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A proof-of-concept, randomized controlled trial of DAR-0100A, a dopamine-1 receptor agonist, for cognitive enhancement in schizophrenia

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Abstract

Background: Evidence from preclinical and human studies indicates the presence of reduced dopamine-1 receptor (D1R) signaling in the cortex, where D1Rs predominate, in patients with schizophrenia (SCZ), which may contribute to their cognitive deficits. Furthermore, studies in nonhuman primates (NHP) have suggested that intermittent administration of low doses of D1R agonists produce long-lasting reversals in cognitive deficits. The purpose of this trial was to test whether a similar design, involving subacute intermittent administration of low doses of a full, selective agonist at D1Rs, DAR-0100A, would improve cognitive deficits in SCZ.

Methods: We randomized 49 clinically stable individuals with SCZ to three weeks of intermittent treatment with 0.5 mg or 15 mg of DAR-0100A, or placebo (normal saline). Functional magnetic resonance imaging (fMRI) BOLD was used to evaluate the effects of drug administration on brain activity during a working memory (WM) task. Effects on cognition were also assessed using the MATRICS and the N-back task as primary endpoints. The CogState battery was used as a secondary endpoint.

Results: There were no observed treatment effects on either the BOLD fMRI signal during WM tasks or the WM domains of the MATRICS. Moderate improvement was detected on the CogState battery and on the attention domain of the MATRICS.

Conclusion: These results suggest that low doses of D1 agonists that do not result in measurable occupancy of the D1R do not reliably improve cognition in SCZ, unlike the observations in NHP. As this drug is limited by its pharmacokinetic profile, better D1R agonists that can achieve adequate levels of D1R occupancy are needed to test the efficacy of this mechanism for cognitive enhancement in SCZ.

Keywords

DAR-0100A, dopamine-1 agonist, cognitive enhancement, schizophrenia, randomized controlled trial

Introduction

While the positive symptoms of schizophrenia (SCZ) relate to dopamine (DA) hyperactivity in striatal regions (Abi-Dargham et al., 2000; Laruelle et al., 1996), the negative symptoms and cognitive deficits of SCZ are thought to relate, at least in part, to a cortical DA deficit, as originally proposed by Pycock et al. (1980), Weinberger (1987), and Davis et al. (1991). An abundance of preclinical data suggests that hypodopaminergia in the prefrontal cortex (PFC), especially dorsolateral PFC (DLPFC), may be associated with cognitive deficits, and that administration of DA agonists may ameliorate some of these deficits. Early work in nonhuman primates (NHPs) demonstrated that selective DA depletion in the DLPFC impaired cognitive function. This effect was reversed by the administration of DA agonists (Brozoski et al., 1979). Later studies focused on dopamine-1 receptors (D1Rs) in the PFC and demonstrated that antagonists, including haloperidol, could impair working memory (WM) performance in animals (Sawaguchi and Goldman-Rakic, 1991; Seamans et al., 1998), while D1R agonists could reverse these deficits (Arnsten et al., 1994). Wass et al. (2013) showed that it is

the sensitivity, but not density, of D1Rs in the prelimbic cortex that determines general cognitive ability and that WM training could modulate general cognitive ability. Positive effects of D1R agonists on WM performance were noted, especially in

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paradigms that included impaired DA function in the PFC (Castner et al., 2000; Roberts et al., 2010), whereas higher doses of D1R agonists impaired WM function (Zahrt et al., 1997). These findings suggested that there is an inverted-U effect of D1R stimulation on cognitive function of the PFC (Arnsten, 1997; Vijayraghavan et al., 2007).

In this context, Castner et al. (2000) performed a seminal study in which trace doses of a D1R agonist were administered to NHPs that displayed haloperidol-induced cognitive deficits. The D1R agonist was administered daily for five days, followed by a 14-day washout period, during which time the NHP subjects continued receiving haloperidol. This cycle was repeated either five or six times in each subject. The authors demonstrated that the D1R agonist ameliorated the haloperidol-induced WM deficits and the improvements in WM persisted for up to a year after the last D1R agonist administration, even though the animals continued to receive haloperidol.

Positron emission tomography (PET) studies of cortical D1R availability in individuals with SCZ have provided inconsistent results, with reports of increases (Abi-Dargham et al., 2002, 2012), decreases (Okubo et al., 1997), or no difference (Karlsson et al., 2002) in cortical D1R availability compared to matched healthy control subjects. More recently, we observed reduced amphetamine-induced DA release in the DLPFC in patients with SCZ compared with controls, further supporting the hypothesis of cortical hypodopaminergia in this disorder (Slifstein et al., 2015).

