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Archival Report

Human Extinction Learning Is Accelerated by an Angiotensin Antagonist via Ventromedial Prefrontal Cortex and Its Connections With Basolateral Amygdala

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ABSTRACT

BACKGROUND: Deficient extinction learning and threat adaptation in the ventromedial prefrontal cortex (vmPFC)-amygdala circuitry strongly impede the efficacy of exposure-based interventions in anxiety disorders. Recent animal models suggest a regulatory role of the renin-angiotensin system in both these processes. Against this background, the present randomized placebo-controlled pharmacologic functional magnetic resonance imaging experiment aimed at determining the extinction enhancing potential of the angiotensin II type 1 receptor antagonist losartan (LT) in humans.

METHODS: Seventy healthy male subjects underwent Pavlovian threat conditioning and received single-dose LT (50 mg) or placebo administration before extinction. Psychophysiological threat reactivity (skin conductance response) and neural activity during extinction served as primary outcomes. Psychophysiological interaction, voxelwise mediation, and novel multivariate pattern classification analyses were used to determine the underlying neural mechanisms.

RESULTS: LT significantly accelerated the decline of the psychophysiological threat response during within-session extinction learning. On the neural level, the acceleration was accompanied and critically mediated by threat-specific enhancement of vmPFC activation. Furthermore, LT enhanced vmPFC-basolateral amygdala coupling and attenuated the neural threat expression, particularly in the vmPFC, during early extinction.

CONCLUSIONS: Overall the results indicate that LT facilitates within-session threat memory extinction by augmenting threat-specific encoding in the vmPFC and its regulatory control over the amygdala. The findings document a pivotal role of angiotensin regulation of extinction learning in humans and suggest that adjunct LT administration has the potential to facilitate the efficacy of exposure-based interventions in anxiety disorders.

Keywords: Angiotensin, Anxiety disorders, Extinction, fMRI, Functional magnetic resonance imaging, Losartan, Ventromedial prefrontal cortex, vmPFC

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Extinction learning refers to the attenuation of a previously learned defensive response when the threat-predictive stimulus is repeatedly encountered in the absence of adverse consequences. Exposure-based interventions capitalize on extinction learning mechanisms to reduce excessive fear in patients with anxiety-related disorders, and they are considered an efficient therapy in these illnesses (1). A significant number of patients, however, do not adequately respond to exposure therapy (2), and impaired extinction processes are considered one key mechanism underpinning the lack of therapeutic efficacy (3). Anxiety-related disorders are highly prevalent and associated with significant psychosocial impairments and societal costs (4). As such, innovative strategies to improve the efficacy or shorten the duration of exposure therapies are urgently needed.

Whereas the behavioral and neural pathomechanisms underlying anxiety disorders are becoming increasingly understood, the translation into efficacious clinical interventions remains inadequate. Notably, the neural mechanisms mediating extinction are extremely well conserved over the course of evolutionary time; hence, the identification in animal models of receptor targets sensitive to pharmacologic modulation that facilitate neural plasticity in pathways supporting extinction learning can feasibly augment the efficacy of exposure-based interventions (5).

Animal models and human neuroimaging research have demonstrated a crucial role of the infralimbic cortex, which is homologous to the human ventromedial prefrontal cortex (vmPFC) and its interactions with the amygdala in extinction learning (6–12). The vmPFC is critically engaged in the reduction of threat expression during extinction (3,12,13), and it governs the

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amygdala inhibition of the conditioned threat response (8,9). Translational models suggest that dysfunctions in amygdala-prefrontal neuroplasticity contribute to extinction failure in anxiety disorders (14). Converging evidence from clinical research suggests that anxiety disorders are characterized by deficient extinction, hypoactivation within the vmPFC, and attenuated vmPFC-amygdala functional connectivity (6,8,10,13,15).

Intriguingly, recent evidence suggests that the renin-angiotensin (RA) system, primarily known for its role as a blood pressure and renal absorption regulator, represents a promising target to facilitate extinction (5). Central angiotensin receptors are densely expressed in limbic and prefrontal brain regions and are critical to changes in neuroplasticity and extinction (16–18). Initial studies in rodents have demonstrated the potential of pharmacologic modulation of RA signaling toward facilitating extinction using the selective competitive angiotensin II type 1 (AT1) antagonist losartan (LT) (19,20). LT is an approved treatment for high blood pressure, and it has an excellent safety record (21,22). Notably, increasing evidence suggests an association between the RA system and anxiety-related disorders [e.g., Terock *et al.* (23)], and initial clinical observations suggested unexpected beneficial effects of LT on memory and anxiety symptomatology (5,21) [see also a recent study demonstrating effects of LT on aversive learning in healthy participants in Pulcu *et al.* (24)]. Accumulating evidence suggests that LT modulation of central RA signaling represents a promising target to enhance extinction learning.

