Exposure Therapy, D-Cycloserine, and Functional Magnetic Resonance Imaging in Patients With Snake Phobia: A Randomized Pilot Study

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ABSTRACT

Objective: D-Cycloserine may enhance fear extinction. The effects of D-cycloserine on human brain function are not well understood, with findings suggesting that D-cycloserine could augment exposure therapy via its effects on the neural substrates of emotional learning and extinction or by acting upon different neural pathways. The aim of this exploratory study was to investigate differences in neural response in patients receiving D-cycloserine or placebo in addition to exposure therapy.

Method: Twenty adults with snake phobia (*DSM-IV* specific phobia) received 50 mg of D-cycloserine or placebo (double-blind, randomized) 1 hour prior to a single session of graded exposure therapy in an outpatient specialty clinic. One week before and after treatment, patients completed a clinical examination and snake-stimuli symptom provocation functional magnetic resonance imaging (fMRI) task (primary outcome measure).

Results: The D-cycloserine and placebo groups responded equally well to treatment, although the D-cycloserine patients reached the top of the exposure hierarchy more quickly ($t = 2.61$, $P < .05$). Only right dorsolateral prefrontal cortex showed an equivalent decrease in hyperactivation to snake stimuli in both groups. Compared to placebo, D-cycloserine augmentation resulted in different ventromedial prefrontal brain activation during processing of phobic stimuli, including enhanced medial orbitofrontal ($F = 11.52$, $P = .001$) and subgenual anterior cingulate activation ($F = 7.41$, $P = .008$) and normalized perigenual cingulate "deactivation" ($F = 3.85$, $P = .05$) to snakes.

Conclusions: A single administration of D-cycloserine combined with exposure therapy can lead to lasting changes in ventromedial and other prefrontal cortex response to phobic stimuli. These changes are qualitatively different from those seen in patients receiving exposure therapy without D-cycloserine.

Trial Registration: ClinicalTrials.gov identifier: NCT01450306

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The partial *N*-methyl-D-aspartate agonist D-cycloserine is thought to enhance or accelerate the effects of exposure therapy.[1] In rats, D-cycloserine enhancement of extinction training has been observed with direct infusion to amygdala,[2,3] a region associated with symptom provocation in phobic humans,[4–6] although phobic activation has also been noted in human insula,[5,7] anterior cingulate cortex,[5–8] and other prefrontal and parahippocampal regions.[6,8–10] During extinction trials, these regions are reengaged,[11,12] and amygdala responses habituate.[13–16] Extinction trials also engage lateral,[15,17,18] dorsomedial and ventromedial,[16,18] and orbitofrontal[13,19,20] regions, which may inhibit learned fearful associations through action on amygdala responsiveness[21] or enhance fear-related cognitive processing.[22] Phobic patients receiving exposure therapy exhibit increases in orbitofrontal cortex activity[8] but decreases in amygdala, insula, anterior cingulate cortex, and parahippocampal gyrus.[10,23,24]

It is possible that D-cycloserine augments exposure efficacy by enhancing exposure-induced neural activity changes in amygdala or in various prefrontal regions. In healthy subjects, D-cycloserine acutely both decreases amygdala response to emotional faces[25] and increases hippocampal acitivity.[26] In phobic patients, D-cycloserine acutely increased activity in anterior prefrontal cortex, anterior cingulate, and insula during symptom provocation.[27] However, acute D-cycloserine effects on fear circuit regions during exposure to anxiety-provoking stimuli do not reflect the ultimate changes to the system following successful treatment.

The present pilot study examined changes in brain response due to D-cycloserine (vs placebo) augmentation of exposure therapy in phobic patients 1 week posttreatment. We predicted exposure treatment effects consistent with previous studies of acute D-cycloserine administration,[25,27] in which activity in amygdala, insula, and anterior cingulate cortex to phobic stimuli would decrease, but ventromedial/orbitofrontal prefrontal activity would increase. We then hypothesized that a quantitative effect of D-cycloserine augmentation would be observed as greater ventromedial increases and greater other fear circuit decreases in patients treated with exposure + D-cycloserine relative to exposure + placebo. Alternatively, if the effect of D-cycloserine was a qualitative change in neural response to previously anxiety-provoking stimuli, we predicted exposure + D-cycloserine–treated patients would engage different brain regions or show different activation profiles.

