RUNNING HEAD: Single-session CBT and d-cycloserine

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Neurocognitive mechanisms of d-cycloserine augmented single-session exposure therapy for anxiety

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Abstract

Drugs targeting the N-Methyl-D-aspartic acid (NMDA) system and learning rates have been proposed as potential adjunct treatments to boost the success of exposure therapy for anxiety disorders. However, the effects of the NMDA partial agonist d-cycloserine on psychological treatment have been mixed. We investigated potential neurocognitive mechanisms underlying the clinical effects of d-cycloserine-augmented exposure, to inform the optimal combination of this and similar agents with psychological treatment. Unmedicated patients with panic disorder were randomised to single-dose d-cycloserine (250mg; N=17) or matching placebo (N=16) 2hrs before one session of exposure therapy. Neurocognitive markers were assessed one day after treatment, including reaction-time based threat bias for fearful faces and amygdala response to threat. Clinical symptom severity was measured using self-report and clinician-rated scales the day before and after treatment, and at 1- and 6-months follow-up. Analysis was by intention-to-treat. One day after treatment, threat bias for fearful faces and amygdala threat response were attenuated in the drug compared to the placebo group. Lower amygdala magnitude predicted greater clinical improvement during follow-up across groups. D-cycloserine led to greater clinical recovery at 1-month follow-up (d-cycloserine 71% versus placebo 25%). D-cycloserine-augmented single-session exposure therapy reduces amygdala threat response, and this effect predicts later clinical response. These findings highlight a neurocognitive mechanism by which d-cycloserine may exert its augmentative effects on psychological treatment and bring forward a marker that may help understand and facilitate future development of adjunct treatments with CBT for anxiety disorders. (D-cycloserine Augmented CBT for Panic Disorder; clinicaltrials.gov; NCT01680107)

Introduction

There has been increasing interest in the combination of exposure-based cognitive-behaviour therapy (CBT) for anxiety disorders with drugs targeting N-methyl-D-aspartate (NMDA) receptors, which play a core role in synaptic plasticity, a key cellular mechanism involved in learning and memory (1). In particular, the NMDA receptor partial agonist d-cycloserine has been investigated for its potential to enhance the effects of psychological treatment. However, after initial enthusiasm and positive trials, a number of further studies indicated no superiority of the drug compared to placebo, or even a worsening of symptoms after unsuccessful exposure (2). These detrimental effects have now been attributed to d-cycloserine not only enhancing fear extinction but also fear reconsolidation, suggesting that augmentative effects may only be evident if exposure is effectively administered (3). Recent meta-analyses emphasise facilitative effects of d-cycloserine on CBT over and above placebo effects (4, 5). Nevertheless, the optimal methods for combination remain to be further identified, and could be informed by characterisation of neurocognitive mechanisms underlying the clinical effects of intervention.

We have recently developed a single-dose CBT testbed that allows the assessment of early effects of treatment on such neurocognitive markers and their contribution to clinical improvement (6). Administering a single session of exposure therapy for panic disorder led to no clinical changes on the day after treatment, but resulted in a substantial decrease in threat bias for fearful faces. At 1-month follow-up and without additional interim treatment, clinical improvement was apparent, with 1/3 of treated patients fulfilling criteria for recovery. Importantly, clinical improvement was driven by the magnitude of threat bias measured on the day after treatment, with lower bias predicting improved clinical outcome. These findings suggest that a reduction of threat bias may be a key mechanism of action in exposure therapy, and they bring forward a single-dose CBT methodology that may help ascertain the mechanisms of action underlying pharmacological-psychological combination treatments.

Such a focus on the mechanisms underpinning recovery with pharmacological exposure therapy can inform optimal combination of different treatment components, and it can provide an experimental medicine model for identification of novel agents.

This study aimed to characterise the effects of d-cycloserine with single-session CBT on neurocognitive markers important in panic disorder. Primary outcome measures were dot probe threat bias previously shown to mediate clinical effects of single-session CBT, as well as automatic panic associations using a task that has also been shown to sensitively capture early effects of CBT in panic disorder (6, 7). The main secondary outcome was amygdala threat response, which has previously been shown to normalise after four sessions of exposure therapy in panic disorder (8). We hypothesised that the d-cycloserine group compared to placebo would show reduced threat bias, panic associations and amygdala threat response after single-session CBT, and that the magnitude of these markers would predict clinical symptom changes during follow-up (6).

Methods and Materials

Participants

Thirty-three patients with a current panic disorder diagnosis and at least moderate agoraphobic avoidance were recruited from the community. Diagnoses were assessed using the Structured Clinical Interview for DSM-IV Axis I Disorders; agoraphobic avoidance was measured using the Structured Panic Assessment Interview (”yes” response to >2 situations listed under “(2) Avoidance – Do you avoid or feel very uncomfortable in …”) (9, 10). Participants were free of CNS-active medication for at last 6 weeks before enrolment. Occasional benzodiazepine or beta-blocker medication (15% of participants) was not an exclusion criterion but patients refrained from these drugs 48 hours before sessions. Participants with contraindication to MRI (e.g. metal implant, left-handedness) were included but not enrolled in the MRI component. (Table 1, Supplementary Materials for CONOSRT

diagram and full list of exclusion criteria).