Against this background, we conducted a preregistered randomized placebo (PLC)-controlled pharmacologic experiment to determine whether targeting the RA system can facilitate extinction in humans. To uncover the underlying neural mechanisms, functional magnetic resonance imaging (fMRI) and psychophysiological threat responses (skin conductance response [SCR]) were acquired simultaneously. The specific goal was to determine the potential of LT (50 mg, single oral dose) as a therapeutic candidate for the clinical augmentation of extinction learning. Previous translational research leads us to expect that LT would accelerate attenuation of the psychophysiological threat responses and that enhanced extinction would be mediated by two neural processes: 1) increased activation in the vmPFC and attenuation of its threat expression in the context of 2) stronger functional interaction of the vmPFC with the amygdala.

METHODS AND MATERIALS

Participants and Experimental Protocols

Seventy healthy male subjects underwent a validated Pavlovian threat acquisition and extinction procedure with simultaneous fMRI and SCR acquisition. To reduce variance related to differences in extinction-related neural activity between men and women (25,26) and sex differences in the responses to RA blockade (27), only male participants were included in the present proof-of-concept study [for a similar approach, see Eckstein *et al.* (28)]. Participants were asked to abstain from caffeinated drinks on the day of the assessment (e.g., coffee, tea, energy beverages). Because of technical issues (SCR recording failure, n = 3) or absence of threat acquisition (n = 8), data from 11 subjects were excluded, leading to 30 LT- and 29 PLC-treated subjects for the evaluation of the primary study hypotheses. For exclusion criteria and a description of the study sample, see Supplemental Methods.

The experiment consisted of 3 sequential stages: 1) acquisition, 2) treatment administration, and 3) extinction. Twenty minutes after acquisition, participants were administered either a single dose (50 mg, by mouth) of LT or PLC, packed in identical capsules. Capsules were dispensed by an independent researcher according to a computer-generated randomization list (n = 2 groups). Consistent with the pharmacodynamic profile of LT [e.g., following oral administration peak plasma levels are reached after 90 minutes, and the terminal elimination half-life of LT proximately ranges from 1.5 to 2.5 hours (29,30–32)], extinction was started 90 minutes after treatment. Although previous studies reported no effects of single-dose LT on cardiovascular activity or mood (18,29,33), these indices were monitored to additionally control primarily for unspecific effects of treatment. The experimental timeline is provided in Figure 1A (for the pharmacodynamic profile and assessment of confounders see Supplemental Methods).

The study was approved by the local institutional ethics committee; it adhered to the Declaration of Helsinki and was a preregistered trial (ClinicalTrials.gov, registration number: NCT03396523; URL: https://clinicaltrials.gov/ct2/show/NCT03396523). The experiments were conducted at the Brain Imaging Center of the Southwest University, Chongqing, China, between January and October 2018.

Experimental Paradigm

During the acquisition stage, participants were repeatedly presented with two different colored squares—the conditioned stimulus (CS). One CS (CS+, 4 seconds) was pseudorandomly paired with a mild electric shock (unconditioned stimulus [US], 2 ms) with 43% contingency, whereas the other CS (CS–, 4 seconds) was never paired with a US. Acquisition was followed by extinction, in which the same cues were presented without US (Supplemental Methods). To enhance threat memory acquisition and to increase the statistical power to determine treatment effects, both learning phases included 2 subsequent runs of the task [for a similar approach, see Feng *et al.* (34)]. The extinction procedure encompassed 2 runs, with each run including 10 CS+ trials without the US, intermixed with 10 presentations of the CS–. No trial-type was repeated more than twice in a row during either acquisition or extinction. Before each run, subjects were informed that "the experimental runs are independent and you may or may not receive the electric shock"; thus, subjects were unable to predict the presence or absence of the US at the beginning of the respective run.