METHOD

Participants

Participants underwent informed consent using procedures approved by Hartford Hospital's Institutional Review Board. Of 26 screened participants, 20 patients, right-handed males and females aged 20–63 years, were randomized. All were diagnosed with specific phobia according to the Mini-International Neuropsychiatric Interview (MINI).[28] Snake phobia severity was measured by using the Snake Questionnaire,[29] a

30-item questionnaire with good internal consistency[29] and sensitivity to treatment effects.[30] All participants scored 18 or higher on the Snake Questionnaire, which is 1.5 standard deviations above normative mean[31] (3 excluded due to low scores); had not had previous treatment for snake phobia (0 excluded due to previous treatment); did not have another psychiatric disorder that was more severe than the snake phobia in the interviewer's judgment (0 excluded due to comorbidity); and were suitable for functional magnetic resonance imaging (fMRI) (3 excluded due to fMRI unsuitability). Clinical Global Impressions-Severity of illness (CGI-S) and -Improvement (CGI-I) scales[32] were used to record global severity of illness and improvement. The study was registered at ClinicalTrials.gov (identifier: NCT01450306).

Study Procedure Overview

The study used a double-blind, placebo-controlled design to test the effects of D-cycloserine on phobia severity and fMRI-measured brain function during a symptom provocation paradigm before and after a 1-session exposure treatment. Participants were randomly assigned to exposure + D-cycloserine or exposure + placebo groups in equal numbers (n = 10 per group). One week before treatment, participants completed the Snake Questionnaire, MINI, CGI-S, and fMRI assessments. On a separate day, they underwent exposure therapy in an outpatient specialty clinic (see Exposure Therapy). Sixty minutes prior to the exposure treatment session, participants were administered 50 mg of D-cycloserine (the most common dose in human fear extinction studies[1]) or an identically packaged placebo capsule. One patient in the exposure + D-cycloserine group reported mild nausea during the hour following administration. One week following this treatment session, participants underwent another clinical and fMRI assessment.

Exposure Therapy

The exposure therapy was modeled after Öst's[33,34] single-session treatment for specific phobias, which includes graded in vivo exposure to a nonvenomous, ~2 foot corn snake in a clear tank and participant modeling for a maximum of 3 hours. A standardized 13-step hierarchy was used in which participants were asked to increase physical proximity and involvement with the snake ranging from sitting in the room to allowing it to lick his/her face. The number of steps completed and minutes required to reach the final step of the standardized hierarchy were recorded. After the session, participants were instructed to continue exposure therapy on their own (eg, viewing snake pictures on the Internet) before returning for posttreatment assessment 1 week later. Two patients from the exposure + D-cycloserine group discontinued at midtreatment due to excessive fear and were withdrawn from further analysis. One exposure + D-cycloserine patient was lost to follow-up and was withdrawn from fMRI analysis (although clinical outcome data were collected). One exposure + placebo patient was considered not to have responded favorably to treatment (based on CGI-I rating) and was not included in fMRI analyses so that D-cycloserine versus placebo-related differences in neural function would not be confounded by treatment response or inadequate dose of exposure.

Clinical Points

- D-Cycloserine may accelerate the effects of exposure-based therapy for anxiety disorders.
- One week after treatment, phobic patients who received exposure plus D-cycloserine exhibited a qualitatively different neural response to feared stimuli compared to patients who received exposure plus placebo.
- D-Cycloserine augmentation of exposure therapy may enhance ventromedial prefrontal brain activity during and after exposures to phobic stimuli.

Posttreatment Assessment and Neuroimaging

One week after treatment, participants again completed the Snake Questionnaire, and the interviewer completed the CGI-S and CGI-I ratings. CGI-I ratings < 3 indicated treatment response. They then performed the fMRI symptom provocation task again.

Functional Neuroimaging

The fMRI symptom provocation task, the study's primary outcome measure, presented participants stimuli slides having 2 pictures of various animals or 2 pictures of various motor vehicles. Participants were instructed to press a button with their right index finger if the 2 pictures contained the same object type, and another button with their middle finger if different. The same/different instruction ensured patients attended to stimuli content. Half of all stimuli were object-type matches. All animal stimuli contained at least 1 picture of a snake, and all transportation stimuli displayed at least 1 car. The order of left- versus right-side, object type, and "same" versus "different" presentation was distributed equiprobably throughout the task. No specific stimulus was repeated. The fMRI block design optimized detection of hemodynamic amplitude difference between 2 conditions (snake stimulus processing vs processing of the transportation control condition). Each condition had 10 blocks of 6 pictures presented at 3 seconds each (18 sec). Each block was interspersed with 12-second crosshair fixation periods for a total of 9.8 minutes' task time. The task was implemented in E-Prime (Psychology Software Tools; Sharpsburg, Pennsylvania) and presented to the participant via high resolution screen seen using a mirror attached to the head coil. A magnetic resonance–compatible fiber optic response device acquired subject responses for offline assessment.