General Procedure

Figure 1 gives an overview of assessments. Participants were randomly allocated to a single oral dose of d-cycloserine (250 mg; King Pharmaceuticals) or placebo capsule (microcrystalline cellulose) (1:1 ratio), administered 2 h before single-session CBT. Previous clinical trials into the add-on effects of d-cycloserine to CBT often used doses of 50mg. Different from our study these trials used longer CBT protocols, and healthy volunteer work indicates an effect of d-cycloserine on single-session hippocampal learning at 250mg but not 50mg (11). Considering that only one session was offered in our protocol, and based on clinical work showing no significant differences in clinical effects of ultra-brief CBT together with d-cycloserine between 50mg and 500mg, we chose a dose of 250mg (12).

All sessions took place in a university setting. After initial screening, participants returned for four study visits, with baseline assessment and intervention taking place on day 1, and outcome testing visits taking place 1 day, 1 month, and 6 months after intervention. During the screening visit, sociodemographic (age, gender, years of education, verbal intelligence) and clinical diagnostic data (primary panic diagnosis, comorbid diagnoses, panic duration) were acquired (13).

Clinical symptoms were assessed on all four visits. On the day after intervention, we also assessed emotional processing using behavioural computer tasks and magnetic resonance imaging (MRI). One of the behavioural tasks was also administered before intervention to secure a baseline measure of emotional processing. Recruitment, obtaining written informed consent, screening for study eligibility, sending enrolment requests to pharmacy, double-blind drug administration, exposure therapy and clinical and neurocognitive testing were carried out by a trained clinical psychologist researcher (AR). All procedures were in accordance with the

Declaration of Helsinki and approved by a research ethics committee. Participants gave written informed consent. Data were collected between November 2013 and April 2016.

Single-session Exposure Therapy

Treatment was delivered by an experienced clinical psychologist and supervised by a senior clinical psychologist involved in graduate training on the Oxford University clinical psychology doctorate programme. The intervention followed a previously published protocol and was a condensed version of routine clinical care (6, 14). It involved one session of exposure-based CBT, based on the well-established cognitive-behavioural theory of panic (15). This approach assumes that anxiety develops as a consequence of normal physical sensations (e.g. increased heart rate) being misperceived as threatening (e.g. I am having a heart attack), and the use of maladaptive safety strategies (e.g. leaving situation) preventing corrective experiences (e.g. I will not die of a heart attack even if I am not leaving the situation.). Session key ingredients: (i) cognitive preparation: explanation of individually relevant learning mechanisms underlying the maintenance of anxiety, especially the role of safety strategies (~15min), (ii) exposure to a fear- provoking situation (e.g. being locked in walk-in closet) and bodily sensations while dropping all safety strategies, to test out catastrophic expectations and break through stimulus-driven response cycles (15min exactly for each participant), (iii) cognitive debriefing, to discuss the patient's experience in a fear situation without safety strategies, and to consolidate new behaviour (~10min).

Randomisation and Masking

Participants and the researcher responsible for treatment, data collection and outcome evaluation remained naïve to drug group allocation until completion of data analysis. Placebo was encapsulated in lactose capsules identical to d-cycloserine. Generation of the randomisation sequence, treatment allocation and drug dispensing were executed by an

external pharmacy not in direct contact with study participants, and the allocation list remained in pharmacy until all steps of data blind-analysis had been completed. The randomization sequence was generated using a random number generator (www.random.org) and was based on blocked randomization (blocks of four) while stratifying for gender and primary diagnosis (panic disorder either with or without agoraphobia).

Outcomes

Cognitive Biomarker Measures. ***i) Faces Dot Probe Task (FDOT).*** Stimuli were coloured photographs of 20 individual faces with a neutral, fearful and happy facial expression each (16). In each of 192 trials, a neutral-neutral, neutral-happy, or neutral-fearful face pair was presented, with one stimulus appearing above and one below a central fixation position. Eight blocks of unmasked versus masked trials each were presented alternatingly. Faces pairs were presented for 100ms in the unmasked and 16ms (plus scrambled face pair for 84ms) in the masked condition, followed by a dot probe ([..] or [:]) that participants categorised as horizontal or vertical using two buttons. Position of an emotional face, probe position and type were fully counterbalanced. Thus, this task design involved congruent trials (dot at the position of an emotional face) and incongruent trials (dot replaces neutral face while emotional face is present).

ii) MRI. Patients completed a 3T MRI scan on the day after treatment, performing an emotion regulation task (17). IAPS images showing negatively valenced panic-related scenes (e.g. funeral) were presented in 8 blocks of 5 images (5s each), alternating with grey fixation baseline blocks (30s) (18). For half of the blocks, participants were instructed to naturally experience the emotional state evoked by the images, without attempting to regulate it (Maintain blocks). For Reappraisal blocks, they were instructed to down-regulate the provoked negative affect by using previously demonstrated strategies of cognitive reappraisal (e.g. reframing, rationalising). Individual contrast images were calculated for Maintain blocks,

Reappraisal blocks, Maintain versus Reappraisal and Reappraisal versus Maintain and then entered to a mixed-effects whole-brain group analysis (19). Based on our previous work which identified the amygdala as hyperactive in Maintain but not Reappraisal blocks in those with panic disorder, we also ran region of interest analyses (ROI), including 10mm radius spherical masks around the peak voxel of a left amygdala region (-14/-6/-8) and its right-hemisphere counterpart, with Maintain BOLD % signal as the main outcome measure (further details: Supplementary Materials) (17).

iii) Implicit Association Task (IAT). 4 panic-related (e.g. anxious), 4 calm-related (e.g. relaxed), 4 me-related (e.g. I) and 4 not-me related words (e.g. them) were presented in random order (7). In one of two blocks, all words belonging to the categories *Panicked* and *Me* were responded to with the left key and *Calm* and *Not Me* stimuli were responded to with the right key, congruent with panic-self associations in patients with panic disorder (fast reaction expected). In a second block, labels were switched, and participants categorised words while pairing *Panicked* with *Not Me* and *Calm* with *Me,* incongruent with panic-self associations characteristic for panic patients (slower reaction expected). 20 practice and 36 experimental trials were presented per block. Block order was counterbalanced across participants.