Skin Conductance Response Analysis

Following previous studies (28,35–37), both the CS+ and CS– stimuli of each run were divided into early (first half) and late (last half) to determine learning-related changes during extinction. Consistent with previous studies examining novel strategies to facilitate extinction (35,36), psychophysiological threat responses were defined as baseline-corrected CS+ by subtracting the mean responses to the CS– (see Supplemental Methods). Treatment effects were determined using a phase

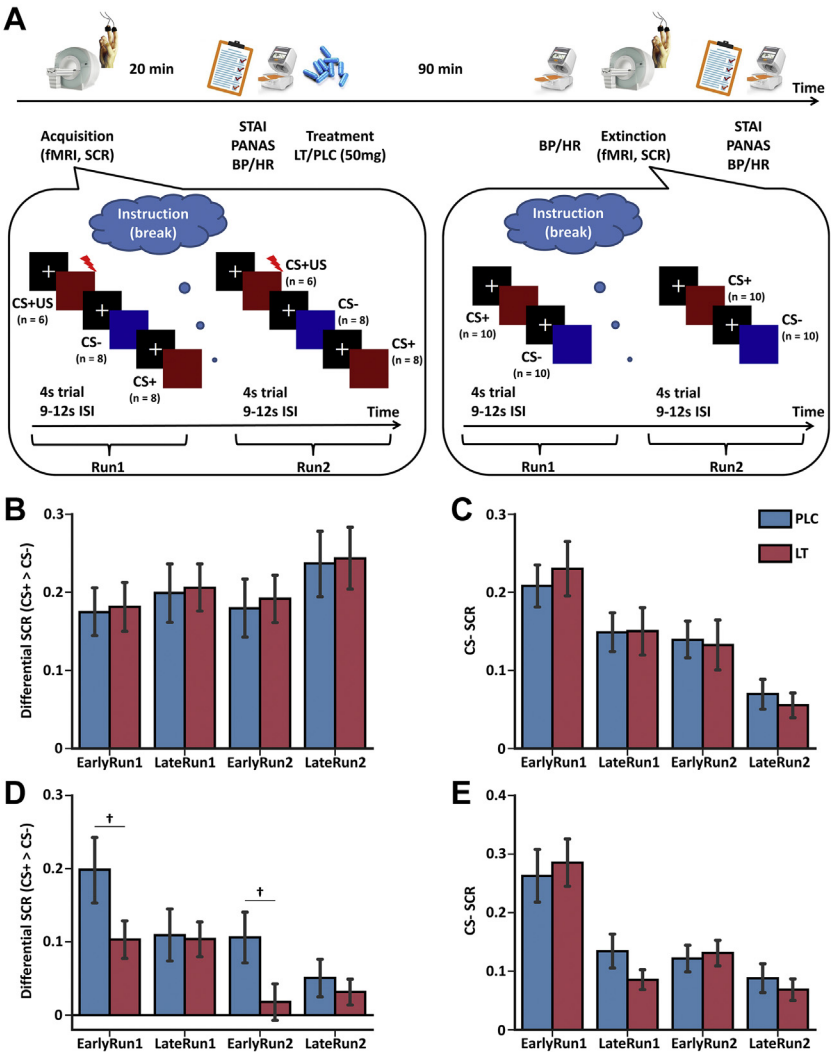

Figure 1. Experimental timeline and losartan (LT) effects on psychophysiological threat responses. **(A)** Experimental timeline and schematic synopsis of functional magnetic resonance imaging (fMRI) tasks. **(B)** Psychophysiological threat responses (conditioned stimulus [CS]+ > CS–) during acquisition demonstrating successful CS discrimination with enhanced skin conductance response (SCR) to the CS+ relative to the CS– in both groups. **(C)** Mean SCR for CS– presentations during acquisition. **(D)** Psychophysiological threat responses (CS+ > CS–) during extinction learning. **(E)** Mean SCR for CS– presentations during extinction learning. Early run, first trials (1–5); late run, last trials (6–10) in each run. $^{\dagger}p < .05$, 1-tailed. BP, blood pressure; HR, heart rate; ISI, interstimulus interval; PANAS, Positive and Negative Affect Schedule; PLC, placebo; STAI, State-Trait Anxiety Inventory; US, unconditioned stimulus.

(early, late) × run (run1, run2) × treatment (LT, PLC) 3-way mixed analysis of variance (ANOVA) with psychophysiological threat responses as the dependent variable. The additional analysis of the CS– served primarily to exclude that LT had an effect on the skin conductance response per se.