Imaging was implemented on a Siemens 3T Allegra scanner (Siemens Medical Solutions; Malvern, Pennsylvania). Head motion was restricted using a custom built apparatus that interfaced with the head coil. Data were realigned using INRIAlign, spatially normalized using custom linear and nonlinear algorithms to standardized Montreal Neurological

Table 1. Sample Characteristics at Pretreatment[a]

Characteristic	Placebo Group (n = 10)	D-Cycloserine Group (n = 10)	*t*	χ^2
Age, mean (SD), y	39.00 (13.91)	34.60 (12.69)	0.74	
Male sex, n (%)	4 (40)	4 (40)		0.00
White, n (%)	8 (80)	6 (60)		0.95
Comorbid anxiety disorder, n (%)	0 (0)	0 (0)		0.00
Comorbid depressive disorder, n (%)	0 (0)	1 (10)		1.05
Taking psychiatric medications, n (%)	3 (30)	2 (20)		0.27
Duration of illness, mean (SD), y	31.80 (14.33)	27.60 (13.87)	0.67	
Snake Questionnaire score, mean (SD)	23.90 (2.24)	22.00 (3.43)	1.47	
CGI-S score, mean (SD)	4.50 (0.71)	4.50 (0.71)	0.00	

[a]All statistical tests were nonsignificant.
Abbreviation: CGI-S = Clinical Global Impressions-Severity of illness scale.

Institute space,[35] smoothed, and analyzed using Statistical Parametric Mapping (Wellcome Trust Centre for Neuroimaging; London, England). Only 1 echo planar imaging time series (for a patient in the exposure + D-cycloserine group at pretreatment) had overall displacement exceeding 1 voxel length in any plane, but it was retained because the statistical maps did not appear contaminated by movement artifact. Head displacement and rotation measurements were used as covariates to minimize the impact of small movements on activation estimates. A 128-second high pass filter was incorporated into each participant's model to remove noise associated with low frequency confounds (eg, respiratory artifact).

RESULTS

Sample Description

Descriptive information about the sample (all randomized patients) is shown in Table 1. The exposure + placebo and exposure + D-cycloserine groups did not significantly differ by age, gender, or race/ethnicity. Only a few participants in each condition took psychiatric medications (1 each of paroxetine and desvenlafaxine in the exposure + D-cycloserine group, 1 each of duloxetine, bupropion, and sertraline in the exposure + placebo group). Medications were reportedly taken in each case for depression, although only 1 patient met criteria for a depressive disorder.

Clinical Outcomes

Treatment clinical outcomes (all randomized patients) are shown in Table 2. All but 3 participants (2 exposure + D-cycloserine and 1 exposure + placebo) were judged by their clinician (CGI-I) to respond to exposure treatment. A 2 (group: exposure + placebo, exposure + D-cycloserine) × 2 (time: pretreatment, posttreatment) mixed factor analysis of variance verified improvement following treatment for both Snake Questionnaire and CGI-S clinical measurements. The group × treatment interaction was not significant, indicating that the exposure + D-cycloserine and exposure + placebo groups showed equivalent reductions in snake phobia severity. The exposure + D-cycloserine group completed the treatment in a significantly shorter amount of time than did the exposure + placebo group.

Identifying Phobia-Specific Brain Regions of Interest

We first identified which brain regions responded more greatly to phobic symptom provocation prior to treatment in all participants using 1-sample *t* tests. Whole-brain correction for multiple comparisons employed false discovery rate methods,[36] $q < .05$. Regions of interest for treatment effect hypothesis testing were defined as 6-mm radius spheres centered on the peak effects from this analysis. To choose our final slate of 21 nonoverlapping regions of interest, we synthesized evidence for acute D-cycloserine effects and brain regions believed to be key mediators of exposure treatment effects (as reviewed in the introduction). These included bilateral amygdala and numerous discrete regions within the frontal lobe (bilateral middle frontal gyrus, inferior frontal gyrus, insula, perigenual and subgenual cingulate, and several ventromedial areas that included 2 discrete orbitofrontal cortex regions in each hemisphere). We also added dorsal anterior cingulate cortex as an a priori region of interest based on previous work.[27] As shown in Figure 1, viewing snake pictures (versus control pictures) was associated with significantly greater activity in numerous brain regions, including amygdala; middle, inferior, and superior frontal gyrus; anterior cingulate cortex; insula; medial orbitofrontal cortex; right inferior parietal lobule; and cerebellum.