Clinical Symptom Measures. *i) Self- and Clinician-Administered Questionnaires.* Before treatment, on the day after, and at 1-month and 6-month follow-up, anxiety and depression symptoms were monitored using established self-report measures: i) State-Trait Anxiety Inventory (STAIS/STAIT), ii) Beck Depression Inventory (BDI), iii) Body Sensations Questionnaire assessing fear of physical sensations (BSQ), iv) Agoraphobic Cognitions Questionnaire (ACQ), v) Mobility Inventory (MI) assessing agoraphobic avoidance, and vi) Panic Attack Scale (PAS) assessing panic frequency (20-24). The clinician-administered Panic Disorder Severity Scale (PDSS) was used at baseline and follow-up appointments (25).

ii) Stress Test. To establish in-vivo stress reactivity, anxiety was measured during 5min

exposure to an individually relevant agoraphobic situation, before and after the day of treatment and at follow-ups (6, 14). This involved being locked in an enclosed walk-in closet (17 CYC/14 PLAC), or a bus or car drive (0 CYC/2 PLAC). Immediately after the test, participants rated their level of anxiety experienced during the test (before, after 1min, after 3min, end of test), using 0-100 visual analogue scales. Baseline ratings were acquired during an earlier part of the sessions.

Demand and Side Effects and Concomitant Treatment

To capture any acute changes, blood pressure and heart rate were measured before and 2hrs after drug administration (expected peak level), and participants completed visual analogue scales rating their mood and physiological symptoms. At the end of the intervention day, participants and experimenter were also asked to guess whether the active capsule had been administered. At follow-ups, we assessed whether patients had accessed additional treatment since the study intervention.

Statistical Analysis

Sample Size. We predicted that the placebo group would show next-day fear bias similar to that seen after single-session CBT (M=5, SD=25), and that the d-cycloserine group would show next-day fear bias similar to that seen in healthy volunteers (M=-15, SD=25) in our previous work (6). With an effect size of d=0.8 and an α-level of 0.05, 16 participants per group would be needed to achieve 70% power. We originally aimed to recruit 50 patients for this study but this was adjusted to 32 based on the above.

Overall Statistical Approach. Data analyses included all randomised subjects and were performed on the intention-to-treat basis (16 PLAC, 17 CYC), except for MRI measures which were analysed per protocol (14 PLAC, 13 CYC). No data was excluded from analysis. To account for participants lost to follow-up (Supplementary Figure 1), missing data were

imputed, assuming data missing at random, by means of multiple imputation (incomplete cases: 1-month follow-up 6% PLAC/ 6% CYC, 6-months follow-up: 19% PLAC/ 18% CYC). Twenty complete data sets were generated using the multiple imputation by chained equations method in the statistical computing software R (R Core Team, 2018), with 20 iterations per imputation (26). Predictors were sociodemographic and clinical baseline characteristics, and baseline and next-day clinical outcome measures; imputed variables were clinical outcomes at follow-ups. Statistical analyses were run in R using the packages nlme and miceadds. In all analyses, the stratification variables gender and primary diagnosis were used as covariates. Statistical tests were two-tailed and based on an alpha-level of significance of 0.05. Treatment effect sizes are reported as Cohen's *d*.

Cognitive Biomarker Measures. In the FDOT, reaction times (RT) below 200 ms and above 2000 ms, or above 2 SDs above the mean, were excluded (6, 27). Bias scores were calculated by subtracting the median RT in congruent trials from the median RT in incongruent trials. For the IAT, scores were computed as Mean-incongruent RT minus Mean-congruent RT, divided by standard deviation across the calm and panicking conditions (28). Group differences were assessed using linear mixed-effects models, including the fixed factors group and condition (happy, fear) for the FDOT, and group and time (baseline, next-day) for the IAT. MRI data were analysed using FSL FEAT 6.0 (FMRIB Software Library; www.fmrib.ox.ac.ul/fsl) with $Z>2.3$ and $p<0.05$. Significant interactions were explored by extracting BOLD % signal changes and entering these into linear mixed-effects models with the factors group and condition (Maintain, Reappraisal).

Clinical Symptom Measures. Group differences on the validated clinical outcome measures and stress test ratings were assessed using linear mixed-effects models, including the factors group and time (next day, 1-month, 6-months) and their interaction, and controlling for pre-treatment scores. In line with recent work, recovery was defined as agoraphobia scores (MI) falling within the range reported for healthy subjects, as this measure most reliably reflects

the impact of the disorder on daily functioning (6, 20). Following established approaches, we also ran eight separate multiple linear regression analyses to predict whether i) BOLD % amygdala signal change (Maintain block) and ii) threat bias (FDOT) on the day after treatment predicted symptom changes at 1-month follow-up on the MI, BSQ, ACQ, or PDSS (6, 29). Next-day symptom severity (baseline for PDSS) on that measure was entered as predictor of no interest to control for its potential influence on the outcome at 1 month. Group, bias, and the group- bias interaction term were entered as predictors of interest.