Given the preclinical and clinical data suggesting that D1R stimulation in the PFC may have procognitive effects in SCZ, a selective D1R agonist was one of two most highly rated targets for drug development for cognitive enhancement in SCZ by the MATRICS Neuropsychopharmacology Selection Committee. However, there has been a dearth of D1R agonist drugs that progressed to clinical testing, and only one available for a proof-of-concept (POC) study. DAR-0100 (dihydropyridine; trans-10,11-dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[a]phenanthridine) is a potent, full agonist at the D1R (Lovenberg et al., 1989; Mottola et al., 1992). It has high affinity for the D1R (IC_{50} =10 nM) and essentially no binding at any other receptor except dopamine-2 receptor (D2R; IC_{50} =130 nM) and α_2 adrenergic receptors (IC_{50} =230 nM; Lovenberg et al., 1989; Mottola et al., 1992). Single doses of DAR-0100 have been given to both individuals with Parkinson's disease, targeting motor improvement (Blanchet et al., 1998), and SCZ (George et al., 2007; Mu et al., 2007). In the SCZ study, patients were randomized in a crossover design to DAR-0100 then placebo, or vice versa (George et al., 2007; Mu et al., 2007). No improvements on the Controlled Oral Word Association Test or the Hopkins Verbal Learning Test were detected. However, side effects were minimal, no orthostatic changes were observed, and increased perfusion (i.e., via functional magnetic resonance imaging [fMRI]) in the frontal cortex was noted (George et al., 2007; Mu et al., 2007). The (+) enantiomer of DAR-0100 (DAR-0100A) is twice as potent as the racemate, and possesses the same functional activity (Knoerzer et al., 1994). Therefore, despite its poor oral bioavailability and short half-life (Blanchet et al., 1998; Buchanan et al., 2007), in the absence of better drugs, we used DAR-0100A to test the D1R hypofunction hypothesis in a POC study. We determined 15 mg administered over 30 minutes as the maximum tolerated dose based on a phase I safety and feasibility study of DAR-0100A in

stable patients with SCZ. We also predicted, from our PET occupancy study in NHPs (Slifstein et al., 2011), that 15 mg would yield approximately 1% occupancy at D1Rs in humans. However, given the evidence from NHP studies that ultra-low doses of D1R agonists lead to cognitive enhancement, that low doses of the racemate DAR-0100 increased perfusion in PFC, and that DAR-0100A is the active enantiomer of DAR-0100, we believed testing low doses would be informative, despite low to negligible occupancy, as these were the same conditions under which a procognitive effect was observed in NHPs. Thus, we performed a phase II, randomized, placebo controlled, POC clinical trial of DAR-0100A for cognitive enhancement in SCZ. Our objective was to test the theory that intermittent, low doses of a D1R agonist would be beneficial, as suggested by Castner et al. (2000). A positive POC study would provide support for the development of D1R agonists as therapeutic agents for cognition in SCZ. Furthermore, information from this study may guide the field in characterizing the level of D1R occupancy needed to target in future drug development of D1 agonists (see supplementary information for additional information).

Patients and methods

All procedures were approved by the Institutional Review Board of the New York State Psychiatric Institute (NYSPI) at Columbia University Medical Center between April 2011 and September 2013. All subjects provided written, informed consent for inclusion in this study. Subjects were medically healthy, adult (age 18–55 years) patients with SCZ or schizoaffective disorder who had been treated as outpatients for at least three months (i.e., no recent inpatient or emergency room visits, no suicidality or homicidality). All subjects had been on haloperidol, aripiprazole, risperidone, paliperidone, or lurasidone for at least one month without changing their dose. Antipsychotic polypharmacy was not permitted in this trial. Concomitant medications, with the exception of medications that affect cognition (e.g., anticholinergics or antihistaminergics, stimulants; benzodiazepines were allowed) were permitted, as long as patients were on stable doses for at least one month (see supplementary information for additional information).

Randomization, masking, and assessment procedures

Baseline assessments (see "Assessments" below), including the baseline fMRI, were completed within two weeks of the first study drug administration. After the baseline procedures, patients were randomized in a double-blind design to placebo or 0.5 mg or 15 mg of DAR-0100A. These doses were chosen because 15 mg was the maximal tolerated dose in our previous single, ascending-dose phase I study ($N=14$; the limiting side effect was a drop in mean arterial pressure >20 mm Hg). In addition, because preclinical studies have shown that very low doses of D1R agonists have procognitive effects (Arnsten et al., 1994; Castner et al., 2000), we included a second, ultra-low-dose treatment group of 0.5 mg. This lower dose approximates the dose of the D1R agent ABT-431 used in the study of Castner et al. (2000). Randomization was stratified by age, sex, and baseline WM performance (3-back adjusted hit rate [AHR] below and above 0.65; chance AHR=0; no subjects were excluded based on their N-back