fMRI Treatment Effects

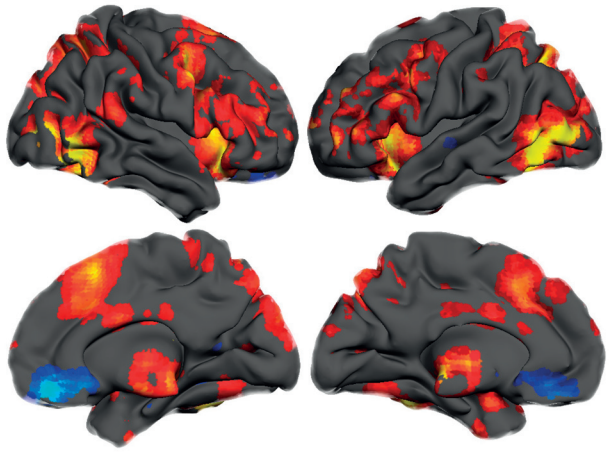
We then used a full factorial design (group × treatment × task) to examine treatment-related differences in the obtained regions of interest. For regions of interest with a significant 3-way interaction, we plotted mean region of interest estimates of brain activity to characterize whether D-cycloserine effects were quantitative or qualitative. Because this was an exploratory study, we reported any evidence for a voxelwise $P < .05$ uncorrected effect (or statistical trend) within each region of interest, noting which regions of interest survived a Holm-Bonferroni correction for multiple comparisons.[37] Treatment effect regions of interest are listed in Table 3, organized by profile of activation changes in the exposure + placebo versus exposure + D-cycloserine groups. Only 1 region of interest, right dorsolateral prefrontal cortex, showed an equivalent decrease in hyperactivation to snake stimuli in both groups. The other 8 brain regions with significant treatment-related reduction of phobic hyperactivity showed a characteristically different type of "reduction" of hyperfunction to snake stimuli in exposure + D-cycloserine versus exposure + placebo. In the exposure + placebo group after treatment, these brain regions typically showed relatively lower activation to snake pictures than to control pictures, sometimes even a "deactivation" to snake pictures (ie, negative signal change) relative to the implicit baseline (not depicted). In contrast, for the exposure + D-cycloserine group, there were no deactivations and no

Table 2. Clinical Outcomes for All Randomized Patients

Variable	Exposure + Placebo (n = 10)		Exposure +D-Cycloserine (n = 10)		Group × Time Effect	Posttreatment Comparison
	Pretreatment	Posttreatment	Pretreatment	Posttreatment		
Snake Questionnaire score, mean (SD)	23.90 (2.24)	9.60 (5.99)	22.00 (3.43)	9.70 (6.18)	$F_{1,18} = 0.64$	
CGI-S score, mean (SD)	4.50 (0.71)	2.70 (0.68)	4.50 (0.71)	3.00 (1.15)	$F_{1,18} = 1.20$	
CGI-I responder, n (%)	…	9 (90)	…	8 (80)		$\chi^2_1 = 0.39$
Duration to treatment completion, mean (SD), min	…	108.10 (27.11)	…	82.80 (14.32)		$t_{18} = 2.61$*

*$P < .05$.
Abbreviations: CGI-I = Clinical Global Impressions-Improvement scale, CGI-S = Clinical Global Impressions-Severity of illness scale.

Figure 1. Brain Regions Showing Significant ($q < .05$, false discovery rate) Differences in Neural Response to Snake Versus Control Pictures, Collapsed Across Study Groups and Evaluations



instances where response to snakes was less than response to control stimuli. Instead, the reductions were reductions in the amount of positive signal change (ie, "activation") to snake stimuli, but the level of this activity never was less than that seen to control stimuli. However, none of these regions of interest demonstrated significantly different 3-way group interaction effects.