Results

Cognitive Biomarker Measures

Behavioural Tasks. Next-day threat bias for fearful faces was significantly lower in the d-cycloserine compared to the placebo group (p=0.042, d=0.77). There were no group differences in implicit panic associations (p=0.93, d=0.11) (Table 2).

MRI. In the whole-brain analysis, significant Group by Task interaction effects were seen in two clusters, including the left frontal orbital cortex (OFC; 499 voxels, MNI -28,12,-14, Z=3.40, p=0.003) and right dorsolateral prefrontal cortex (DLPFC; 379 voxels, MNI 36,36,30, Z=4.06, p=0.017). Interactions were driven by the d-cycloserine group showing a significant decrease in activation during Maintain (OFC: p=0.019; DLPFC: p=0.037) and a significant increase in activation during Reappraisal (OFC: p=0.0090; DLPFC: p=0.013) compared to placebo (Figure 2 A/B).

Compared to placebo, d-cycloserine led to a significant decrease of right amygdala activation in Maintain (p=0.023) but not in Reappraisal blocks (mean difference 0.02, 95% CI –0.23 to 0.27; p=0.88). These differential effects for Maintain and Reappraisal blocks are in line with our previous work showing that patients compared to controls show increased threat response during Maintain but not Reappraisal blocks (17). No drug effects were found in the left

amygdala (Maintain: p=0.54; Reappraisal: mean difference -0.08, 95% CI –0.47 to 0.30; p=0.66) (Table 2; Figure 2 C).

Clinical Symptom Measures

There were no significant group differences in fear of physical symptoms (BSQ), agoraphobia severity (MI), agoraphobic cognitions (ACQ), panic attack frequency (PAS) or panic severity (PDSS) on the day after treatment or at follow-ups (all p>0.095) (Table 2; Figure 3). On the day after treatment, the d-cycloserine compared to placebo group reported lower state anxiety (STAIS; p=0.0041) and depression scores (BDI p=0.0037). The STAIS group difference remained sustained throughout follow-ups (both p<0.0046); the BDI group difference vanished at 1-month follow-up due to the placebo group achieving additional gains (p=0.54) but reappeared at 6-month follow-up (p=0.035) due to the d-cycloserine group showing further response. Trait anxiety was significantly lower after d-cycloserine- versus placebo-supported CBT at 1- and 6-months follow-ups (both p<0.0036). In the stress test, the d-cycloserine group reported lower anxiety on the day after treatment and at 6-months follow-up (both p<0.0021), but the group difference failed to reach statistical significance 1-month after treatment (p=0.057).

Recovery rates were still low and similar between groups on the day after treatment (PLAC 4/16 (25%), CYC 5/17 (29.4%), *RR* 1.06, *CI* 0.70-1.61, p=0.98). At 1-month follow-up, the d-cycloserine group showed significantly greater treatment response than the placebo group (PLAC 4/16 (25.0%), CYC 12/17 (70.1%), *RR* 2.55, *CI* 1.16-5.61, p=0.015). At 6-months follow-up, the placebo group showed improved clinical gains, closer to those of the d-cycloserine group (PLAC 7/16 (43.8.0%), CYC 12/17 (70.6%), *RR* 1.91, *CI* 0.81-4.49, p=0.17).

Prediction of Clinical Symptom Changes

FDOT threat bias was not predictive of change on any of the clinical measures (all R^2<0.20, all p>0.25). Amygdala responsivity on the day after treatment predicted 1-month follow-up changes in agoraphobia severity (MI) across groups (R^2=0.48; main effect bias: p=0.0070; all other clinical outcome measures: all R^2<0.38, all p>0.11), with those patients showing the lowest amygdala response achieving greater reduction in agoraphobic avoidance during follow-up (Figure 2D).

Demand and Side Effects and Concomitant Treatment

No serious adverse events were reported. D-cycloserine caused no acute differential changes in blood pressure, heart rate and mood (Supplementary Table 1). Neither experimenter nor participants were able to correctly guess group allocation (d-cycloserine guesses; experimenter PLAC 44%, CYC 41%, p=0.88; participant PLAC 25%, CYC 53% p=0.10), suggesting that double-blindness was maintained. Two participants accessed additional CBT during 6-month follow-up (PLAC: N=2; CYC: N=0; p=0.22).

Discussion

Threat bias for fearful faces (FDOT) and amygdala response to aversive images were significantly lower in the d-cycloserine compared to the placebo group on the day after treatment. Greater next-day reduction in amygdala threat response was associated with greater improvement in agoraphobic avoidance during 1-month follow-up. Along with this reduced amygdala threat response after cycloserine, we also observed decreased activation in prefrontal-cortical areas associated with fear response inhibition, suggesting diminished need for emotion regulation. D-cycloserine led to significantly greater clinical recovery at 1-month follow-up than placebo (71% vs 25%), but recovery rates were not statistically different at 6-months follow-up (71% vs 44%). There were no group differences on clinical measures specific to panic disorder, but the drug improved response to an in-vivo stress test and

outcome on more global measures of mental health, including state-trait anxiety and depression, with medium to large effect sizes. Overall, d-cycloserine had no effects on heart rate, blood pressure, or self-reported mood and side effect changes, recommending it as a well-tolerated drug to use in single-dose settings.