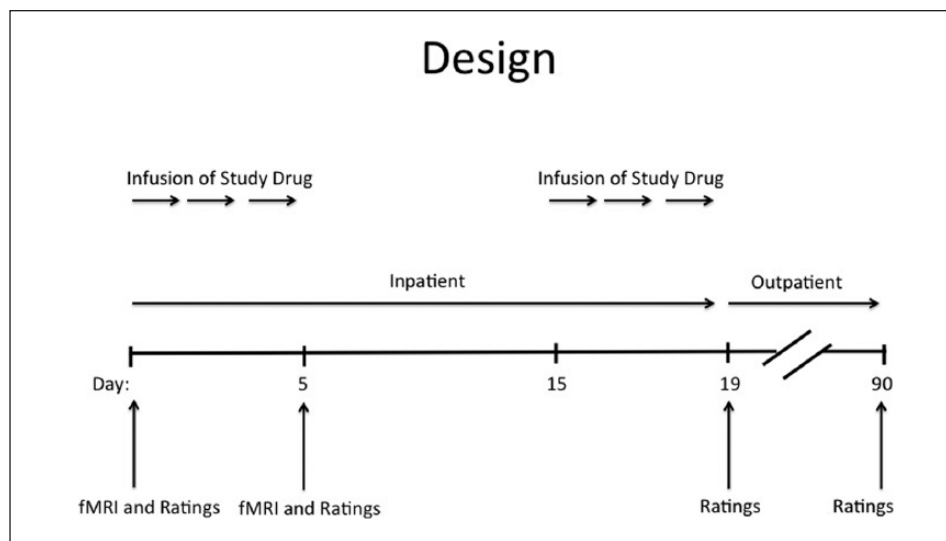


Figure 1. Overall trial design.

AHR). We randomized by age, given the evidence that DIR density decreases with age (Abi-Dargham et al., 2002).

After randomization, patients were admitted to the 5-South Inpatient Unit of NYSPI. Each subject received five daily doses of IV DAR-0100A or placebo on days 1–5 (Figure 1). On day 5, subjects were administered DAR-0100A during fMRI. After their fMRI session, all subjects completed the assessment battery (see below). Subjects remained in the hospital on days 6–14 without receiving the study drug. They then received five more daily doses of the study drug on days 15–19. After infusion on day 19, subjects completed the assessment battery again, and were discharged. Subjects had follow-up visits on days 30, 60, and 90 for safety assessments, and completed the assessment battery a final time on day 90.

DAR-0100A/placebo administration

On day 1, 50 mL of study drug was administered as a constant IV drip over 30 minutes. Hemodynamic parameters, including orthostatic measures, were monitored by an ACLS-certified physician every five minutes during drug infusion and every 15 minutes for one hour thereafter. A second IV was inserted on days 1, 4, 15, and 19 to obtain blood samples for antipsychotic and DAR-0100A drug-level monitoring.

fMRI participants

Of the 49 participants who were included in the intent-to-treat (ITT) sample, we obtained high-quality, baseline, and day 5 fMRI data for 36 participants for the self-ordered WM task (SOT; Curtis et al., 2000) and 29 for the N-back (Supplementary Tables 1 and 2; see the online supplementary material for additional information).

fMRI task procedures

SOT. Task procedures were identical to those described elsewhere (see the online supplementary material for additional information; Van Snellenberg et al., 2014).

Nonverbal N-back. Stimuli used for the nonverbal N-back were the same as those employed for the SOT (see the online supplementary material for additional information).

fMRI methods

Data acquisition. Imaging was carried out on a Philips 1.5 Tesla Intera scanner at the Columbia Radiology MRI Center at the Neurological Institute of New York. Participants lay supine on the scanner bed while viewing stimuli projected onto a screen located at the foot of the scanner bed through a mirror mounted on the head coil. The cursor was controlled with a handheld fiber optic trackball with buttons on either side, making it functionally similar to a computer mouse. T1-weighted images were obtained with an SPGR sequence with a 256 mm FOV, 200 slices, and 1 mm isotropic voxels. Whole-brain functional EPIs were obtained using an eight-channel SENSE coil with a SENSE factor of 1.5, 2 s TR, 28 ms TE, 77° flip angle, 192 mm field of view, 40 slices, and 3 mm isotropic voxels (see the online supplementary material for additional information on fMRI data processing, artifact removal, and modeling).

Assessments: non-fMRI cognitive measures and self-reported symptoms

The assessment battery that was performed at baseline and on days 5, 19, and 90, included the NIMH MATRICS Neurocognitive Battery (Marder and Fenton, 2004), N-Back WM task, Columbia Suicide Severity Rating Scale (C-SSRS) (Posner et al., 2007), Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987), Scale for the Assessment of Negative Symptoms (SANS) (Andreassen, 1982), Calgary Depression Rating Scale for Schizophrenia (CDSS) (Addington et al., 1992), Trails B, and Cogstate Schizophrenia Battery (CSSB) (Pietrzak et al., 2009). These measures were chosen to assess effects of DAR-0100A on cognition and symptoms with a focus on WM, negative symptoms, depressive symptoms, and positive symptoms. Our

primary behavioral outcome was the score on the WM domain of the MATRICS. Notably, due to issues with subject rating fatigue and a desire to prioritize WM tests, we discontinued the MSCEIT from the MATRICS battery after the first 12 subjects. In addition, assessments were completed in the following order (verbal N-Back, MATRICS and Trails B, clinical ratings, CSSB), as our a priori hypotheses focused on WM performance as measured by the MATRICS and verbal N-Back.

