American College of Neuropsychopharmacology

ARTICLE

Single doses of a highly selective inhibitor of phosphodiesterase 1 (lenrispodun) in healthy volunteers: a randomized pharmaco-fMRI clinical trial

Sahib S. Khalsa[1,2]✉, Teresa A. Victor[1], Rayus Kuplicki[1], Hung-Wen Yeh[1,3,4], Kimberly E. Vanover[5], Martin P. Paulus[1,2] and Robert E. Davis[6]

Lenrispodun is a potent and highly selective inhibitor of phosphodiesterase (PDE) type 1, which is thought to prolong intracellular second messenger signaling within cortical and subcortical dopaminergic brain regions. This is the first study of a PDE1 inhibitor in healthy volunteers using behavioral and neuroimaging approaches to examine its effects on neural targets and to provide a safety and tolerability assessment. The primary objectives were to determine whether lenrispodun induces changes in BOLD fMRI signals in the inferior frontal gyrus (IFG) during the stop signal task, and the dorsal anterior insula (dAI) during the extinction phase of a fear conditioning/extinction task. Using a double-blind, placebo-controlled, within-subjects design, 26 healthy individuals (22 completed all fMRI sessions) received in random order a single oral dose of placebo, lenrispodun 1.0 milligram (mg) or lenrispodun 10.0 mg and completed several tasks in the scanner including the stop signal ($n = 24$) and fear conditioning/extinction tasks ($n = 22$). Prespecified region-of-interest analyses for the IFG and dAI were computed using linear mixed models. Lenrispodun induced increases in IFG activity during the stop signal task at 1.0 mg (Cohen's $d = 0.63$) but not 10.0 mg (Cohen's $d = 0.07$) vs. placebo. Lenrispodun did not induce changes in dAI activity during fear extinction at either dose. Exploratory outcomes revealed changes in cardiac interoception. Lenrispodun administration was well-tolerated. These results provide evidence that 1.0 mg lenrispodun selectively improved neural inhibitory control without altering fear extinction processing. Future investigations should determine whether lenrispodun improves inhibitory control in target populations such as individuals with attention deficit hyperactivity disorder. Trial registration: ClinicalTrials.gov identifier: NCT03489772.

Neuropsychopharmacology (2022) 47:1844–1853; https://doi.org/10.1038/s41386-022-01331-3

INTRODUCTION

The cyclic nucleotides 3′,5′-cyclic adenosine monophosphate (cAMP) and guanosine monophosphate (cGMP) are important intracellular second messengers that play key roles in regulating numerous biochemical processes in the brain including certain cognitive functions. These are synthesized by adenylyl or guanylyl cyclases and degraded (hydrolyzed) by phosphodiesterases (PDEs) allowing cell type specific intracellular regulation. There are 11 main PDE members with PDE4, PDE7, and PDE8 being highly selective for cAMP and PDE5, PDE6, and PDE9 for cGMP. The remaining PDEs, including PDE1, can hydrolyze both cyclic nucleotides.

Prolongation and amplification of cyclic nucleotide signaling can be accomplished by small molecule inhibitors of PDEs [1]. PDE family members that selectively regulate cAMP or cGMP appear to exert prominent influence in different phases of memory formation in animal models [2]. Inhibition of PDE family members, like PDE4, that selectively regulate levels of cAMP appears to be involved in promoting consolidation of memories at late (i.e., 1 to 3 h post-stimulus) time scales. Inhibition of PDE family members that selectively regulate levels of cGMP, like PDE5, appears to be involved in promoting consolidation of memories at immediate time scales. Several PDE inhibitors (PDE2: BAY 60–7550; PDE4: rolipram; PDE5: sildenafil; PDE10A: papaverine) attenuate cognitive deficits in extra-dimensional shift paradigms, a measure of executive functioning in rodents [3], and PDE2 and PDE10 inhibition reverses MK-801-induced memory deficits [4] as well as modulate auditory information processing during a sensorimotor gating paradigm [5]. Taken together, there is evidence that PDE inhibitors affect several distinct cognitive processes in animals. These findings have generated strong interest in developing PDE inhibitors as potential therapeutics for psychiatric disorders, particularly those associated with neurocognitive deficits.

Recently, Intra-Cellular Therapies Inc. has revealed a series of selective phosphodiesterase 1 (PDE1) inhibitors. Inhibitors of PDE1 block the degradation of cAMP and cGMP and amplify downstream intracellular signaling. Lenrispodun exhibits picomolar affinity for PDE1, possesses exquisite selectivity against all other PDE families, demonstrates favorable brain pharmacokinetics, and shows good efficacy in vivo in animal models consistent with a centrally active mechanism of action [6]. In a preclinical study with

[1]Laureate Institute for Brain Research, Tulsa, OK, USA. [2]Oxley College of Health Sciences, The University of Tulsa, Tulsa, OK, USA. [3]Health Services and Outcomes Research, Children's Mercy Hospital, Kansas City, MO, USA. [4]School of Medicine, University of Missouri—Kansas City, Kansas City, MO, USA. [5]Engrail Therapeutics, San Diego, CA, USA. [6]Intra-Cellular Therapies Inc., New York, NY, USA. ✉email: skhalsa@laureateinstitute.org

Received: 17 December 2021 Revised: 11 April 2022 Accepted: 15 April 2022
Published online: 29 April 2022

rats focused on memory performance using the novel object recognition paradigm, lenrispodun (0.1–10.0 mg/kg, oral administration) resulted in enhanced acquisition, consolidation, and retrieval memory processes [7]. Consistent with modulation of both cAMP and cGMP, lenrispodun enhanced both immediate and late memory consolidation [7]. In the periphery, PDE1 inhibition may also hold promise for the treatment of cardiovascular disease [8]. For example, lenrispodun (0.1–10.0 mg/kg, oral administration) increased cardiac output without altering systemic blood pressure in animal models of heart failure [9]. More recently, single-doses of lenrispodun were shown to be well-tolerated and to confer cardiac inodilator effects in humans with heart failure with reduced ejection fraction [10]. Moreover, lenrispodun has been shown to exhibit anti-inflammatory effects both centrally and peripherally [11, 12]. Thus, the unique profile of PDE1 inhibition may provide a rationale for emphasizing not just central (i.e., cognitive and affective) processes but also for potential utility in improving cardiovascular function.

Building upon these preclinical and clinical studies, the current trial applied a pharmacological-fMRI approach to evaluate the neural effects of acute administration of lenrispodun on cognitive and affective processing in healthy humans. The stop signal task (SST), a classic approach for measuring impulse control, was utilized as a probe of cognitive processing with an emphasis placed on activity within the inferior frontal gyrus (IFG) during the exertion of response inhibition. Previous studies with the SST have shown that modulation of the inferior frontal cortex is important for regulation of behavioral and cognitive inhibition. For example, individuals with attention deficit hyperactivity disorder (ADHD) exhibited lower activation in the IFG and insula during the SST than medicated individuals [13] and abnormal activity in the dorsolateral prefrontal and anterior cingulate cortices relative to healthy individuals [14]. A fear conditioning/extinction task, a classic approach for measuring associative learning in which participants learn to associate a neutral stimulus with an aversive stimulus, was selected as a probe of affective processing with an emphasis placed on activity within the insular cortex during the extinction phase of the task. This task was chosen based on (1) the clinical relevance of modulating associative fear learning, which requires phasic dopamine signaling in limbic (particularly anterior insular [15, 16] as well as amygdala [17]), prefrontal [18], and striatal [19] brain circuits, and (2) other dopamine modulating agents, such as methylphenidate, have been consistently observed to improve cognitive function via enhanced extinction of contextual fear in animal models [20] and via enhancement of fear extinction learning in humans [21].