These findings highlight a possible neuropsychological mechanism of action of exposure-based CBT that might also be a crucial interface for d-cycloserine action. Across participants, lower amygdala response on the day after treatment predicted lower symptom severity one month later, suggesting that this neural effect might be a key mechanism by which exposure therapy exerts its clinical effects. The amygdala is thought to be crucial in threat processing, and increased responsivity is characteristic of anxiety disorders where information processing is biased towards threat (30). In contrast, a reduction in amygdala hypersensitivity is seen after exposure therapy, where threat stimuli lose their potential to automatically signal danger (30, 31). Our results suggest that this change in amygdala function occurs very rapidly during CBT, after only one session. Anxiety patients may already start to process threat information differently at this point. Over time and in interaction with everyday challenges, this reduced threat sensitivity presumably translates into more distinct symptom improvement. These findings add to our previous work showing that attentional bias for fearful faces changes early during treatment and mediates clinical outcome (6).

Even though this study identified no specific neuropsychological mechanisms of d-cycloserine action, we found that the drug lead to significant reduction in behavioural threat bias and amygdala threat responsivity than placebo one day after treatment. These findings corroborate the idea that threat processing might be a landmark parameter for treatment enhancement with d-cycloserine. The drug is thought to enhance NMDA receptor functioning and neuroplasticity in the amygdala and hippocampus, areas relevant to fear extinction and threat processing (1). It is possible that d-cycloserine exerts its augmentative clinical effects by further amplifying changes in amygdala sensitivity taking place during exposure. As our

results show, these changes occur early during treatment, providing a rationale as to why d-cycloserine has particularly beneficial effects on clinical outcome when applied early in the therapeutic process.

Although our clinical results remain to be replicated in a large-scale clinical trial, this study also replicates earlier findings that 25% of patients reach recovery one month after single-session CBT (6). The present results add to this observation, indicating that these clinical effects are not only stable during a follow-up of 6 months, but that additional clinical gains may be achieved during this time-frame. This study also provides preliminary evidence that d-cycloserine may improve clinical response to single-session CBT. 71% of participants in the d-cycloserine group fulfilled criteria for recovery at 1- and 6-month follow-up, a rate that is comparable to standard longer-term CBT courses (32). These findings point to the possibility that if used in an optimal way, targeting very early therapeutic learning, d-cycloserine may lead to substantially improved clinical effects.

While these results are promising for the development of more compact psychological-pharmacological combination treatments, there are limitations. First, while we previously found that FDOT threat bias one day after single-session CBT predicted clinical improvement, this observation was not replicated in this study. Recent work into the psychometric properties of the dot probe task suggests weak test-retest reliability, indicating that alternative measures of threat bias might be preferable (33). In line with this recommendation, we found that amygdala threat response was more sensitive to brief treatment than the behavioural measures used, leading to large effect sizes of $d>1$ and predicting clinical recovery. Also, MRI and threat bias measures were only applied after but not before treatment, limiting our ability to evaluate to what degree observed between-group differences on the day after treatment in fact relate to within-subject change. Furthermore, this study was designed to detect neurocognitive rather than clinical effects of d-cycloserine, and even though our clinical observations indicate efficaciousness of single-session CBT with d-cycloserine, these findings need to be validated

in a large-scale trial to reliably evaluate clinical efficacy of this combination intervention. While our participants are representative of those seen in routine clinical care regarding symptom severity and sociodemographic markers, they were recruited from the community rather than being treatment-seekers, and they were unmedicated (34). Future trials will have to investigate whether the facilitative effects of d-cycloserine on single-session CBT found here translate to these cases.

Taken together, this is the first study to identify a neurocognitive mechanism by which d-cycloserine may deploy its augmentative effects on clinical outcomes during exposure therapy for anxiety. The drug substantially amplifies changes in amygdala threat reactivity taking place early in psychological treatment, and the magnitude of these changes predicts clinical improvement across time. Our findings suggest that threat processing might be a landmark parameter for treatment enhancement with d-cycloserine, and they provide a possible explanation as to why d-cycloserine particularly affects clinical outcome when applied early in treatment.

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Figure 1. Overview of study visits and assessments throughout the trial.

Figure 2. A./B. Whole-brain analysis Group x Task interaction: Compared to placebo, d-cycloserine-augmented single-session CBT led to significantly stronger activation in areas of cognitive control during Reappraise versus Maintain on the day after treatment. **C.** Region-of-interest analysis Group x Task interaction: Compared to placebo, cycloserine lead to reduced activation of the right amygdala during Maintain block. **D.** The relationship between amygdala responsivity on the day after single-session CBT and symptom improvement at 1-month follow-up: lower amygdala responsivity on the day after treatment predicts stronger reduction in agoraphobia severity as measured using the self-report Mobility Inventory (MI) across groups. Images thresholded at $Z>2.3$, $p<0.05$, corrected. Note: Error bars show SEM.

Figure 3. Self-report and clinician-rated symptom scores for the two groups at baseline, on the day after treatment and at follow-ups, after multiple imputation of missing data (error bars show SEM). D-cycloserine lead to stronger improvements in state and trait anxiety (STAIS, STAIT), depressivity (BDI) and agoraphobic cognitions (ACQ) on the day after treatment, with group differences remaining significant throughout follow-ups.

Table 1. Demographic and Clinical Key Data in placebo versus d-cycloserine group.