MRI Acquisition and Analysis

MRI data were acquired using a Siemens TRIO 3T system with a 12-channel head coil (Siemens, Erlangen, Germany). Functional time-series were processed using SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). On the first level, the CS+ and CS– stimuli were modeled, and condition-specific regressors for early (first half) and late (second half) of extinction were defined (see Supplemental Methods).

Whole-Brain Analyses

Effects of LT on extinction were assessed using a whole-brain phase (early, late) × run (run1, run2) × treatment (LT, PLC) 3-way mixed ANOVA with the CS+ > CS– contrasts as the dependent variable. Significant interaction effects were further disentangled by 2 independent post hoc approaches to warrant both high regional specificity (whole-brain voxelwise post hoc *t* tests) and high robustness (leave-one-subject-out cross-validation [LOSO-CV] procedure) (38). Group-level analyses (including LOSO-CV) were conducted using FSL Randomise (FMRIB Software Library; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) with permutation-based inferences (10,000 permutations). Significant clusters were determined using a height threshold of $p < .001$ (2-tailed) and an extent threshold of $p < .05$ (2-tailed) with cluster-based familywise error (FWE) correction.

Region of Interest: vmPFC Contributions During Early Extinction

Because of the critical role of the vmPFC in extinction (8,12,37,39,40) and our a priori regional hypothesis, we explored LT effects on stimulus-specific vmPFC activation during early extinction. To this end, activity estimates were extracted from an

Table 1. Participant Demographics and Control Measures

Measure	Time	LT Group (n = 30)	PLC Group (n = 29)	t_{57}	p Value
Age, Years		20.50 (1.80)	20.86 (1.68)	–0.80	.43
Body Mass Index, kg/m^2		22.33 (2.51)	21.34 (2.52)	1.51	.14
Systolic Blood Pressure	Before drug administration	110.03 (6.42)	107.59 (5.49)	1.57	.12
	Before extinction	111.17 (6.07)	108.79 (6.18)	1.49	.14
	After extinction	111.00 (5.69)	109.72 (5.76)	0.86	.40
Diastolic Blood Pressure	Before drug administration	71.70 (7.86)	71.10 (5.95)	0.33	.74
	Before extinction	72.70 (7.51)	70.17 (4.88)	1.53	.13
	After extinction	72.50 (8.57)	72.34 (4.15)	0.09	.93
Heart Rate	Before drug administration	76.93 (9.27)	76.03 (10.09)	0.36	.72
	Before extinction	75.33 (10.06)	74.17 (10.04)	0.44	.66
	After extinction	77.27 (9.44)	76.76 (10.93)	0.19	.85
STAI State Anxiety Score	Before extinction	41.90 (8.88)	41.24 (10.51)	0.26	.80
	After extinction	37.23 (8.48)	38.14 (8.16)	–0.42	.68
PANAS Negative Affect Score	Before extinction	16.17 (6.95)	16.34 (9.08)	–0.08	.93
	After extinction	14.43 (6.46)	14.59 (7.60)	–0.08	.93
PANAS Positive Affect Score	Before extinction	22.73 (7.55)	23.07 (6.83)	–0.18	.86
	After extinction	23.70 (7.67)	22.34 (6.02)	0.75	.45

Values are presented as mean (SD).
LT, losartan; PANAS, Positive and Negative Affect Schedule; PLC, placebo; STAI, Spielberger State-Trait Anxiety Inventory.

anatomically defined vmPFC region of interest (Supplemental Methods) and subjected to a stimulus (CS+, CS–) × run (run1, run2) × treatment (LT, PLC) 3-way mixed ANOVA.

Neural Threat Expression: Multi-Voxel Pattern Analysis

Following Reddan *et al.* (39), a neural pattern of threat was developed to differentiate CS+ versus CS– (trained and tested on the acquisition data) and subsequently applied to early extinction activation to determine treatment effects on the neural threat expression (Supplemental Methods).

Voxelwise Mediation Analyses

To determine whether treatment effects on vmPFC activation (CS+ > CS–) during early extinction critically contributed to the accelerated attenuation of the psychophysiological threat responses (SCR, CS+ > CS–), voxelwise mediation analyses were conducted (Mediation Toolbox: https://github.com/canlab/MediationToolbox) (41,42). Mediation effects were inferred using bootstrapping (10,000 replacements) and false discovery rate (FDR) correction.

