cramps	0.0	----	5.3	3.0
Sensitivity to light	0.0	----	5.3	1.0
Trouble sleeping	8.7	1.5	0.0	----
Dizziness	17.4	2.0	0.0	----
Bladder irritation	4.3	2.0	0.0	----