		Placebo		*D-cycloserine*	
		N=16		*N=17*	
		M	*SD*	*M*	*SD*
Sociodemographic Data					
Sex	- Female	13 (81%)		12 (71%)	
	- Male	3 (19%)		5 (29%)	
Age		41.9	13.7	42.1	16.7
Years of education		15.7	3.0	15.5	2.4
Verbal IQ (NART)		112.9	5.9	112.9	6.8
Clinical Key Data					
Panic Duration in years		10.3	12.8	9.8	11.7
Primary Diagnosis	- PD without agoraphobia	6 (38%)		6 (35%)	
	- PD with agoraphobia	10 (62%)		11 (65%)	
Comorbid social phobia		1		3	
Comorbid specific phobia		1		4	
Comorbid OCD		1		0	

Note: NART = National adult reading test; PD = panic disorder without agoraphobia; PDA = panic disorder with agoraphobia

Table 2. Primary and secondary outcomes at baseline, on the day after treatment, and at 1-month and 6-months follow-ups.

		PLACEBO	D-CYCLOSERINE	Adjusted mean diff (SE)	95% CI, p-value, Cohen's d
FDOT (fear)	Next-Day	21.3 (30.8)	2.1 (17.2)	-19.0 (9.3)	-38.04 to 0.14; **p=0.042**; d=0.77
IAT	Baseline	-0.42 (0.52)	-0.27 (0.48)	0.17 (0.15)	-0.13 to 0.48; p=0.25; d=0.30
	Next-Day	-0.62 (0.36)	-0.66 (0.34)	-0.01 (0.15)	-0.32 to 0.29; p=0.93; d=0.11
Right Amygdala	Next-Day	0.25 (0.27)	-0.08 (0.35)	-0.32 (0.14)	-0.61 to -0.03; **p=0.023**; d=1.06
Left Amygdala	Next-Day	0.17 (0.33)	0.05 (0.68)	-0.12 (0.19)	-0.50 to 0.27; p=0.54; d=0.22
STAI-S (20-80)	Baseline	42.8 (7.3)	43.9 (12.5)	..	..
	Next-Day	44.9 (8.8)	34.6 (11.0)	-10.67 (3.57)	-17.83 to -3.51; **p=0.0041;** d=1.01
	1-month	39.7 (9.2)	32.2 (10.9)	-7.92 (3.71)	-15.30 to -0.54; **p=0.036**; d=0.74
	6-months	38.9 (12.8)	31.4 (9.7)	-8.05 (3.95)	-15.94 to -0.16; **p=.046**; d=0.66
STAI-T (20-80)	Baseline	52.2 (6.7)	52.4 (9.4)	..	..
	Next-Day	50.8 (8.3)	47.1 (10.7)	-3.53 (3.60)	-10.74 to 3.68; p=0.33; d=0.39
	1-month	46.3 (11.8)	38.2 (12.6)	-7.99 (3.74)	-15.44 to -0.54; **p=0.036**; d=0.66
	6-months	44.4 (12.8)	35.2 (10.3)	-9.20 (3.89)	-16.96 to -1.44; **p=0.021**; d=0.79
BDI (0-63)	Baseline	16.8 (9.4)	17.3 (11.7)	..	..
	Next-Day	18.3 (10.1)	13.2 (10.0)	-5.26 (2.46)	-10.19 to -0.33; **p=0.037**; d=0.51
	1-month	12.5 (8.0)	11.1 (10.4)	-1.57 (2.56)	-6.66 to 3.53; p=0.54; d=0.15
	6-months	12.0 (10.1)	6.3 (6.6)	-5.86 (2.72)	-11.29 to -0.42; **p=0.035**; d=0.67
ACQ (1.0-5.0)	Baseline	2.5 (0.5)	2.4 (0.7)	..	..
	Next-Day	2.6 (0.5)	2.3 (0.6)	-0.21 (0.19)	-0.60 to 0.17; p=0.27; d=0.30
	1-month	2.2 (0.7)	1.9 (0.7)	-0.29 (0.20)	-0.69 to 0.11; p=0.16; d=0.43
	6-months	2.0 (0.7)	1.6 (0.6)	-0.37 (0.22)	-0.81 to 0.07; p=0.095; d=0.61
BSQ (1.0-5.0)	Baseline	3.2 (0.8)	3.2 (0.7)	..	..
	Next-Day	3.2 (0.8)	3.0 (0.8)	-0.12 (0.27)	-0.66 to 0.41; p=0.65; d=0.14
	1-month	2.6 (0.8)	2.5 (0.8)	-0.06 (0.27)	-0.60 to 0.48; p=0.82; d=0.13
	6-months	2.4 (1.0)	2.2 (1.0)	-0.12 (0.29)	-0.71 to 0.47; p=0.68; d=0.20
MI (1.0-5.0)	Baseline	2.3 (0.7)	2.1 (0.9)	..	..
	Next-Day	2.2 (0.5)	2.1 (0.8)	-0.038 (0.16)	-0.35 to 0.27; p=0.81; d=0.15
	1-month	1.9 (0.6)	1.7 (0.8)	-0.12 (0.16)	-0.43 to 0.20; p=0.46; d=0.28
	6-months	1.8 (0.5)	1.5 (0.6)	-0.18 (0.18)	-0.54 to 0.19; p=0.34; d=0.54
PAS (0-4)	Baseline	1.8 (1.1)	1.7 (1.2)	..	..
	Next-Day	1.8 (1.0)	1.5 (1.0)	-0.26 (0.27)	-0.79 to 0.27; p=0.33; d=0.30
	1-month	0.7 (0.8)	0.5 (1.0)	-0.22 (0.30)	-0.81 to 0.37; p=0.46; d=0.22
	6-months	0.5 (0.8)	0.3 (0.7)	-0.19 (0.30)	-0.79 to 0.40; p=0.52; d=0.27
PDSS (0-28)	Baseline	11.8 (4.0)	11.6 (4.6)	..	..
	1-month	6.1 (4.4)	4.8 (5.3)	-1.31 (1.39)	-4.11 to 1.48, p=0.35, d=0.27
	6-months	4.6 (3.8)	3.4 (3.8)	-1.19 (1.45)	-4.12 to 1.73, p=0.41; d=0.24
Stress (0-100)	Baseline	41.1 (25.9)	43.4 (24.7)	..	..
	Next-Day	29.2 (21.5)	17.8 (14.6)	-14.09 (4.55)	-23.0 to -5.1; **p=0.0021**; d=0.62
	1-month	19.1 (17.3)	13.0 (15.2)	-8.84 (4.63)	-17.9 to 0.3; p=0.057; d=0.37
	6-months	20.0 (22.0)	11.5 (15.6)	-11.20 (4.67)	-20.4 -2.0; **p=0.017**; d=0.45