Network-Level Effects: Functional Connectivity Analysis

Given that both human and animal studies strongly implicate vmPFC-mediated inhibition of the amygdala as a key extinction mechanism (9–12), a functional connectivity analysis (43) was used to determine treatment effects on the vmPFC-amygdala coupling during early extinction. We hypothesized that LT-induced extinction enhancement would be accompanied by stronger functional interaction between these regions (1-sided).

RESULTS

Participants

Consistent with previous studies (18,29,33), no effects of drug or placebo on cardiovascular and affective indices were observed, which, together with the chance level guesses for treatment, argues against unspecific confounding effects of treatment (Table 1). During the pretreatment acquisition phase, both groups exhibited successful threat acquisition on the psychophysiological (Figure 1B, C) and neural (Supplemental Results and Supplemental Figure S1) levels. Importantly, 2-sample t tests did not reveal between-group activation differences during this stage.

Reduced Psychophysiological Threat Responses During Early Extinction

A mixed ANOVA model using the psychophysiological threat response during extinction learning as a dependent variable demonstrated a significant main effect of run ($F_{1,57}$ = 17.063; $p < .001$; partial η^2 = .230; η^2 indicates effect size in terms of eta squared) reflecting decreased psychophysiological threat responses in run2 compared to run1 and a marginally significant main effect of phase ($F_{1,57}$ = 3.387; p = .071; partial η^2 = .056) reflecting decreased psychophysiological threat responses during late extinction. Together, these results demonstrated successful extinction learning. Moreover, a significant treatment and phase interaction effect ($F_{1,57}$ = 5.017, p = .029, partial η^2 = .081) was observed, with post hoc 2-sample t tests indicating that relative to the PLC group, the LT group exhibited decreased psychophysiological threat responses during early extinction learning across both runs (t_{57} = –2.179; p = .034, d = –0.567; d indicates effect size in terms of Cohen's d), suggesting accelerated extinction learning. Exploratory run-specific