The primary objectives of the study were (1) to determine whether lenrispodun induced changes in blood-oxygen-level-dependent functional magnetic resonance imaging (BOLD fMRI) signals in the IFG during the SST; (2) to determine whether lenrispodun induced an attenuating effect on BOLD fMRI signals in the anterior insula during the extinction phase of a fear conditioning/extinction task. The secondary objectives of the study were (1) to determine whether lenrispodun affected BOLD fMRI signals elicited by the Stop vs. Go signal in (a) dorsolateral prefrontal cortex, (b) dorsal anterior cingulate (dAC) cortex; (2) to determine whether lenrispodun affected BOLD fMRI signals elicited by fear conditioning stimulus (CS+ vs. CS−) in (a) amygdala, (b) prefrontal cortex, and (c) insula; and (3) to assess safety and tolerability of lenrispodun. Exploratory objectives examined lenrispodun effects on cardiovascular function, interoception, and neural responses to emotional faces.

MATERIALS AND METHODS

Study overview

This single center, randomized, double-blind, placebo-controlled, within-subjects study evaluated brain activation patterns using BOLD fMRI following the administration of three single oral doses of study drug (placebo, lenrispodun 1.0 mg, and lenrispodun 10.0 mg) in healthy human participants at the Laureate Institute for Brain Research in Tulsa, Oklahoma, USA. After providing written informed consent, participants underwent screening procedures. All study procedures were approved by the Western Institutional Review Board (Study number 1184787). Within 14 days, eligible individuals returned to the clinical site for the first of three neuroimaging sessions (scheduled 14 ± 2 days apart). In randomized order, participants received a single oral dose at each neuroimaging visit of: Drug A (Placebo), Drug B (1.0 mg lenrispodun) or Drug C (10.0 mg lenrispodun). Pharmacodynamic effects included evaluation of brain activation patterns using BOLD fMRI during cognitive or emotion-provoking tasks (SST; fear conditioning task (including extinction); emotional face processing task; visceral interoceptive attention (VIA) task); and during the resting state. A standard fMRI sequence protocol was followed (see "Image analysis" section). Self-report questionnaires evaluated changes in mood. Blood samples were collected for determination of lenrispodun and metabolites as well as potential biomarkers. The End-of-Study safety visit took place on day 60 ± 5 days. Total study duration was ~ 80 days. The study design and schedule of assessments are represented in Fig. 1A, B (The results of several assessments are not reported here including blood biomarkers, certain BOLD fMRI tasks (resting state and the monetary incentive delay) and the behavioral session tasks (bandit task and the implicit approach/avoidance task)).

PARTICIPANTS

Enrollment of up to 25 healthy participants, 18 to 45 years of age, inclusive, was planned, with the expectation that 20 individuals would complete all phases of the study including the 1-month follow-up (sample size estimation in Supplementary Materials). The period of recruitment and follow-up was between July 2018 to August 2019. The trial ended upon completion of the recruitment goals and final follow-up visit. Volunteers were recruited from the Tulsa, Oklahoma area via print flyers, social media, and radio advertisements. Detailed inclusion/exclusion criteria and study discontinuation procedures are described in the Supplementary Materials.

Study medication

Lenrispodun was provided to the clinical site pharmacy as a bulk drug. Purified water was also supplied. An unblinded pharmacist prepared the liquid solution of lenrispodun for oral administration to subjects, which was dispensed in a blinded, single-dose container labeled with a randomization code. Detailed information on the randomization process is described in the Supplementary Materials. The oral placebo solution was purified water, identical in appearance, that did not contain lenrispodun. The study drug was administered orally by squirting a 1 milliliter (ml) liquid solution into the participant's mouth. Listerine strips given before and after administration provided blinding of taste.

Safety assessments

Safety and tolerability were evaluated for all participants. Measures included adverse events across all time points, clinical laboratory evaluations, electrocardiogram, vital signs, and physical examination including the neurological component during the screening and end-of-study visits. Vital sign assessments included blood pressure, heart rate, respiratory rate, and oral temperature. Blood pressure and heart rate were measured in both the supine and standing positions. The supine measurement was performed after at least 10 minutes (min) of lying down. The standing measurement was performed at 1, 3, and 5 min intervals after rising from the supine to standing position. Additional safety assessments are detailed in the Supplementary Materials.

Study assessments

The study assessments described in the Supplementary Materials were performed at the Screening Visit (Visit 1) only.

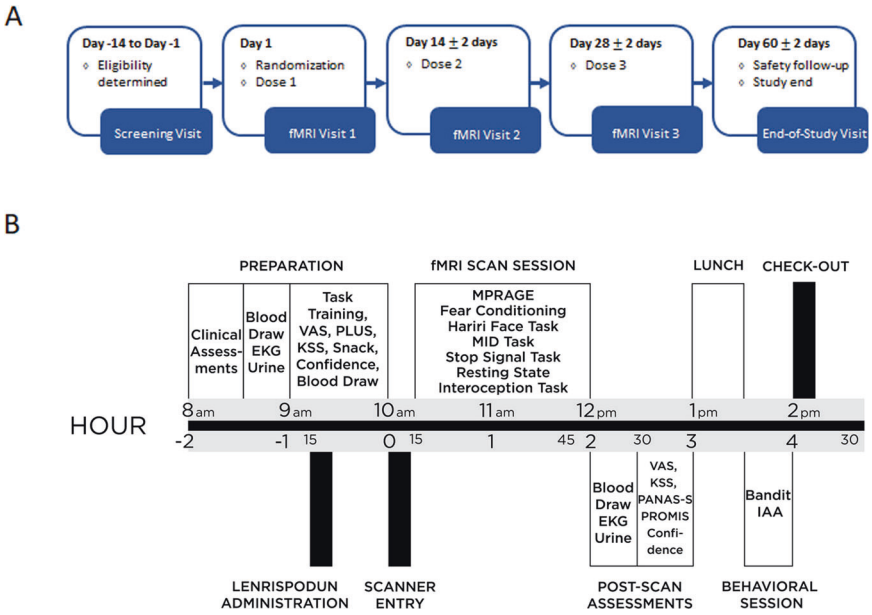

Fig. 1 Study design and schedule of assessments at neuroimaging visits. A Study design, **B** Schedule of assessments at neuroimaging visits. An alternative scheduling option allowed for a 10 am start time. EKG electrocardiogram, VAS visual analog scale, KSS Karolinska Sleepiness Scale, PLUS participant last use summary, MPRAGE magnetization-prepared rapid gradient echo, MID monetary incentive delay task, PANAS-S positive and negative affect scheduled-state, PROMIS patient-reported outcomes measurement information system, Bandit Bandit task, IAA implicit approach/avoidance task.

Scanning assessments

The primary scanning assessments are described below for the stop signal and fear conditioning tasks. Additional scanning assessments are described in the Supplementary Materials for the emotional face processing, monetary incentive delay, and VIA tasks.