Note: FDOT = Faces Dot Probe Task; IAT = Implicit Association Task; STAIS/ STAIT = state trait anxiety inventory; BDI = Beck Depression Inventory; ACQ = Agoraphobic Cognitions Questionnaire; BSQ = Body

Sensations Questionnaire; MI = Mobility Inventory; PAS = Panic Attack Scale; PDSS = Panic Disorder Severity Scale.

Figure 1

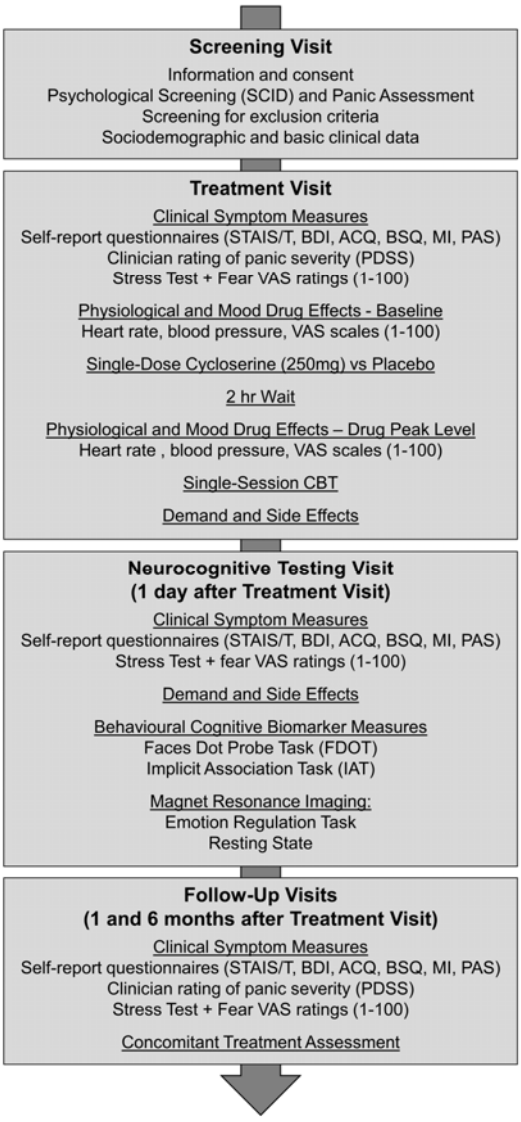
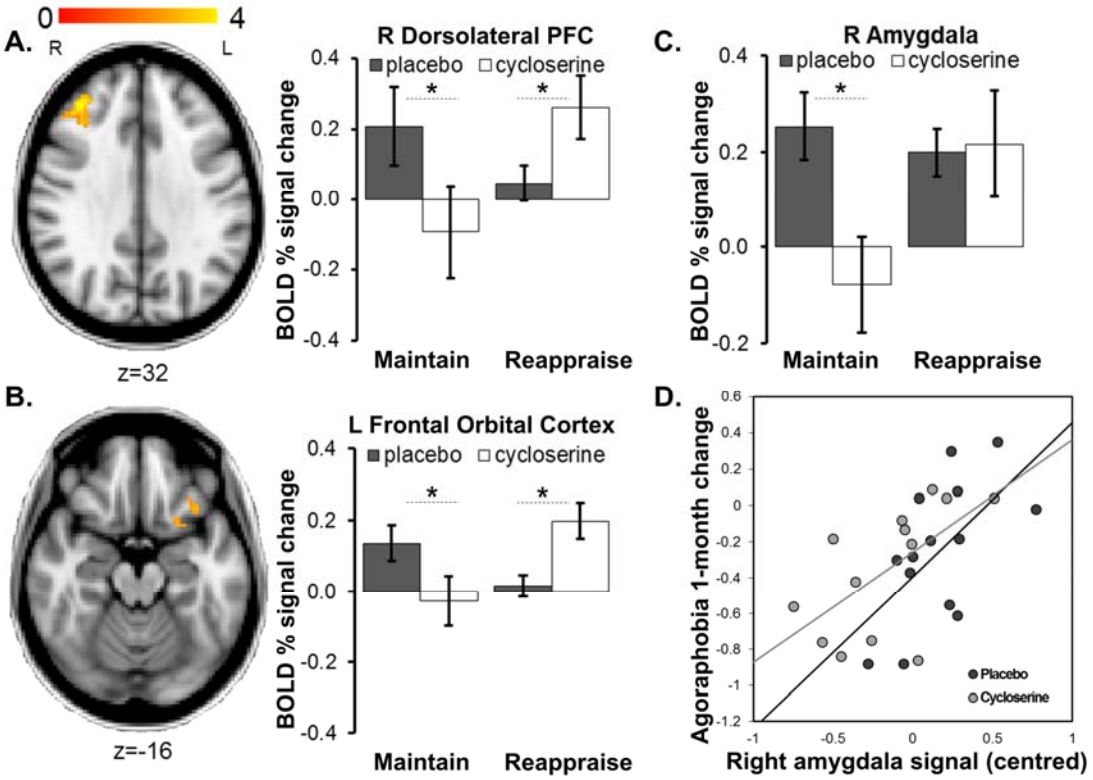