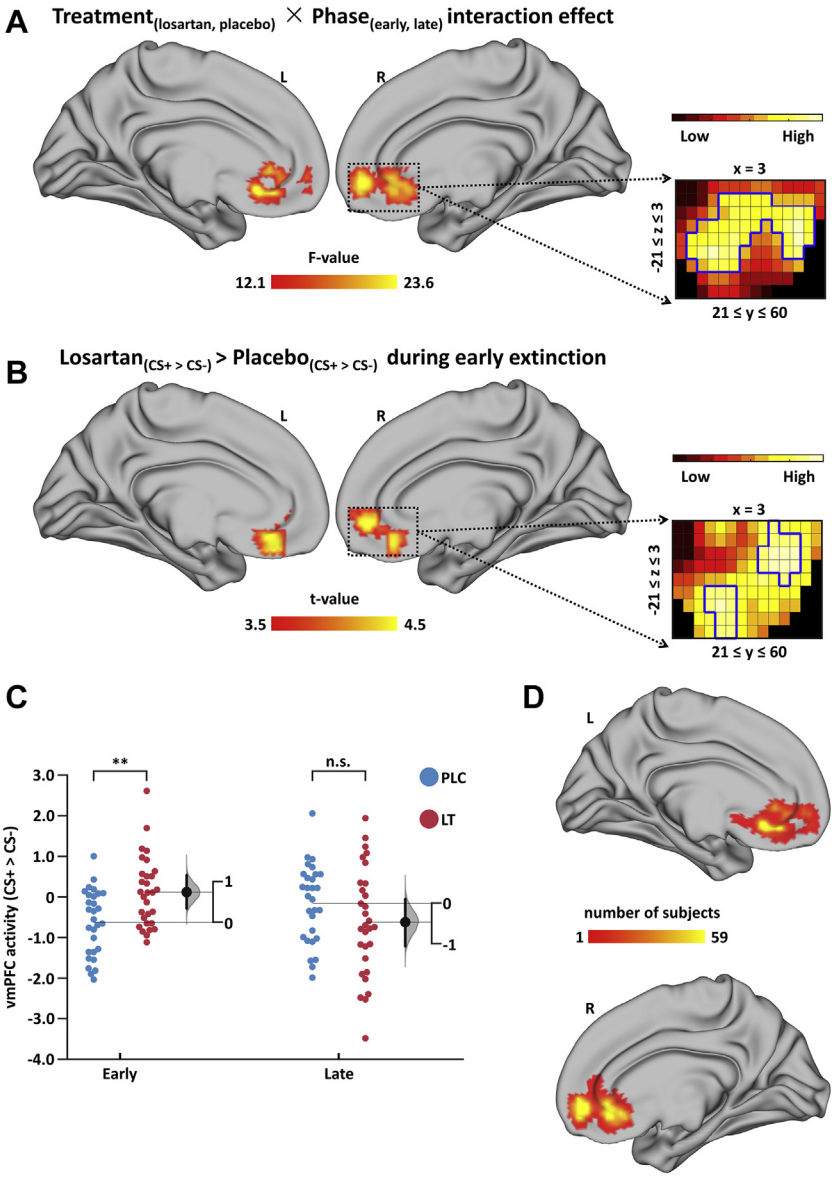

Figure 2. Losartan (LT) treatment effects on brain activity (conditioned stimulus [CS]+ > CS–) during extinction learning. **(A)** Ventromedial prefrontal cortex (vmPFC) activity showed significant treatment by phase interaction effect. **(B)** LT specifically increased vmPFC activity during early extinction learning. **(C)** Mean vmPFC activity (CS+ > CS–) extracted from the regions of interest depicted in **(D)** showed that LT increased vmPFC activity during early, but not late, extinction learning. **(D)** Overlay of all 59 leave-one-subject-out cross-validation regions of interest. All regions of interest were created leaving out one subject at the group-level statistic (cluster-level familywise error [FWE]-corrected). Statistical images were thresholded at $p < .05$ (2-tailed), cluster-level FWE-corrected with a cluster-forming threshold of $p < .001$ (2-tailed). Examples of unthresholded patterns are presented in the inserts; small squares indicate voxel statistical weight; red-outlined squares indicate significance at $p_{clusterFWE} < .05$. The filled curve indicates the null-hypothesis distribution of the difference of means (Δ), and the 95% confidence interval of Δ is illustrated by the black line. $^{**}p < .01$. L, left; n.s., not significant; PLC, placebo; R, right.

analyses confirmed that LT treatment enhanced early extinction learning during both initial and repeated extinction learning (run1 $t_{57} = 1.805$, $p = .038$, $d = 0.470$; run2 $t_{57} = 2.012$, $p = .024$, $d = 0.525$; 2-sample t tests comparing the treatment groups, 1-tailed; Figure 1D). No further significant main or interaction effects were observed on the psychophysiological threat responses (all $p > .4$), nor were any effects of treatment observed on the safety signal (CS–; all $p > .15$; Figure 1E), confirming the threat-specific effects of LT and the absence of unspecific treatment effects on the SCR psychophysiological signal.

To determine whether LT generally reduced the SCR to threat signals rather than facilitating extinction learning, we also explored between-group differences in the SCR reactivity to the first CS+ occurrence in each run. The lack of significant differences in the SCR reactivity to the first CS+ trials (run1 $t_{57} = -1.305$, $p = .197$, $d = -0.338$; run2 $t_{57} = -0.8975$, $p = .3732$, $d = -0.233$) confirmed specific effects on extinction learning.

Increased Threat-Specific vmPFC Engagement During Early Extinction

Whole-brain analysis revealed a significant treatment and phase interaction effect on extinction-related neural activity (CS+ > CS–) in the vmPFC (peak Montreal Neurological Institute (MNI) x, y, z = –3, 27, –12; $F_{1,57} = 23.582$; $p_{clusterFWE} = .011$; $k = 187$; Figure 2A). Regional specificity of treatment effects was examined with voxelwise whole-brain comparisons demonstrating increased vmPFC activity following LT relative to PLC during early (peak MNI x, y, z = 6, 48, –3; $t_{57} = 4.505$;