Stop signal task

To measure behavioral and neural responses to inhibitory processing, participants completed a SST based closely on a previous version [22]. At each trial onset either an "X" or an "O" appeared on a black background. Participants were instructed to press, as quickly and accurately as possible, the left button when an "X" appeared, and the right button when an "O" appeared. They were also instructed not to press either button whenever they heard a tone during a trial (stop trials). Each trial lasted 1300 milliseconds (ms) and was separated by 200 ms inter-stimulus intervals (blank screen). Six blocks were performed, each containing 48 trials (12 stop and 36 nonstop trials in each block). Trial order was pseudo-randomized. A practice run was conducted prior to scanning to determine their mean reaction time (MRT) from "X" and "O" stimulus onset. These individual measures were used to determine the stop signal delay for the six different stop trial types. Specifically, stop signals were delivered at 0 (MRT-0), 100 (MRT-100), 200 (MRT-200), 300 (MRT-300), 400 (MRT-400), or 500 (MRT-500) ms prior to their MRT, yielding a range of difficulty levels [22]. For analysis, trials were divided into "easy" (MRT-500, MRT-400, MRT-300) and "hard" (MRT-200, MRT-100, MRT-0) conditions.

Fear conditioning/extinction task

The fear conditioning measure was based closely on a previously used task [23]. The stimuli consisted of two abstract images as conditioned stimuli (CS), presented for 2 s at a time. CS+ images (paired with the unconditioned stimulus (US) during fear acquisition) and CS− images (never paired with the US) were counter-balanced across participants. The US was a 1 s scream beginning 500 ms after image onset. In the 9–15 s between CS image presentations, participants began a continuous performance task requiring a right- or left button press in response to right- or left-facing arrows. This increased engagement and attention during inter-trial intervals. The task had three components: a familiarization period, fear acquisition, and fear extinction. The familiarization phase (2.5 min) involved five presentations of each CS with no instances of the US to provide a baseline and allow familiarization to the scanner environment. The acquisition phase was divided into two 8-min runs. Each run consisted of 15 presentations of the CS− and 20 presentations of the CS+: five with (CS+ paired) and 15 without (CS+ unpaired) the US, matching the previous study [23] and allowing for an equal number of trials to be included in the analysis (CS+ paired trials were excluded from analysis so as to not confound processing of the CS+ with reactivity to the US). Finally, the extinction phase (12.3 min) involved 25 presentations of each CS with no instances of the US. Participants rated their experienced valence, arousal, and anxiety level to each CS at four times during the task: after familiarization, halfway through acquisition, after acquisition, and after extinction. Trials were presented in a fixed, pseudo-randomized order, constrained so that no more than two identical trials occurred in a row and runs were presented consecutively, so there was never more than a few min between them.

Interoceptive attention task

To measure the impact of lenrispodun on brain activity during interoceptive signal processing, participants completed the VIA task. During this task, participants alternated their focus of attention between two conditions: the interoception condition and the exteroception condition. During the interoception condition, the word "HEART" or "STOMACH" was presented on the screen and participants were instructed to focus their attention on interoceptive sensations from that organ. For example, upon seeing the word "HEART," participants focused their attention on how intensely they felt the sensation of their heart beating. During the exteroception (control) condition, the word "TARGET" was presented in the middle of the screen and the color of the word alternated from black to a lighter shade of gray

every second (s). Participants were instructed to focus their attention on the intensity of these color changes. Each task condition was presented in 10-s blocks, and half of the blocks were followed immediately by a 5-s response period during which the participant used a visual scale (0 = "no sensation" to 6 = "extreme sensation") to rate the intensity of interoceptive sensations or exteroceptive color changes experienced during the preceding trial. Blocks were often separated by a variable inter-stimulus interval, during which participants looked at a fixation mark. Each run of the task began with a 10-s initial fixation period and ended with a 10-s final fixation period. Participants performed two scanning runs, each lasting 360 s (including initial and final fixation periods). By requiring participants to focus their attention on internal sensations from their heart and viscera, this task made use of the attentional spotlight effect to amplify the signal within cortical regions underlying viscero-sensory perception, thereby serving as a functional localizer for mapping interoceptive activity in the insula. It has previously served as a means of identifying group differences in the dorsal mid-insula's BOLD response during the deployment of goal-directed interoceptive attention [24–27].

Hariri face task–modified

This task has been shown previously to sensitively probe amygdala activity and was a modified version of the Hariri face task [28]. It required the participant to match one of two faces to a target face based on the emotion expressed on the target face. The key component of the task was an emotional face discrimination task that consisted of a control condition and an experimental condition. The participant was presented with three ellipses, one centered at the top of the screen and two at the lower corners of the screen. Participants were instructed to select the ellipse in the lower half of the screen that matched the ellipse in the upper of the screen. The number of correct matches on the left and the right side were matched in each block. There were three emotional discrimination trial types each with a similar format as the control task except the faces were matched on the emotion expressed on the face, where faces were either angry, fearful, or happy.

Structural brain MRI

A T1-weighted magnetization-prepared rapid acquisition with gradient echo (TR/TE = 5/2.012 ms, FOV/slice = 240 × 192/0.9 mm, 186 axial slices) structural MRI scan was performed on days 1, 14, and 28 during the fMRI scan to allow co-registration of functional and structural brain images.

Image acquisition

All fMRI data were acquired on a GE Discovery MR750 3T scanner using an 8-channel GE phased array coil (TR/TE = 2000/27 ms, FOV/slice = 240/2.9 mm, 128 × 128 matrix, 39 axial slices) with varying numbers of TRs depending on the task.

Functional neuroimaging analysis

Analysis of fMRI data varied depending on the task. However, all preprocessing was conducted in AFNI [29] using common subject-level processing, described in the Supplementary Materials. Following preprocessing, each subject had contrasts of interest averaged over prespecified regions of interest selected from the Brainnetome atlas [30], including dorsal anterior insula, IFG, amygdala, medial prefrontal cortex, anterior insula, dorsolateral prefrontal cortex, dAC cortex and mid and posterior insula (Supplementary Table 1). These contrasts were used in the subsequent group-level analyses. Descriptive statistics for fMRI variables were summarized in units of percent signal change. Each imaging objective was assessed using a linear mixed effects model with a task-specific contrast as its outcome. Task-specific processing and statistical analysis details are described in the Supplementary Materials.

Exploratory objectives

Exploratory objectives examined lenrispodun effects on cardiovascular function (orthostatic heart rate), interoception, neural responses to emotional faces, behavioral performance, and drug/metabolite concentration levels.

RESULTS

Participants

Thirty-three participants were screened. Twenty-six participants were randomized (Table 1), and 7 participants were screen-failed. Most (73.1–76.9%) completed each cross-over period (Fig. 2 and Supplementary Fig. 1, Supplementary Table 2).

FUNCTIONAL NEUROIMAGING TASKS

Stop signal task

Omnibus tests showed varying support for a main effect of lenrispodun across prespecified regions of interest, i.e., the IFG ($p = 0.07$), dorsolateral prefrontal cortex ($p = 0.2$), dAC ($p = 0.09$), and anterior insula ($p = 0.006$). Post hoc tests showed consistent support when comparing the 1.0 mg dose to placebo, where all regions showed increased NOGOvGO activation (p's 0.01–0.09, d from 0.57 to 0.87), but there was no support for an effect of 10.0 mg (p's > 0.66, d < 0.14) (Table 2 and Fig. 3A).

Fear conditioning/extinction

None of the a priori hypothesized subregions showed a statistically significant difference between treatment groups, either during acquisition or extinction of fear conditioning. Relating to the first primary objective, lenrispodun had no effect on the CSPLUSvCSMINUS contrast during early extinction in the dorsal anterior insula (main effect of drug $p = 0.94$, $d = -0.05$ for 1.0 mg and 0.24 for 10.0 mg). Similarly, for secondary objectives evaluating related brain regions, there was no significant drug by time interaction during conditioning in the amygdala ($p = 0.84$), medial prefrontal cortex ($p = 0.98$), or anterior insula ($p = 0.31$) (see Supplementary Fig. 4).