Data analysis

fMRI second-level statistical modeling. Several second-level statistical tests were conducted in order to ensure that no treatment effects were missed. All analyses were tested for significance using robust regression (Wager et al., 2005) and thresholded at $p < 0.05$ after false discovery rate (FDR) correction (Benjamini and Hochberg, 1995).

For the SOT, contrast images of overall task-related activation were calculated for each participant by taking the mean activation during correct trials at each voxel across steps 1–7 in the SOT (step 8 was omitted because poor performance on this step left very few correct trials for many participants) and subtracting the activation in the corresponding voxel in the control task, for each of the baseline and day 5 scans. Within each participant, the day 5–baseline contrast was calculated, and between-group comparisons of this were calculated for the 0.5 mg dose–placebo and 15 mg dose–placebo groups. For the nonverbal N-back task, the 2-back–1-back contrast was calculated for each participant at baseline and day 5, and similar to the SOT the day 5–baseline difference in these contrasts was calculated for each participant for use in between-group tests of 0.5 mg dose–placebo and 15 mg dose–placebo.

Analysis of non-fMRI cognitive measures and self-reported symptoms

The primary outcome measure was the WM subdomain of the MATRICS. Secondary outcome measures were the other domains and subdomains of the MATRICS, subdomains and composite scores of CSSB, domains of PANSS, SANS, and CDRS, and N-Back (this was a verbal version of the N-Back using letters rather than the nonverbal version of the N-Back used in fMRI that utilized blocks) measures. Ratios of the verbal N-Back were also used as secondary outcome measures (3-Back AHR/1-Back AHR; 2-Back AHR/1-Back AHR).

Change scores were calculated at each assessment time point during the study by subtracting the baseline measure of the outcome from each subsequent measure of the outcome. The change score outcomes were analyzed using longitudinal mixed effects models with random intercept and autoregressive covariance structure (Fitzmaurice et al., 2004) to account for the within-subject correlation among repeated measures. Each outcome was modeled as a function of time, treatment, and the interaction between time and treatment. Cohen's *d* effect sizes were calculated by taking the mean difference in change from baseline between groups averaged across time, divided by the baseline standard deviation of the measure.

All analyses were conducted based on the ITT principle. All statistical tests were two-tailed, and the significance level was set

at 0.05. No correction was made to *p*-values for tests of multiple secondary outcomes (Feise, 2002). PROC MIXED in SAS[®] was used to conduct these analyses.

Results

As shown in Figure 2, of the 69 subjects enrolled in this trial, 55 were randomized, but because six either withdrew consent or had no follow-up assessments, only 49 were included in the ITT analysis. The demographics of the complete ITT sample are provided in Table 1.

fMRI results

SOT. None of the between-group contrasts of differences in the change of activation from baseline to day 5 showed any significant regions of four or more voxels after FDR correction, even after restricting analyses to voxels that showed significant activation across all groups at baseline.

N-back. Like the SOT, none of the between-group contrasts of differences in the change of activation from baseline to day 5 showed any regions of four or more voxels after FDR correction, even after restricting analyses to voxels showing significant activation at baseline.

Behavioral results

MATRICES results. There was a significant treatment group \times time interaction for the primary outcome WM domain ($p = 0.03$). However, upon further examination of the estimates and contrasts between treatment groups, this result was primarily due to differences in the change between day 19 and day 90 between the 0.5 mg dose group (which improved between days 19 and 90) and the 15 mg dose group (which worsened between days 19 and 90). There were no significant differences at day 5, day 19, or day 90 between placebo and 0.5 mg dose, or between placebo and 15 mg dose groups (Table 2). There were significant advantages for both the 0.5 mg dose ($p = 0.01$) and 15 mg dose groups ($p = 0.01$) compared with placebo on the attention subdomain of the MATRICS as measured by the Continuous Performance Test, as well as for the 15 mg dose group compared with placebo on the Fluency test of the MATRICS ($p = 0.02$; Table 2). Significant differences were not detected between treatment groups on the other domains or individuals tests.

CSSB results. In contrast to the MATRICS results, there were significant treatment effects between the 15 mg dose group and placebo group on the CSSB global cognition composite score and the composite memory score, as well as on the tests for visual attention (Identification), learning (One Card Learning), WM (One Back), and social emotional cognition (Table 3).