Qualitative differences in hemodynamic activity to snake and control stimuli also were detected in 9 regions of interest, including right insula, bilateral inferior frontal gyrus, all 4 medial orbitofrontal cortex regions of interest, and subgenual cingulate (Figure 2). In the exposure + placebo group, left and right ventrolateral prefrontal cortex (inferior frontal gyrus) and right insula showed a reduction of pretreatment hyperactivation to snakes to an inhibited profile. In contrast, these brain regions continued to overengage to snakes in the exposure + D-cycloserine group. In 2 regions of orbitofrontal cortex, D-cycloserine actually enhanced the hyperfunction to snakes. In the other 2 medial orbitofrontal cortex regions of interest, snakes deactivated these regions, while control stimuli activated them prior to treatment. Following treatment, the exposure + placebo group showed little signal change to either stimulus class, but the exposure + D-cycloserine group activated to snakes. Finally, subgenual anterior cingulated cortex showed greater deactivation to snakes than to control stimuli prior to treatment in both study groups. This profile was unaffected by exposure + placebo, but deactivation to snakes reversed in the exposure + D-cycloserine group. Left amygdala reduced activation to snakes in the exposure + placebo group but not in the exposure + D-cycloserine group, though this interaction was only at a statistical trend level ($P < .08$).

Post hoc tests indicated that there were some differences in the exposure + D-cycloserine and exposure + placebo groups' baseline brain response to the task. The pretreatment group × task interaction was significantly different for right middle frontal gyrus (Brodmann area [BA] 9/6) ($F_{8,64} = 5.36$, $P < .03$), left insula ($F_{8,64} = 5.54$, $P < .02$), right insula/inferior frontal gyrus (BA 47) ($F_{8,64} = 3.92$, $P < .05$), right medial orbitofrontal ($F_{8,64} = 6.57$, $P < .01$), left superior frontal gyrus (BA 10) ($F_{8,64} = 4.73$, $P < .03$), and right amygdala ($F_{8,64} = 4.02$, $P < .05$), with the exposure + placebo group having slightly less hemodynamic response to snake stimuli.

Correlations Between Posttreatment Snake-Elicited Hemodynamic Activity and Snake Questionnaire Scores

In the exposure + placebo group, reduced Snake Questionnaire score was significantly ($P < .05$) and positively correlated with reduction in snake-elicited hemodynamic activity in right middle frontal gyrus ($r = 0.615$), right insula/inferior frontal gyrus ($r = 0.581$), right superior frontal gyrus ($r = 0.579$), left ($r = 0.611$) and right ($r = 0.584$) medial orbitofrontal cortex, left orbitofrontal cortex ($r = 0.494$), and left ($r = 0.593$) and right ($r = 0.506$) amygdala. In contrast, for the exposure + D-cycloserine group, significant ($P < .05$) correlations were found only for perigenual cingulate cortex ($r = -0.794$) and left ventrolateral prefrontal cortex ($r = 0.266$) regions of interest.

DISCUSSION

Whereas animal research has primarily emphasized D-cycloserine effects in amygdala, the primary finding of the present study was that D-cycloserine augmentation results in different ventromedial prefrontal cortex activation during processing of phobic stimuli, including enhanced medial orbitofrontal cortex and subgenual anterior cingulate cortex activation and normalized perigenual cingulate deactivation to snakes. Some researchers have proposed that fear extinction is a form of automatic cognitive control,[38] wherein orbitofrontal regions in conjunction with perigenual and subgenual cingulate regulate amygdala respond in fear

Table 3. Profile of Treatment Effects for Patients Receiving Placebo Versus D-Cycloserine in Addition to Exposure Therapy in Brain Regions of Interest Defined by Symptom Provocation