Subjective and behavioral assessment

Lenrispodun had no effect on self-reported mood including PROMIS Anxiety ($p = 0.15$, partial $\eta^2 = 0.093$), Anger ($p = 0.82$, partial $\eta^2 = 0.010$), and Depression ($p = 0.60$, partial $\eta^2 = 0.028$) (Supplementary Fig. 2). Lenrispodun did not induce dose-related

Table 1. Demographic characteristics of the randomized participants.

Demographic	
Number (*n*) of participants	26
Age	
Mean ± Standard deviation (SD)	23.5 ± 4.7 years
Gender, *n* (%)	
Male	15 (57.7%)
Female	11 (42.3%)
Race/ethnicity, *n* (%)	
White or Caucasian	14 (53.8%)
Black or African American	6 (23.1%)
Asian	2 (7.7%)
Native or Indigenous	0 (0%)
Unknown/refused	3 (11.5%)
Height	
Mean ± SD	172.3 ± 8.8 centimeters
Weight	
Mean ± SD	76.8 ± 14.0 kilograms

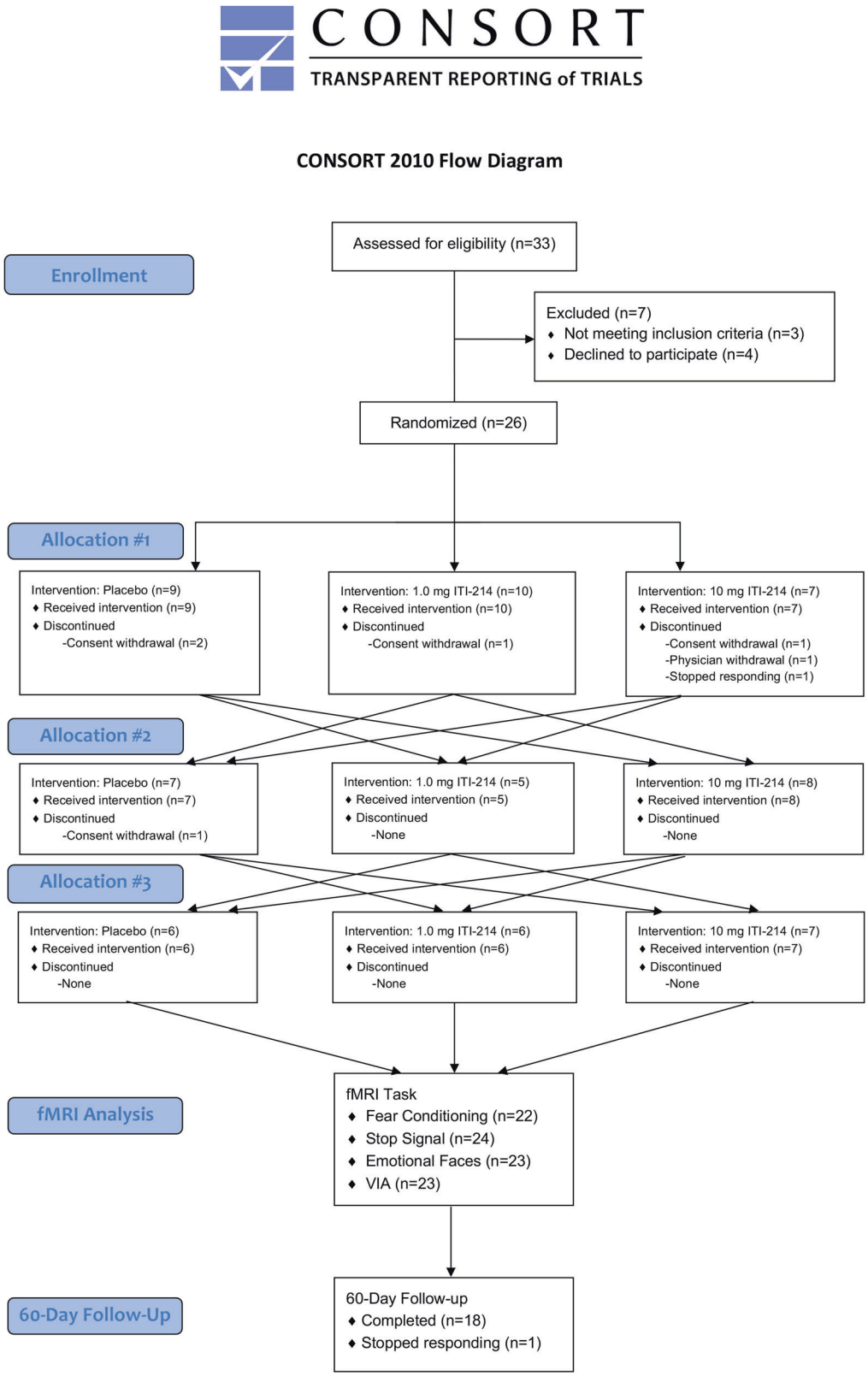

Fig. 2 Study consort diagram. Consort diagram illustrating participant randomization and number of completers for each fMRI task and visit.

effects on behavioral performance measures collected in the MRI (reaction time, response accuracy) during (1) fear conditioning and extinction, (2) SST, or (3) emotional face processing task. For these three tasks there were some effects of visit such that participants were less accurate and slower at later visits.

EXPLORATORY AIMS

Visceral interoceptive attention

There was no effect of drug on the prespecified Intero-Extero contrast (i.e., HEART + STOMACHvTARGET) in the VIA task for the amygdala ($p = 0.97$), dorsal anterior insula ($p = 0.54$), mid and

Table 2. Statistical significance and effect sizes for the ANOVA test and fixed effects (dose and timepoint) analyses examining the influence of lenrispodun on neural activity during the stop signal, fear conditioning, emotional face processing and visceral interoception attention task as well as orthostatic heart rate changes.

Task	Region of interest	ANOVA test		Fixed effects							
				Lenrispodun: 1.0 mg		Lenrispodun: 10.0 mg		T2		T3	
		Drug (*p*)	Visit (*p*)	*p*	Cohen's *d*	*p*	Cohen's *d*	*p*	Cohen's *d*	*p*	Cohen's *d*
Stop signal	Inferior frontal gyrus	0.068	0.584	0.062	0.628	0.833	−0.068	0.359	−0.298	0.412	−0.268
	Dorsolateral prefrontal cortex	0.195	0.487	0.089	0.572	0.660	0.141	0.430	0.257	0.255	0.374
	Dorsal anterior cingulate	0.086	0.711	0.047*	0.690	0.795	0.085	0.419	−0.269	0.650	−0.152
	Anterior insula	0.006	0.128	0.012*	0.871	0.760	−0.099	0.059	−0.634	0.176	−0.452
Fear conditioning	Dorsal anterior insula	0.626	0.684	0.876	−0.052	0.460	0.242	0.942	−0.024	0.430	−0.263
Emotional face processing	Amygdala	0.754	0.480	0.591	−0.182	0.480	−0.238	0.480	−0.243	0.241	−0.401
	Dorsal anterior insula	0.327	0.377	0.358	−0.254	0.151	−0.401	0.185	−0.369	0.756	−0.086
	Medial prefrontal cortex	0.440	0.937	0.283	−0.298	0.276	−0.302	0.724	−0.097	0.841	−0.055
Visceral interoceptive attention: Interoception-exteroception	Amygdala	0.971	0.889	0.883	−0.042	0.812	−0.068	0.632	0.137	0.796	0.074
	Dorsal anterior insula	0.544	0.361	0.334	0.377	0.975	0.012	0.262	0.425	0.879	−0.056
	Mid and posterior insula	0.911	0.183	0.829	−0.062	0.826	0.063	0.853	−0.053	0.105	−0.474
	Medial prefrontal cortex	0.844	0.188	0.648	0.159	0.927	−0.031	0.606	0.176	0.226	−0.409
Visceral interoceptive attention: Heart	Amygdala	0.225	0.526	0.123	−0.553	0.189	−0.460	0.611	−0.175	0.552	0.201
	Dorsal anterior insula	0.122	0.536	0.161	−0.518	0.059	−0.691	0.384	−0.309	0.901	0.043
	Mid and posterior insula	0.039	0.559	0.023*	−0.934	0.069	−0.716	0.623	−0.187	0.585	0.204
	Medial prefrontal cortex	0.070	0.892	0.065	−0.724	0.052	−0.741	0.809	−0.089	0.828	0.079
Orthostatic heart rate	Supine	<0.001	0.807	0.036*	0.718	<0.001*	2.016	0.533	−0.201	0.890	−0.045
	Standing 1 min	<0.001	0.536	0.001*	1.212	<0.001*	2.004	0.449	0.251	0.734	−0.113
	Standing 3 min	<0.001	0.962	0.038*	0.725	<0.001*	1.278	0.784	0.091	0.917	0.035
	Standing 5 min	0.038	0.545	0.138	0.510	0.016*	0.828	0.480	0.235	0.701	−0.128