Additional analyses. We also examined potential treatment effects on negative symptoms, positive symptoms, general psychopathology symptoms, and depressive symptoms, as well as on other measures of WM (Supplementary Table 3). None of these analyses was significant. Exploratory analyses examining

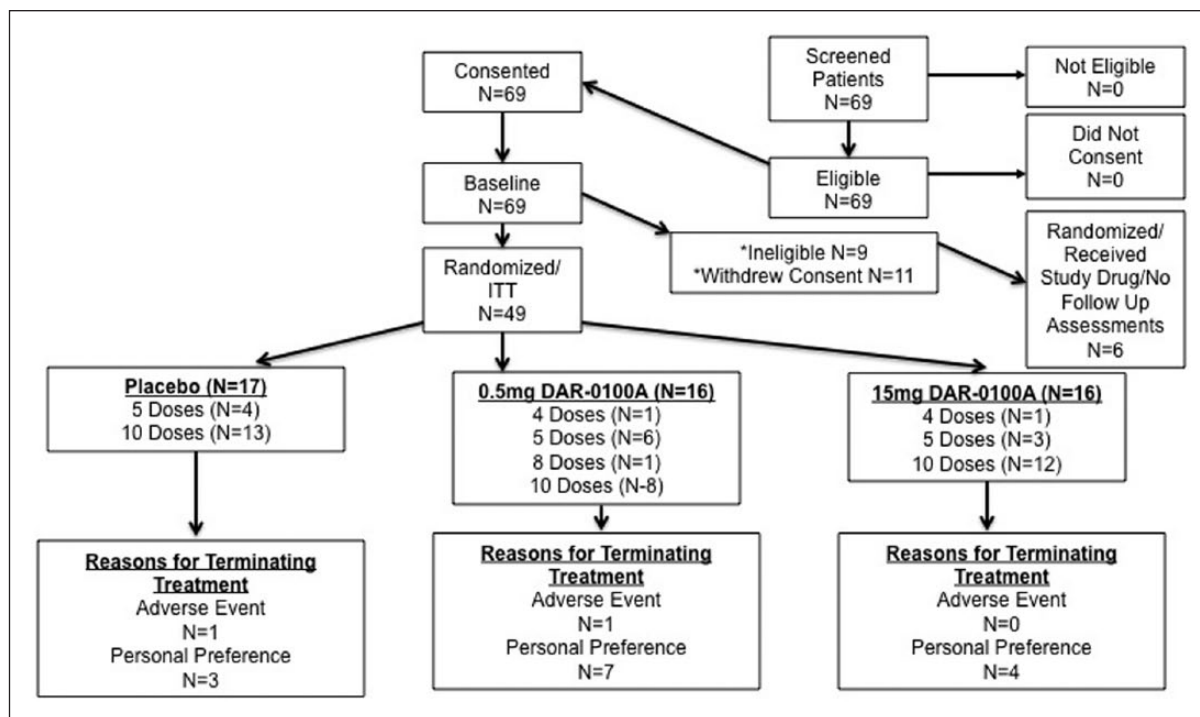


Figure 2. CONSORT diagram.

Table 1. Demographic information for the complete ITT sample.

	Placebo (N=17)	0.5 mg dose (N=16)	15 mg dose (N=16)
Age (SD)	40.4 (9.5)	38.6 (8.8)	39.5 (9.2)
Sex	8M, 9F	8M, 8F	9M, 7F
Baseline PANSS total (SD)	58.2 (18.3)	54.6 (17.6)	52.3 (9.0)
Antipsychotic	6A, 6R, 2L, 1P, 2H	7A, 5R, 3L, 0P, 1H	6A, 7R, 2L, 0P, 1H
Smoking	7Y, 10N	8Y, 8N	4Y, 12N
Race/ethnicity	5C, 11AA, 1As, 0M (3H)	3C, 10AA, 2As, 1M (2H)	7C, 8AA, 0As, 1M (4H)
Verbal 3-back adjusted hit rate [AHR]	11 ≤0.65, 6 >0.65	10 ≤0.65, 6 >0.65	11 ≤0.65, 5 >0.65

M: male; F: female; A: aripiprazole; R: risperidone; L: lurasidone; P: paliperidone; H: haloperidol; Y: yes; N: no; C: Caucasian; AA: African American; As: Asian; M: Mixed; H: Hispanic.

differential treatment effects on all measures in subgroups split by the stratification factors (age, sex, and baseline WM performance as measured by the verbal 3-Back AHR > or <0.65) showed similar results between the subgroups.

Discussion

Overall, the results from primary assessment measures of cognitive functions, symptoms, and imaging were negative. In secondary analyses, we observed strong effects on the CSSB in the higher dose DAR-0100A group on measures of learning, WM, visual attention, and social emotional cognition, as well as on composite measures of memory and overall cognition and on the attention measure of the MATRICS.