Screening Visit
Information and consent
Psychological Screening (SCID) and Panic Assessment
Screening for exclusion criteria
Sociodemographic and basic clinical data
Treatment Visit
Clinical Symptom Measures
Self-report questionnaires (STAIS/T, BDI, ACQ, BSQ, MI, PAS)
Clinician rating of panic severity (PDSS)
Stress Test + Fear VAS ratings (1-100)
Physiological and Mood Drug Effects - Baseline
Heart rate, blood pressure, VAS scales (1-100)
Single-Dose Cycloserine (250mg) vs Placebo
2 hr Wait
Physiological and Mood Drug Effects – Drug Peak Level
Heart rate , blood pressure, VAS scales (1-100)
Single-Session CBT
Demand and Side Effects
Neurocognitive Testing Visit
(1 day after Treatment Visit)
Clinical Symptom Measures
Self-report questionnaires (STAIS/T, BDI, ACQ, BSQ, MI, PAS)
Stress Test + fear VAS ratings (1-100)
Demand and Side Effects
Behavioural Cognitive Biomarker Measures
Faces Dot Probe Task (FDOT)
Implicit Association Task (IAT)
Magnet Resonance Imaging:
Emotion Regulation Task
Resting State
Follow-Up Visits
(1 and 6 months after Treatment Visit)
Clinical Symptom Measures
Self-report questionnaires (STAIS/T, BDI, ACQ, BSQ, MI, PAS)
Clinician rating of panic severity (PDSS)
Stress Test + Fear VAS ratings (1-100)
Concomitant Treatment Assessment

Figure 2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R
L
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placebo
cycloserine
BOLD % signal change
Maintain
Reappraise
C.
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placebo
cycloserine
BOLD % signal change
Maintain
Reappraise
B.
z=-16
L Frontal Orbital Cortex
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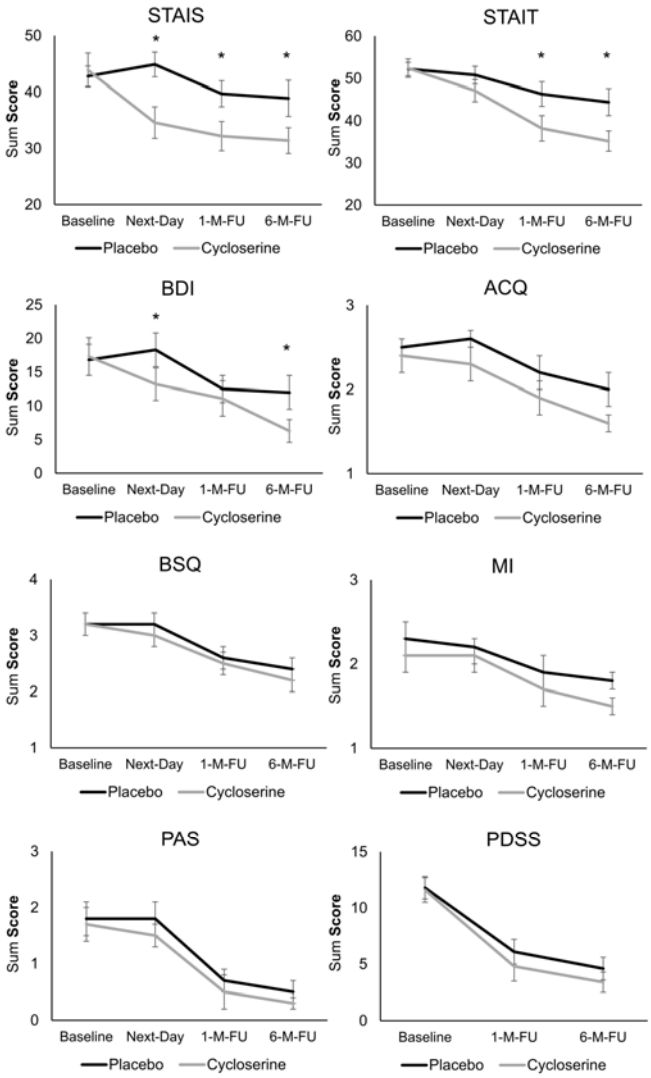
Maintain
Reappraise
D.
Agoraphobia 1-month change
Placebo
Cycloserine
Right amygdala signal (centred)

Figure 3


STAIS
STAIT
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PDSS
Sum Score
Baseline
Next-Day
1-M-FU
6-M-FU
Placebo
Cycloserine