Significant results are marked for post hoc pairwise tests. *$p < 0.05$

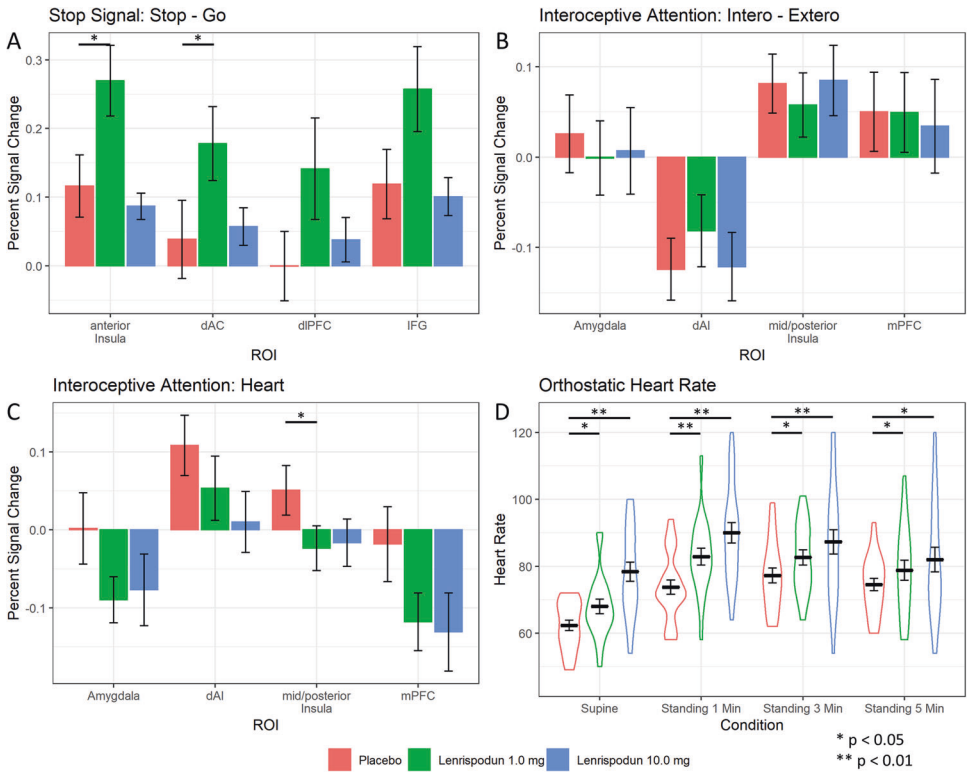

Fig. 3 Brain activity as a function of lenrispodun dose (1.0 or 10.0 mg) vs. placebo. A Stop signal task, **B** interoceptive-exteroceptive attention contrast, **C** heart attention, **D** positional heart rate changes. ROI region of interest, dAC dorsal anterior cingulate cortex, dlPFC dorsolateral prefrontal cortex, IFG inferior frontal gyrus, dAI dorsal anterior insula, mPFC medial prefrontal cortex, Min minutes.

posterior insula ($p = 0.91$), or medial prefrontal cortex ($p = 0.84$) (Table 2 and Fig. 3B). However, based on the emerging data supporting a cardiovascular effect of lenrispodun, we decided post hoc to test for an effect of drug on the response to heart attention only. In this case, we observed evidence for an effect of drug in the mid and posterior insula (main effect $p = 0.039$; 1.0 mg $p = 0.023$, $d = -0.93$; 10.0 mg $p = 0.069$, $d = -0.72$) and medial prefrontal cortex (main effect $p = 0.07$; 1.0 mg $p = 0.065$, $d = -0.724$; 10.0 mg $p = 0.05$, $d = -0.74$) (Table 2 and Fig. 3C).

Orthostatic heart rate

Lenrispodun increased heart rate while supine in a dose-dependent manner, and this effect gradually weakened while participants stood for 5 min (Table 2 and Fig. 3D).

Emotional face processing

There were no statistically significant effects of drug on BOLD fMRI contrast between faces and shapes in the amygdala ($p = 0.75$), dorsal anterior insula ($p = 0.33$) or medial prefrontal cortex ($p = 0.44$) or on behavior (see Supplementary Fig. 5).

Pharmacokinetic results

Lenrispodun and three metabolites were present in blood samples taken ~45 and 210 min after administration (Supplementary Fig. 3). Linear mixed effects models confirmed significant main effects of drug and time, and a significant drug*time interaction (all p's <0.001). Post hoc tests confirmed a dose-dependent response at each timepoint after administration (Supplementary Table 3).

Safety results

No serious adverse events or deaths were reported during the study. During the study, seven participants reported treatment-emergent adverse events (TEAE). All TEAEs were mild in severity and no TEAE was considered related to the study drug. The number of participants with TEAE were: 2 with the 1 mg dose, 2 with the 10 mg dose, and 3 with placebo. The following TEAEs were reported: procedural nausea, procedural anxiety, dizziness, claustrophobia, motion sickness, seasonal allergy, and a road traffic accident during the follow-up period (i.e., several weeks after the final dose administration).

DISCUSSION

This study used a within-subjects design in healthy volunteers to examine whether selective phosphodiesterase 1 inhibition via lenrispodun significantly influences cognitive, affective, and interoceptive processing yielding three main results. First, 1.0 mg lenrispodun increased BOLD fMRI signals in the IFG during the SST consistent with a cognitive effect. Second, lenrispodun did not induce an attenuating effect on BOLD fMRI signals in the dorsal anterior insula during the extinction phase of a fear conditioning task, suggesting a lack of effect on emotional processing. Third, lenrispodun increased resting and orthostatic heart rate in a dose-dependent manner yielding significant differences in insula activation during heart-focused attention. Lenrispodun was considered safe and well-tolerated at both doses. Collectively, these results support the hypothesis that PDE1 inhibition selectively affects inhibitory control and cardiovascular interoceptive processing without affecting basic fear processing in healthy volunteers.