	Placebo Time × Task (n = 9)				D-Cycloserine Time × Task (n = 7)				Group × Time × Task			
Region of Interest	Peak Region of Interest x,y,z Coordinates	*F*	*P*	Effect Size (Cohen *d*)	Peak Region of Interest x,y,z Coordinates	*F*	*P*	Effect Size (Cohen *d*)	Peak Region of Interest x,y,z Coordinates	*F*	*P*	Effect Size (Cohen *d*)
Reduction of snake activity in both placebo and D-Cycloserine												
R middle frontal gyrus (BA 9/6)	54,15,45	7.64	.007	0.66	54,6,48	4.53	.04	0.51	54,15,45	1.62	NS	…
Placebo inhibition of snake activity versus D-Cycloserine reduction												
L inferior frontal gyrus (BA 47)	−39,21,−6	10.23[a]	.002	0.76	−42,24,−6	8.04	.006	0.68	−42,24,−3	0.38	NS	…
L insula (BA 13)	−39,12,−9	13.51[a]	.0005	0.88	−42,6,−6	8.05	.006	0.68	−42,9,−12	2.85[b]	.09	0.40
R insula	39,9,3	14.09[a]	.0004	0.90	42,12,6	4.83	.03	0.53	48,12,6	1.55	NS	…
L insula/inferior frontal gyrus (BA 47)	−36,18,−9	13.97[a]	.0004	0.89	−36,24,−15	6.23	.02	0.60	−33,15,−12	2.53	NS	…
R insula/inferior frontal gyrus (BA 47)	33,24,−15	11.93[a]	.001	0.83	39,24,−21	4.68	.03	0.52	36,21,−12	1.31	NS	…
L superior frontal gyrus (BA 10)	−36,54,24	12.49[a]	.001	0.84	−30,54,30	7.48	.008	0.65	−33,54,21	2.64	NS	…
R superior frontal gyrus (BA 10)	24,57,30	14.76[a]	.0003	0.92	30,57,30	3.13	.08	0.42	27,54,27	3.44[b]	.07	0.44
Dorsal anterior cingulate	3,27,33	13.00[a]	.001	0.86	0,21,33	2.15	NS	…	0,30,30	3.06[b]	.08	0.42
Qualitatively different activation profiles to snake or control stimuli in placebo versus D-cycloserine groups												
L middle/inferior frontal gyri (BA 9)	−60,6,30	5.27	.025	0.55	−60,9,33	2.25	NS	…	−57,9,27	1.09	NS	…
R inferior frontal gyrus/insula (BA 13/45)	42,30,3	12.44[a]	.001	0.84	42,36,9	1.11	NS	…	39,33,6	5.36	.02	0.55
R inferior frontal gyrus	27,24,0	24.27[a]	.00001	1.18	24,27−3	0.90	NS	…	27,27,0	11.95[a]	.001	0.83
L inferior frontal gyrus (BA 46)	−48,42,9	11.46[a]	.001	0.81	−48,45,6	2.54	NS	…	−48,39,9	6.99	.01	0.63
L medial orbitofrontal-1	−21,36,−15	2.31	NS	…	−15,33,−15	12.83[a]	.001	0.86	−15,30,−15	3.91	.05	0.47
R medial orbitofrontal-1	18,27,−18	1.79	NS	…	21,30,−18	20.95[a]	.00002	1.09	21,33,−15	11.52[a]	.001	0.81
L orbitofrontal-2	−27,21,−15	10.22[a]	.002	0.76	−24,30,−18	8.06	.006	0.68	−21,27,−15	4.41	.04	0.50
R orbitofrontal-2	33,30,−12	14.15[a]	.0004	0.90	24,33,−15	10.94[a]	.002	0.79	24,33,−9	9.45	.003	0.73
Perigenual anterior cingulate	3,30,−12	1.16	NS	0.26	6,27,−9	7.07	.01	0.64	3,30,−6	3.85	.05	0.47
Subgenual anterior cingulate	0,9,0	3.87	.05	0.47	3,9,−6	5.47	.02	0.56	3,9,−3	7.41	.008	0.65
L amygdala	−21,3,−21	7.00	.01	0.63	−21,0,−15	2.03	NS	…	−21,3,−21	3.01[b]	.08	0.41
R amygdala	21,−6,−18	2.60	NS	…	24,−3,−24	1.71	NS	…	21,−6,−15	1.56	NS	…