Our negative results should be compared and contrasted with the results of a previous, single-dose trial of the racemic mixture of dihydrexidine in SCZ (George et al., 2007). In that study,

George et al. administered single doses of DAR-0100A and observed no significant effects on neuropsychological performance or symptom measures. Using fMRI in the same subjects, Mu et al. (2007) observed an increase in gadolinium-based perfusion in frontal cortex. While these results are not consistent with our own, it should be noted that the imaging techniques used in these two studies are different and do not necessarily reflect the same neurobiological phenomena. Similarly, Rosell et al. administered three doses of 15 mg of DAR-0100A or placebo to 16 individuals with schizotypal personality disorder and observed improvement in WM on the Paced Auditory Serial Audition Task (PASAT) and on the 2-Back to 0-back ratio (Rosell et al., 2015). Differences between the study of Rosell et al. and the current study may relate to differences in patient populations (SCZ vs. schizotypal personality disorder), antipsychotic medication status (antipsychotic free in the study of Rosell et al.), length of treatment with DAR-0100A, and the outcome measures used.

Table 2. Behavioral effects on subdomains of the MATRICS.

Outcome	<i>p</i> -Value for treatment×day (df=4)	<i>p</i> -Value for overall treatment (df=2)	Placebo versus 0.5 mg dose (Cohen's d)	Placebo versus 0.5 mg dose (<i>p</i> -value)	Placebo versus 15 mg dose (Cohen's d)	Placebo versus 15 mg dose (<i>p</i> -value)
Working Memory: Domain	0.03	N/A	Day 5: 0.11 Day 19: -0.14 Day 90: -0.16	Day 5: 0.60 Day 19: 0.59 Day 90: 0.47	Day 5: -0.09 Day 19: -0.16 Day 90: 0.18	Day 5: 0.67 Day 19: 0.49 Day 90: 0.41
Speed of Processing: Domain	0.14	0.25	0.16	0.39	-0.15	0.39
Trail Making Test	0.11	0.40	0.25	0.21	0.03	0.88
Brief Assessment of Cognition in Schizophrenia	0.20	0.38	0.37	0.17	0.21	0.43
Category Fluency	0.62	0.06	-0.21	0.38	-0.57	0.02
Verbal Learning: Hopkins Verbal Learning Test-Revised	0.68	0.28	0.12	0.61	0.36	0.12
Wechsler Memory Scale® 3rd Ed.	0.16	0.98	0.02	0.95	-0.03	0.90
Letter-Number Span	0.48	0.93	0.05	0.84	-0.04	0.86
Reasoning and Problem Solving: Mazes	0.64	0.63	-0.16	0.48	-0.20	0.37
Visual Learning: Brief Visuospatial Memory Test-Revised	0.29	0.70	-0.01	0.97	-0.17	0.45
Attention/Vigilance Continuous Performance Test	0.38	0.01	-0.45	0.01	-0.40	0.01
Verbal: Domain	0.81	0.25	0.07	0.75	0.36	0.11

Table 3. Behavioral effects on CSSB outcomes.

Outcome variable	<i>p</i> -Value for treatment×day (df=4)	<i>p</i> -Value for overall treatment (df=2)	Placebo versus 0.5 mg dose (Cohen's d)	Placebo versus 0.5 mg dose (<i>p</i> -value)	Placebo versus 15 mg dose (Cohen's d)	Placebo versus 15 mg dose (<i>p</i> -value)
International Shopping List Task (ISLT)	0.86	0.49	-0.005	0.99	-0.26	0.29
Groton Maze Learning Test (GMLT)	0.16	0.25	-0.02	0.92	0.30	0.16
Detection Task	0.12	0.19	0.26	0.15	0.29	0.10
Identification	0.33	0.03	0.34	0.08	0.49	0.01
One Card Learning	0.18	0.09	-0.18	0.35	-0.39	0.03
One Back	0.32	0.04	-0.09	0.72	-0.60	0.02
Social Emotional Cognition Task (SECT)	0.37	0.09	-0.11	0.37	-0.26	0.03
Memory Composite	0.20	0.03	-0.26	0.56	-0.56	0.01
Global Cognitive Composite	0.23	0.003	-0.26	0.14	-0.60	0.001

CSSB: Cogstate Schizophrenia Battery.

The primary limitation of this trial is the narrow therapeutic index of DAR-0100A and its minimal target engagement at the D1R. Our negative trial needs to be interpreted within the context of negligible receptor occupancy targeted by the doses we were restricted to, due to poor tolerability of higher doses. While agonist activity does not necessarily require substantial receptor occupancy, we were constrained by the side effects of DAR-0100A from testing if higher-target engagement might have led to different results. In the current study, hemodynamic and pharmacokinetic data clearly indicate that DAR-0100A was well distributed and pharmacologically active. However, we also observed unquantifiable blood levels of DAR-0100A (although nonspecific metabolites were qualitatively observed at levels commensurate with the two doses, and not at all in the placebo group) and no effect of DAR-0100A on BOLD signal (i.e., a measure of functional target engagement). These observations, coupled with the primarily negative behavioral results from this trial, suggest that D1R agonist treatment at negligible receptor occupancy (Castner et al., 2000) is ineffective in patients with SCZ and that future trials of D1R stimulation in SCZ require a drug with a broader therapeutic index able to achieve higher levels of target engagement.