Lenrispodun increased BOLD fMRI signals in the IFG during the SST. Although there was evidence for an effect lenrispodun at 1.0 mg, with an effect size of 0.63, given the number of participants this effect was not significant at the $p = 0.05$ threshold ($p = 0.062$). Examination of the ancillary data revealed that the lenrispodun dose effect at 1.0 mg was driven by an increased IFG response to Stop trials, without affecting the Go trials. This dose-, task-, and brain region-selective central nervous system engagement—

consistent with the mechanism of action of central PDE1 inhibition by lenrispodun—was substantiated by the results related to the SST as measured by the secondary objectives. It is unclear why the effects were observed with 1.0 mg, but not 10.0 mg lenrispodun. To the extent that lenrispodun may enhance dopamine 1 (D1) receptor function, a nonlinear, "inverted-U" dose-response has been well established for D1-mediated cognitive processes [31]. Nonetheless, the effects of 1.0 mg were consistent across brain regions involved in inhibitory control, providing internally consistent evidence of a clear central effect of lenrispodun at a relatively low dose; important findings that can inform dose selection for future clinical studies.

The effect of lenrispodun on inhibitory cognitive control is consistent with the distribution of PDE1 effects in the brain. Specifically, the neural focus of PDE1 inhibition has been linked primarily to D1 receptors in the brain, which are most densely distributed in humans in the basal ganglia, amygdala, mamillary bodies, as well as the insula and cingulate among a broad distribution in the cortex [32]. A recent study showed that PDE1 inhibition via lenrispodun increased second messenger concentrations and facilitated neurotransmission in the mouse prefrontal cortex [33]. Moreover, in the same study these effects were mirrored by the D1 agonist SKF38393, which also improved working memory and attentional performance, providing a direct link to dopaminergic function. In another study, the PDE1 inhibitor Lu AF64196 did not increase neuronal cyclic nucleotide levels in general but blocked the NMDA-induced reduction in cyclic nucleotides. PDE1 inhibition also down-regulated the D1-receptor mediated increase in cAMP and increased long-term potentiation in rat ventral striatum. A third orally available PDE1 inhibitor, DSR-141562, reversed social interaction and novel object recognition deficits induced by repeated treatment with an N-methyl-D-aspartate receptor antagonist, phencyclidine in mice and rats [34]. Taken together, these preclinical studies provide support for a general hypothesis that PDE1 inhibition may modulate glutamatergic and dopaminergic signaling in brain areas enriched in D1 receptors [35].

The selective increase in stop-related activation for the low dose lenrispodun in executive control and salience areas point toward the possibility of this drug to selectively modulate neural aspects of inhibitory cognitive control. Action-stopping, which is a canonical executive function, activates both the right inferior frontal cortex (rIFC) and the anterior insula [36, 37], and these were precisely the regions whose activity was modulated by low dose lenrispodun in addition to the dAC. The rIFC (which contains the rIFG) is particularly relevant for triggering stopping in concert with a wider network. However, the evidence for inhibitory cognitive control deficits in target populations is mixed. Some studies have reported ADHD-related behavioral effects on the SST [38], with meta-analyses showing a moderate effect size on the stop signal reaction time [39]. Similarly, individuals with ADHD show some brain-related processing dysfunctions, i.e., outcome-related impairment in the medial prefrontal cortex [40], as well as reduced activation in performance monitoring areas of dorsomedial and left ventrolateral prefrontal cortices, thalamus, cingulate, and parietal regions [41]. However, there is limited evidence for deficits on stop signal behavior in depression or anxiety [42]. Traditional pharmacological modulation of neural systems involved in the SST has also yielded complex results. For example, in one study methylphenidate reduced activation within the right IFG/insula during successful inhibition, failed inhibition, and attentional capture, but increased activation within the superior frontal, dAC, and parieto-occipital cortices [43]. Another study found an increase in right inferior frontal, left middle frontal, left angular gyri and right caudate during inhibition in addition to increased pregenual cingulate and dAC activity [44]. Overall, based on the observed dose-, task-, and brain region-selective central nervous system engagement by lenrispodun, the present findings suggest that investigational modulation of dysfunctional inhibitory control may be warranted in target populations such as ADHD. Such improvement of inhibitory control by PDE1 inhibition may translate to therapeutic benefit in individuals with attentional and inhibitory control deficits.

The lack of effects of lenrispodun during fear conditioning (primary outcome) or emotional face presentation does not support the hypothesis that PDE1 inhibition modulates associative learning or basic affective processing. The initial hypothesis was that PDE1 inhibition might affect learning processes similar to methylphenidate [45] and be related to methylphenidate's beneficial effect in PTSD [21]. This was not supported by the present results which may indicate that PDE1 inhibition alone is not sufficient to alter these processes. PDE1 inhibition is calcium calmodulin-dependent which requires sufficient dopaminergic tone to modulate intracellular signaling. Thus, one possible interpretation of the null result with respect to fear conditioning is that there may not have been sufficient endogenous dopaminergic tone in this assay. It is possible that PDE1 inhibition would enhance the effects of dopamine agonists or might show a signal in a patient population with enhanced dopaminergic tone, or during co-administration of a dopamine-releasing agent, but those conditions were beyond the scope of the present experiment. Another possibility is that the fear conditioning fMRI assay was insufficient to trigger threat-related processing, a problem that has been raised in relation to key threat-related brain regions such as the amygdala [46]. Nevertheless, these results are consistent with a lack of PDE1-related modulation of basic affective processes and point to a selective effect of PDE1 mediated cognitive processes in healthy volunteers.

The modulating effect of lenrispodun on resting and orthostatic heart rate is consistent with the peripheral action of this compound [9], and in secondary and exploratory analyses this effect was reflected by significant shifts in insular activation during an interoceptive attention task. We have shown that the same sectors of the insular cortex are sensitive to inodilator modulation during peripheral beta-adrenergic stimulation with isoproterenol [47, 48], thus these results provide convergent evidence for the insula's role in cardiac interoception. The main effect of lenrispodun was independent of dose for the mid/posterior insula, whereas the anterior insula showed a dose-dependent reduction, implying a heterogeneous response in subregions implicated in the attentional mapping of ongoing vs. anticipated changes [49, 50]. These secondary results underscore the potential utility of lenrispodun in modulating cardiovascular function at the level of the autonomic and central autonomic nervous systems, which could yield novel targets related to peripheral and central cardiovascular regulation, for example, at the level of limbic [51] and brainstem-associated structures such as the hypothalamus [52].

This study has limitations that should be noted. First, it was not designed to differentiate between the acute and chronic effects of lenrispodun, which may have shed light on adaptive changes that contribute to the possible therapeutic effects of this mechanism of action. Second, this study was conducted with healthy volunteers, who may have a different subjective and circuit level baseline than target populations as it relates to fear learning, thereby limiting generalizability of the findings [53]. For example, while lenrispodun did not modulate stress or anxiety ratings in these healthy volunteers, we cannot exclude the possibility of such an effect in target (patient) populations. Strengths of this study include the hypothesis-guided use of an a priori ROI-based approach and the reporting of effect size estimates, which allows for power calculations in subsequent studies, and is increasingly reflective of a best-practice methodology in fMRI research [54, 55]. Based on the favorable safety/tolerability profile and encouraging effects in the brain, future investigations could focus on acute and chronic administration of PDE1 inhibitors and their effect on inhibitory

control processing in healthy volunteers as well as in target populations such as individuals with ADHD.

CONCLUSION

This is the first study to provide evidence of central nervous system engagement by lenrispodun, consistent with its PDE1 inhibition mechanism of action. Lenrispodun selectively improved neural inhibitory cognitive control in a manner that may translate to therapeutic benefit in patient populations with impaired inhibitory processing, such as ADHD. These data also provide further evidence of the safety and tolerability of lenrispodun and support its future development for the treatment of central nervous system disorders.