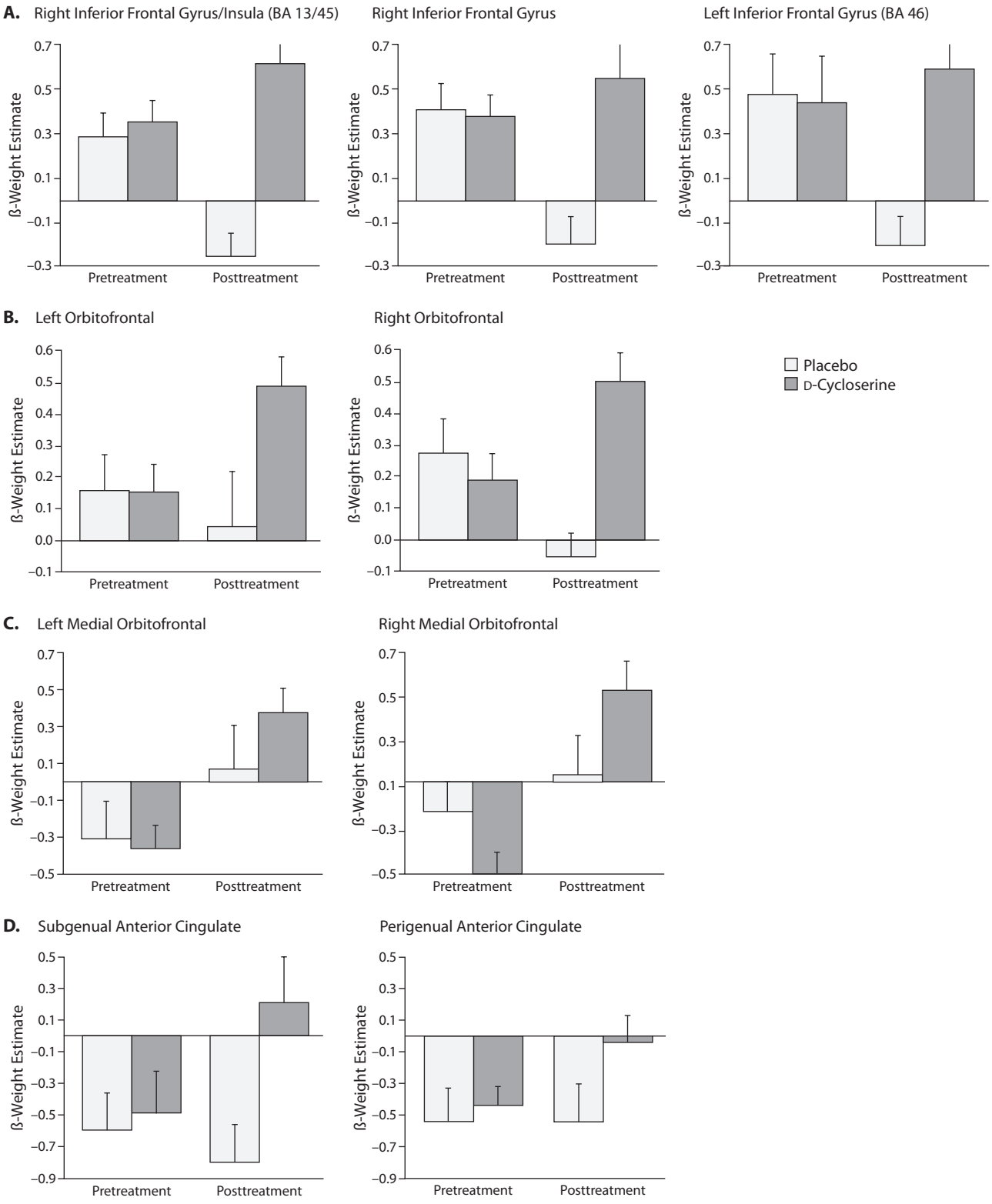
[a]Indicates effect survives Holm-Bonferroni correction for multiple comparisons. [b]Indicates a statistical trend.
Abbreviations: BA = Brodmann area, L = left, NS = nonsignificant, R = right.

extinction trials.[12,16] Indeed, ventromedial prefrontal cortex lesions in animals result in resistance to fear extinction.[39,40] Others[41] have proposed that ventromedial prefrontal cortex helps integrate information from separate limbic structures used to suppress irrelevant neural representations, making it an ideal point of augmentation for exposure treatment. It appears that a single administration of D-cycloserine, combined with exposure therapy, can lead to characteristically different, lasting changes in ventromedial response to (previously) phobic stimuli. The present findings extend previous work[25–27] showing acute D-cycloserine effects on anterior cingulate cortex, insula, and anterior prefrontal cortex by demonstrating that D-cycloserine–related neural changes are evident a week after treatment.