Additional limitations of this study include its relatively small sample size, as well as the difficulties translating nonhuman WM tasks (such as performed in Castner et al., 2000) into human studies (Dudchenko et al., 2013). Specifically, different tasks were utilized in the two studies, which may have used different underlying circuitry. Therefore, it may not be a species difference that is driving the lack of translation, but rather the inability to use the same tasks in NHPs and humans.

The combination of DAR-0100A's evanescent pharmacokinetics and the cardiovascular side effects that limited the dose produced results that fell short of their therapeutic goal. We can conclude from this trial that using D1 agonists at doses that produce negligible receptor occupancy fails to produce therapeutic effects in SCZ. Future studies with similar agents that have longer durations of action and better tolerability at doses which can achieve adequate measurable levels of receptor occupancy (above a minimal threshold of 5–10%) are needed to determine whether the D1 receptor is a viable therapeutic target for SCZ.

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Dr Lieberman had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Conflict of interest

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References

- Abi-Dargham A, Mawlawi O, Lombardo I, et al. (2002) Prefrontal dopamine D1 receptors and working memory in schizophrenia. *J Neurosci* 22: 3708–3719.
- Abi-Dargham A, Rodenhiser J, Printz D, et al. (2000) Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. *Proc Natl Acad Sci U S A* 97: 8104–8109.
- Abi-Dargham A, Xu X, Thompson JL, et al. (2012) Increased prefrontal cortical D1 receptors in drug naive patients with schizophrenia: a PET study with [¹¹C]NNC112. *J Psychopharmacol* 26: 794–805.
- Addington D, Addington J, Maticka-Tyndale E, et al. (1992) Reliability and validity of a depression rating scale for schizophrenics. *Schizophr Res* 6: 201–208.
- Andreasen NC (1982) Negative symptoms in schizophrenia. Definition and reliability. *Arch Gen Psychiatry* 39: 784–788.
- Arnsten AF (1997) Catecholamine regulation of the prefrontal cortex. *J Psychopharmacol* 11: 151–162.
- Arnsten AF, Cai JX, Murphy BL, et al. (1994) Dopamine D1 receptor mechanisms in the cognitive performance of young adult and aged monkeys. *Psychopharmacology (Berl)* 116: 143–151.
- Benjamini Y and Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B Stat Methodol* 57: 289–300.
- Blanchet PJ, Fang J, Gillespie M, et al. (1998) Effects of the full dopamine D1 receptor agonist dihydrexidine in Parkinson's disease. *Clin Neuropharmacol* 21: 339–343.
- Brozoski TJ, Brown RM, Rosvold HE, et al. (1979) Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. *Science* 205: 929–932.
- Buchanan RW, Freedman R, Javitt DC, et al. (2007) Recent advances in the development of novel pharmacological agents for the treatment of cognitive impairments in schizophrenia. *Schizophr Bull* 33: 1120–1130.
- Castner SA, Williams GV and Goldman-Rakic PS (2000) Reversal of antipsychotic-induced working memory deficits by short-term dopamine D1 receptor stimulation. *Science* 287: 2020–2022.
- Curtis CE, Zald DH and Pardo JV (2000) Organization of working memory within the human prefrontal cortex: a PET study of self-ordered object working memory. *Neuropsychologia* 38: 1503–1510.
- Davis KL, Kahn RS, Ko G, et al. (1991) Dopamine in schizophrenia: a review and reconceptualization. *Am J Psychiatry* 148: 1474–1486.
- Dudchenko PA, Talpos J, Young J, et al. (2013) Animal models of working memory: a review of tasks that might be used in screening drug treatments for the memory impairments found in schizophrenia. *Neurosci Biobehav Rev* 37: 2111–2124.
- Feise RJ (2002) Do multiple outcome measures require p-value adjustment? *BMC Med Res Methodol* 2: 8.
- Fitzmaurice GM, Laird NM and Ware JH (2004) *Applied Longitudinal Analysis*. Hoboken, NJ: Wiley.
- George MS, Molnar CE, Grenesko EL, et al. (2007) A single 20 mg dose of dihydrexidine (DAR-0100), a full dopamine D1 agonist, is safe and tolerated in patients with schizophrenia. *Schizophr Res* 93: 42–50.
- Karlsson P, Farde L, Halldin C, et al. (2002) PET study of D(1) dopamine receptor binding in neuroleptic-naive patients with schizophrenia. *Am J Psychiatry* 159: 761–767.
- Kay SR, Fiszbein A and Opler LA (1987) The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull* 13: 261–276.
- Knoerzer TA, Nichols DE, Brewster WK, et al. (1994) Dopaminergic benzo[a]phenanthridines: resolution and pharmacological evaluation of the enantiomers of dihydrexidine, the full efficacy D1 dopamine receptor agonist. *J Med Chem* 37: 2453–2460.
- Laruelle M, Abi-Dargham A, van Dyck CH, et al. (1996) Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. *Proc Natl Acad Sci U S A* 93: 9235–9240.
- Lovenberg TW, Brewster WK, Mottola DM, et al. (1989) Dihydrexidine, a novel selective high potency full dopamine D-1 receptor agonist. *Eur J Pharmacol* 166: 111–113.
- Marder SR and Fenton W (2004) Measurement and treatment research to improve cognition in schizophrenia: NIMH MATRICS initiative to support the development of agents for improving cognition in schizophrenia. *Schizophr Res* 72: 5–9.
- Mottola DM, Brewster WK, Cook LL, et al. (1992) Dihydrexidine, a novel full efficacy D1 dopamine receptor agonist. *J Pharmacol Exp Ther* 262: 383–393.
- Mu Q, Johnson K, Morgan PS, et al. (2007) A single 20 mg dose of the full D1 dopamine agonist dihydrexidine (DAR-0100) increases prefrontal perfusion in schizophrenia. *Schizophr Res* 94: 332–341.
- Okubo Y, Suhara T, Suzuki K, et al. (1997) Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET. *Nature* 385: 634–636.
- Pietrzak RH, Olver J, Norman T, et al. (2009) A comparison of the CogState Schizophrenia Battery and the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) Battery in assessing cognitive impairment in chronic schizophrenia. *J Clin Exp Neuropsychol* 31: 848–859.
- Posner K, Melvin GA, Stanley B, et al. (2007) Factors in the assessment of suicidality in youth. *CNS Spectr* 12: 156–162.
- Pycock CJ, Kerwin RW and Carter CJ (1980) Effect of lesion of cortical dopamine terminals on subcortical dopamine receptors in rats. *Nature* 286: 74–76.
- Roberts BM, Seymour PA, Schmidt CJ, et al. (2010) Amelioration of ketamine-induced working memory deficits by dopamine D1 receptor agonists. *Psychopharmacology (Berl)* 210: 407–418.
- Rosell DR, Zaluda LC, McClure MM, et al. (2015) Effects of the D1 dopamine receptor agonist dihydrexidine (DAR-0100A) on working memory in schizotypal personality disorder. *Neuropsychopharmacology* 40: 446–453.
- Sawaguchi T and Goldman-Rakic PS (1991) D1 dopamine receptors in prefrontal cortex: involvement in working memory. *Science* 251: 947–950.
- Seamans JK, Floresco SB and Phillips AG (1998) D1 receptor modulation of hippocampal-prefrontal cortical circuits integrating spatial memory with executive functions in the rat. *J Neurosci* 18: 1613–1621.