REFERENCES

1. Rose GM, Hopper A, De Vivo M, Tehim A. Phosphodiesterase inhibitors for cognitive enhancement. Curr Pharm Des. 2005;11:3329–34.
2. Rutten K, Prickaerts J, Hendrix M, van der Staay FJ, Sik A, Blokland A. Time-dependent involvement of cAMP and cGMP in consolidation of object memory: studies using selective phosphodiesterase type 2, 4 and 5 inhibitors. Eur J Pharmacol. 2007;558:107–12.
3. Rodefer JS, Saland SK, Eckrich SJ. Selective phosphodiesterase inhibitors improve performance on the ED/ID cognitive task in rats. Neuropharmacology. 2012;62:1182–90.
4. Reneerkens OA, Rutten K, Bollen E, Hage T, Blokland A, Steinbusch HW, et al. Inhibition of phoshodiesterase type 2 or type 10 reverses object memory deficits induced by scopolamine or MK-801. Behav Brain Res. 2013;236:16–22.
5. Reneerkens OA, Sambeth A, Blokland A, Prickaerts J. PDE2 and PDE10, but not PDE5, inhibition affect basic auditory information processing in rats. Behav Brain Res. 2013;250:251–6.
6. Li P, Zheng H, Zhao J, Zhang L, Yao W, Zhu H, et al. Discovery of potent and selective inhibitors of phosphodiesterase 1 for the treatment of cognitive impairment associated with neurodegenerative and neuropsychiatric diseases. J Med Chem. 2016;59:1149–64.
7. Snyder GL, Prickaerts J, Wadenberg ML, Zhang L, Zheng H, Yao W, et al. Preclinical profile of ITI-214, an inhibitor of phosphodiesterase 1, for enhancement of memory performance in rats. Psychopharmacology. 2016;233:3113–24.
8. Humphrey JM, Movsesian M, Am Ende CW, Becker SL, Chappie TA, Jenkinson S, et al. Discovery of potent and selective periphery-restricted quinazoline inhibitors of the cyclic nucleotide phosphodiesterase PDE1. J Med Chem. 2018;61:4635–40.
9. Hashimoto T, Kim GE, Tunin RS, Adesiyun T, Hsu S, Nakagawa R, et al. Acute enhancement of cardiac function by phosphodiesterase type 1 inhibition. Circulation. 2018;138:1974–87.
10. Gilotra NA, DeVore AD, Povsic TJ, Hays AG, Hahn VS, Agunbiade TA, et al. Acute hemodynamic effects and tolerability of phosphodiesterase-1 inhibition with ITI-214 in human systolic heart failure. Circ Heart Fail. 2021;14:e008236.
11. Golshiri K, Ataei Ataabadi E, Rubio-Beltran E, Dutheil S, Yao W, Snyder GL, et al. Selective phosphodiesterase 1 inhibition ameliorates vascular function, reduces inflammatory response, and lowers blood pressure in aging animals. J Pharmacol Exp Ther. 2021;378:173–83.
12. O'Brien JJ, O'Callaghan JP, Miller DB, Chalgeri S, Wennogle LP, Davis RE, et al. Inhibition of calcium-calmodulin-dependent phosphodiesterase (PDE1) suppresses inflammatory responses. Mol Cell Neurosci. 2020;102:103449.
13. Congdon E, Altshuler LL, Mumford JA, Karlsgodt KH, Sabb FW, Ventura J, et al. Neural activation during response inhibition in adult attention-deficit/hyperactivity disorder: preliminary findings on the effects of medication and symptom severity. Psychiatry Res. 2014;222:17–28.
14. Pliszka SR, Glahn DC, Semrud-Clikeman M, Franklin C, Perez R 3rd, Xiong J, et al. Neuroimaging of inhibitory control areas in children with attention deficit hyperactivity disorder who were treatment naive or in long-term treatment. Am J Psychiatry. 2006;163:1052–60.
15. Fullana MA, Albajes-Eizagirre A, Soriano-Mas C, Vervliet B, Cardoner N, Benet O, et al. Fear extinction in the human brain: a meta-analysis of fMRI studies in healthy participants. Neurosci Biobehav Rev. 2018;88:16–25.
16. Heimer L, Van Hoesen GW. The limbic lobe and its output channels: implications for emotional functions and adaptive behavior. Neurosci Biobehav Rev. 2006;30:126–47.
17. Fadok JP, Dickerson TM, Palmiter RD. Dopamine is necessary for cue-dependent fear conditioning. J Neurosci. 2009;29:11089–97.
18. Abraham AD, Neve KA, Lattal KM. Dopamine and extinction: a convergence of theory with fear and reward circuitry. Neurobiol Learn Mem. 2014;108:65–77.
19. Ikegami M, Uemura T, Kishioka A, Sakimura K, Mishina M. Striatal dopamine D1 receptor is essential for contextual fear conditioning. Sci Rep. 2014;4:3976.
20. Abraham AD, Cunningham CL, Lattal KM. Methylphenidate enhances extinction of contextual fear. Learn Mem. 2012;19:67–72.
21. McAllister TW, Zafonte R, Jain S, Flashman LA, George MS, Grant GA, et al. Randomized placebo-controlled trial of methylphenidate or galantamine for persistent emotional and cognitive symptoms associated with PTSD and/or traumatic brain injury. Neuropsychopharmacology. 2016;41:1191–8.
22. Schuckit MA, Tapert S, Matthews SC, Paulus MP, Tolentino NJ, Smith TL, et al. fMRI differences between subjects with low and high responses to alcohol during a stop signal task. Alcohol Clin Exp Res. 2012;36:130–40.
23. Ball TM, Knapp SE, Paulus MP, Stein MB. Brain activation during fear extinction predicts exposure success. Depress Anxiety. 2017;34:257–66.
24. Avery JA, Drevets WC, Moseman SE, Bodurka J, Barcalow JC, Simmons WK. Major depressive disorder is associated with abnormal interoceptive activity and functional connectivity in the insula. Biol Psychiatry. 2014;76:258–66.
25. Simmons WK, Rapuano KM, Kallman SJ, Ingeholm JE, Miller B, Gotts SJ, et al. Category-specific integration of homeostatic signals in caudal but not rostral human insula. Nat Neurosci. 2013;16:1551–52.
26. Stewart JL, Khalsa SS, Kuplicki R, Puhl M, Investigators T, Paulus MP. Interoceptive attention in opioid and stimulant use disorder. Addiction Biol. 2020;25:e12831.
27. Burrows K, DeVille DC, Cosgrove KT, Kuplicki RT, Tulsa I, Paulus MP, et al. Impact of serotonergic medication on interoception in major depressive disorder. Biol Psychol. 2022;169:108286.
28. Hariri AR, Tessitore A, Mattay VS, Fera F, Weinberger DR. The amygdala response to emotional stimuli: a comparison of faces and scenes. Neuroimage. 2002;17:317–23.
29. Cox RW. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. Computers Biomed Res. 1996;29:162–73.
30. Fan L, Li H, Zhuo J, Zhang Y, Wang J, Chen L, et al. The human brainnetome atlas: a new brain atlas based on connectional architecture. Cereb Cortex. 2016;26:3508–26.
31. Cools R, Frobose M, Aarts E, Hofmans L. Dopamine and the motivation of cognitive control. Handb Clin Neurol. 2019;163:123–43.
32. Cortés R, Gueye B, Pazos A, Probst A, Palacios JM. Dopamine receptors in human brain: autoradiographic distribution of D1 sites. Neuroscience. 1989;28:263–73.
33. Pekcec A, Schülert N, Stierstorfer B, Deiana S, Dorner-Ciossek C, Rosenbrock H. Targeting the dopamine D(1) receptor or its downstream signalling by inhibiting phosphodiesterase-1 improves cognitive performance. Br J Pharmacol. 2018;175:3021–33.
34. Enomoto T, Tatara A, Goda M, Nishizato Y, Nishigori K, Kitamura A, et al. A novel phosphodiesterase 1 inhibitor DSR-141562 exhibits efficacies in animal models for positive, negative, and cognitive symptoms associated with schizophrenia. J Pharmacol Exp Ther. 2019;371:692–702.
35. Betolngar DB, Mota É, Fabritius A, Nielsen J, Hougaard C, Christoffersen CT, et al. Phosphodiesterase 1 bridges glutamate inputs with NO- and dopamine-induced cyclic nucleotide signals in the striatum. Cereb Cortex. 2019;29:5022–36.
36. Bartoli E, Aron AR, Tandon N. Topography and timing of activity in right inferior frontal cortex and anterior insula for stopping movement. Hum Brain Mapp. 2018;39:189–203.
37. Aron AR, Fletcher PC, Bullmore ET, Sahakian BJ, Robbins TW. Stop-signal inhibition disrupted by damage to right inferior frontal gyrus in humans. Nat Neurosci. 2003;6:115–16.
38. Schachar RJ, Chen S, Logan GD, Ornstein TJ, Crosbie J, Ickowicz A, et al. Evidence for an error monitoring deficit in attention deficit hyperactivity disorder. J Abnorm Child Psychol. 2004;32:285–93.
39. Lipszyc J, Schachar R. Inhibitory control and psychopathology: a meta-analysis of studies using the stop signal task. J Int Neuropsychol Soc. 2010;16:1064–76.
40. Hauser TU, Iannaccone R, Ball J, Mathys C, Brandeis D, Walitza S, et al. Role of the medial prefrontal cortex in impaired decision making in juvenile attention-deficit/hyperactivity disorder. JAMA Psychiatry. 2014;71:1165–73.
41. Rubia K, Halari R, Mohammad AM, Taylor E, Brammer M. Methylphenidate normalizes frontocingulate underactivation during error processing in attention-deficit/hyperactivity disorder. Biol Psychiatry. 2011;70:255–62.
42. Spechler PA, Stewart JL, Kuplicki R, Paulus MP, Tulsa I. Parsing impulsivity in individuals with anxiety and depression who use Cannabis. Drug Alcohol Depend. 2020;217:108289.
43. Pauls AM, O'Daly OG, Rubia K, Riedel WJ, Williams SC, Mehta MA. Methylphenidate effects on prefrontal functioning during attentional-capture and response inhibition. Biol Psychiatry. 2012;72:142–9.
44. Nandam LS, Hester R, Bellgrove MA. Dissociable and common effects of methylphenidate, atomoxetine and citalopram on response inhibition neural networks. Neuropsychologia. 2014;56:263–70.
45. Howlett JR, Huang H, Hysek CM, Paulus MP. The effect of single-dose methylphenidate on the rate of error-driven learning in healthy males: a randomized controlled trial. Psychopharmacology. 2017;234:3353–60.