Another difference between the exposure + placebo versus exposure + D-cycloserine groups was that many prefrontal cortex regions did not reduce activation in D-cycloserine–treated patients as they did for those who received placebo. As seen in previous reports,[10,23,24] nearly all regions examined responded to exposure + placebo by reducing activation to snake stimuli. Individual variation in treatment success also was linearly related to reduction of snake-elicited hyperactivation in these regions. Indeed, only bilateral medial orbitofrontal cortex and right amygdala failed to reduce snake hyperactivity relative to control stimuli in the exposure + placebo group after treatment. In contrast, snake-elicited hyperactivity for the exposure + D-cycloserine group in left dorsolateral prefrontal cortex, 2 regions of right inferior frontal gyrus, and bilateral orbitofrontal gyrus remained unchanged, or even appeared to increase activity relative to pretreatment levels. These effects were statistically different between groups. Left amygdala also showed this profile, albeit at a trend significance level despite its notable medium effect size (Cohen $d = 0.41$). As noted by Aupperle et al,[27] increased activity in these regions may signal enhanced attention to cognitive representations of the feared stimulus,[22,42] more complete integration of information regarding the fear experience and stronger cognitive representation of fear, or more adaptive self-appraisals during exposure.[43]

Nine of 21 regions of interest showed decreases of snake-elicited hyperactivity in both exposure + placebo and exposure + D-cycloserine groups. However, only 1 region of interest examined (right dorsolateral prefrontal cortex) showed reduced activation in both groups. For the other 8 regions of interest, hemodynamic response to snakes in exposure + placebo was actually *lower* than to control items, whereas, in exposure + D-cycloserine, the amount of hyperactivation to snakes simply lessened. Half of the regions of interest showing this effect contained insular cortex, known to integrate somatic signals for interoceptive awareness and

Figure 2. Regions of Interest With Statistically Significant Differences in Neural Response to Snake Versus Control Pictures for Patients Receiving Exposure + D-Cycloserine or Exposure + Placebo[a]



[a]The y-axis of each subfigure represents β-weight estimate differences between general linear model-estimated hemodynamic response to snakes versus control condition for each region: (A) ventrolateral prefrontal regions showing increased hemodynamic response to snakes in exposure + D-cycloserine, (B) orbitofrontal regions showing increased hemodynamic response to snakes in exposure + D-cycloserine, (C) medial orbitofrontal regions showing a switch from negative signal change (ie, "deactivation") to snakes to activation and positive change to control items with little or no negative signal change posttreatment in exposure + D-cycloserine, (D) ventral anterior cingulate subregions showing normalized deactivation in exposure + D-cycloserine. Positive numbers indicate a hemodynamic response of snake > control.

Abbreviation: BA = Brodmann area.

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participate in networks needed to determine salience and attentional focus on external stimuli.[44–47] If replicated by future studies having greater statistical power, regions showing this profile might represent a mechanism important to typical exposure treatment, whereby previously established links between intense fear and conscious perception of the phobic target are inhibited (ie, relative to control items) to prevent an anxiety response.

This was an exploratory study, limited by the small sample size (therefore, the study could have been underpowered for some contrasts), the presence of medications in some patients, and reliance on voxelwise *P* values, which can capitalize on chance. Although we noted which effects survived corrections for multiple comparisons, some results must be viewed cautiously until replication. Although the phobic participants exhibited meaningful changes in brain activation from pretreatment to posttreatment, it would be helpful to determine if these changes reflect normalization relative to nonphobic controls. It is possible that D-cycloserine affects other brain systems outside study regions of interest that might be important to therapeutic effects. Despite random assignment, patients in the exposure + D-cycloserine group had greater baseline response to snakes in several regions. Future work should clarify differences in both pretreatment and posttreatment brain activity for treatment nonresponders, which was not possible given this study's sample size. In contrast to previous studies showing that D-cycloserine adds to the efficacy of exposure therapy,[43,48,49] we found equivalent outcomes on the Snake Questionnaire between study groups, possibly due to a ceiling effect imposed by strong overall single-session exposure treatment outcomes.[33,34] It is also possible that a single dose of D-cycloserine is insufficient to elicit substantial changes in brain function and behavior; previous single-dose studies in student volunteers failed to show a clinical effect of D-cycloserine.[50,51] However, D-cycloserine did appear to accelerate fear reduction in the present study; this finding is consistent with previous research[52–54] and might be expected to result in greater acceptability and cost-effectiveness of treatment.[55] Thus, the altered ventromedial prefrontal cortex activity seen in the D-cycloserine group might contribute to more rapid fear extinction or other learning processes, although additional research is needed to examine the relationship between hemodynamic activity and degree/rate of treatment response. It is also possible that the obtained changes in neural functioning would lead to detectable behavioral changes later on, such as accelerated fear extinction in a subsequent exposure session.

Drug names: bupropion (Aplenzin, Wellbutrin, and others), desvenlafaxine (Pristiq), duloxetine (Cymbalta), paroxetine (Paxil, Pexeva, and others), sertraline (Zoloft and others).

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