- Slifstein M, Suckow RF, Javitch JA, et al. (2011) Characterization of in vivo pharmacokinetic properties of the dopamine D1 receptor agonist DAR-0100A in nonhuman primates using PET with [11C] NNC112 and [11C] raclopride. *J Cereb Blood Flow Metab* 31: 293–304.
- Slifstein M, van de Giessen E, Van Snellenberg JX, et al. (2015) Deficits in prefrontal cortical and extra-striatal dopamine release in schizophrenia: a PET fMRI study. *JAMA Psychiatry* 72: 316–324.
- Van Snellenberg JX, Conway AR, Spicer J, et al. (2014) Capacity estimates in working memory: Reliability and interrelationships among tasks. *Cogn Affect Behav Neurosci* 14: 106–116.
- Vijayraghavan S, Wang M, Birnbaum SG, et al. (2007) Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. *Nat Neurosci* 10: 376–384.
- Wager TD, Keller MC, Lacey SC, et al. (2005) Increased sensitivity in neuroimaging analyses using robust regression. *Neuroimage* 26: 99–113.
- Wass C, Pizzo A, Sauce B, et al. (2013) Dopamine D1 sensitivity in the prefrontal cortex predicts general cognitive abilities and is modulated by working memory training. *Learn Mem* 20: 617–627.
- Weinberger DR (1987) Implications of normal brain development for the pathogenesis of schizophrenia. *Arch Gen Psychiatry* 44: 660–669.
- Zahrt J, Taylor JR, Mathew RG, et al. (1997) Supranormal stimulation of D1 dopamine receptors in the rodent prefrontal cortex impairs spatial working memory performance. *J Neurosci* 17: 8528–8535.