46. Fullana MA, Albajes-Eizagirre A, Soriano-Mas C, Vervliet B, Cardoner N, Benet O, et al. Amygdala where art thou? Neurosci Biobehav Rev. 2019;102:430–31.
47. Hassanpour MS, Simmons WK, Feinstein JS, Luo Q, Lapidus RC, Bodurka J, et al. The insular cortex dynamically maps changes in cardiorespiratory interoception. Neuropsychopharmacology. 2018;43:426–34.
48. Teed AR, Feinstein JS, Puhl M, Lapidus RC, Upshaw V, Kuplicki RT, et al. Association of generalized anxiety disorder with autonomic hypersensitivity and blunted ventromedial prefrontal cortex activity during peripheral adrenergic stimulation: a randomized clinical trial. JAMA Psychiatry. 2022;79:323–32.
49. Barrett LF, Simmons WK. Interoceptive predictions in the brain. Nat Rev Neurosci. 2015;16:419–29.
50. Lovero KL, Simmons AN, Aron JL, Paulus MP. Anterior insular cortex anticipates impending stimulus significance. Neuroimage. 2009;45:976–83.
51. Oppenheimer S, Cechetto D. The insular cortex and the regulation of cardiac function. Compr Physiol. 2016;6:1081–133.
52. Manuel J, Farber N, Gerlach DA, Heusser K, Jordan J, Tank J, et al. Deciphering the neural signature of human cardiovascular regulation. Elife. 2020;9:e55316.
53. Lebron-Milad K, Abbs B, Milad MR, Linnman C, Rougemount-Bucking A, Zeidan MA, et al. Sex differences in the neurobiology of fear conditioning and extinction: a preliminary fMRI study of shared sex differences with stress-arousal circuitry. Biol Mood Anxiety Disord. 2012;2:7.
54. Geuter S, Qi G, Welsh RC, Wager TD, Lindquist MA. Effect size and power in fMRI group analysis. bioRxiv. 2018. https://doi.org/10.1101/295048.
55. Szucs D, Ioannidis JP. Sample size evolution in neuroimaging research: an evaluation of highly-cited studies (1990-2012) and of latest practices (2017-2018) in high-impact journals. Neuroimage. 2020;221:117164.

ACKNOWLEDGEMENTS

We thank Lisa Augustine, RN, Megan Cole, RN and Dara Crittenden for assistance with participant recruitment, lenrispodun administration, and safety monitoring; Nigel Negm for assistance with blood and urine sample processing and shipment; Saint Francis Health System Cardiology for assistance with EKG review; and the LIBR MRI technologists for assistance with imaging data acquisition.

AUTHOR CONTRIBUTIONS

Conceptualization: MPP, KEV, and RED; Design of the work: MPP, KEV, HY, and RED; Acquisition, analysis or interpretation of the data for the work: SSK, TAV, RK, KEV, MPP, and RED; Writing—original draft: SSK, TAV, and RK; Writing—critical revisions/editing: all authors; Final approval: SSK, KEV, MPP, and RED. Agreement to be accountable for all aspects of the work, ensuring accuracy/integrity: SSK, TAV, RK, KEV, MPP, and RED. The full trial protocol may be accessed by contacting SSK.

FUNDING

Funding for the study was provided by Intra-Cellular Therapies, Inc. SSK is supported by The William K. Warren Foundation, and the National Institute of Mental Health Grant Award Number (K23MH112949). MPP is supported by The William K. Warren Foundation, the National Institute on Drug Abuse (U01 DA041089), and the National Institute of General Medical Sciences Center Grant Award Number (1P20GM121312). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

COMPETING INTERESTS

RED and KEV were employees of Intra-Cellular Therapies, Inc during the design and collection of study data. RED is a current employee of Intra-Cellular Therapies and may hold stock or stock options. KEV may hold stock of Intra-Cellular Therapies and is a current employee of Engrail Therapeutics and paid advisor to Evolution Research Group. MPP is an advisor to Spring Care, Inc., a behavioral health startup, he has received royalties for an article about methamphetamine in UpToDate. SSK, TAV, RK, and HY have no conflicts-of-interest related to this manuscript to disclose.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41386-022-01331-3.

Correspondence and requests for materials should be addressed to Sahib S. Khalsa.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.