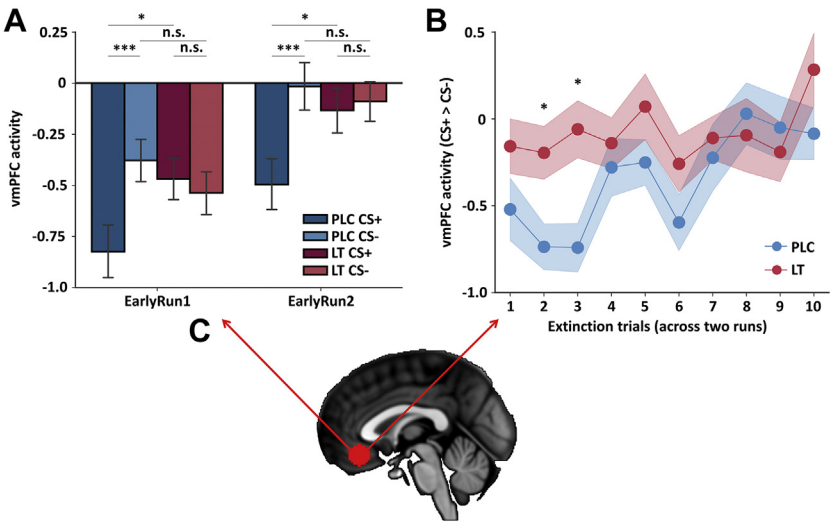

Figure 3. Losartan (LT) treatment specifically increased ventromedial prefrontal cortex (vmPFC) activity to threat stimulus (conditioned stimulus [CS]+) during early extinction learning. **(A)** LT increased vmPFC activity to CS+ but not CS– in the early extinction phase in both runs. *$p < .05$ and ***$p < .001$; error bars represent standard errors. **(B)** Single-trial analysis confirmed that LT increased vmPFC activity to CS+ in early trials during extinction learning. *$q < .05$, false discovery rate corrected. Data are represented as group mean ± SEM. **(C)** vmPFC region of interest. n.s., not significant; PLC, placebo.

$p_{clusterFWE}$ = .013; 2-tailed k = 139) but not late extinction (Figure 2B). Post hoc analyses with the LOSO-CV procedure further demonstrated that LT—relative to PLC—increased vmPFC activation during early (t_{57} = 3.417; p = .001; d = 0.890) but not late (t_{57} = –1.556; p = .125; d = –0.405) extinction (Figures 2C and 2D display an overlay of all leave-one-subject-out regions of interest). Note that the vmPFC exhibited robust early extinction learning–induced treatment and phase interaction effect.

Further exploration of vmPFC contributions during early extinction by means of extraction of beta estimates revealed significant main effects of stimulus type and run, and a significant treatment and stimulus type interaction effect (see Supplemental Results). Exploratory post hoc 2-sample t tests demonstrated that LT enhanced threat-specific vmPFC reactivity during both the initial (run1 t_{57} = 2.141; p = .037; d = 0.557) and the repeated (run2 t_{57} = 2.126; p = .038; d = 0.554) extinction in the absence of effects on the safety signal (CS–, $p > .29$; Figure 3A).

Consistent with our hypothesis on accelerated extinction by LT, an exploratory single trial analysis (Supplemental Methods) revealed that LT specifically increased vmPFC activation during initial trials of re-exposure to the threat stimulus ($q < .05$, FDR corrected; Figure 3B).

Reduced Neural Threat Expression During Early Extinction

The threat-predictive pattern reliably evoked neural threat reactivity during acquisition [comparable to Reddan *et al.* (39)] (see Supplemental Results and Supplemental Figures S2A, S2B). Applying the threat-predictive pattern to early extinction activation (CS+ > CS–) using a LOSO-CV procedure demonstrated that first, in the entire sample, higher neural threat expression was associated with stronger psychophysiological threat reactivity (r_{57} = 0.571; $p < .001$) and confirmed functional relevance of the neural expression of threat (39). Second, relative to PLC, LT significantly decreased the magnitude of the threat-predictive pattern expression (t_{57} = –2.091; p = .041; d = –0.544, 2-sample t test), confirming attenuated neural threat expression during early extinction. Based on our a priori regional hypothesis, and the key role of the vmPFC in extinction (3,8,13,35), a vmPFC-focused partial threat expression analysis was conducted. In concordance with the whole-brain results, LT significantly attenuated the vmPFC partial threat pattern expression (CS+ > CS–) during early extinction (t_{57} = –3.410; p = .001; d = –0.888; Supplemental Results and Supplemental Figure S2C).

vmPFC Activation Drives LT-Induced Accelerated Extinction

The voxelwise mediation analysis aimed at further determining the relationship between treatment, psychophysiological threat attenuation, and the underlying neural basis. Conjunction effects (paths a, b, and a × b) were observed in a vmPFC cluster (peak MNI x, y, z = –3, 45, –15; z = –3.692; $q < .05$; FDR-small volume corrected in the anatomically defined vmPFC, k = 138), demonstrating that LT increased vmPFC activation (path a), while activation in this region was associated with stronger suppression of psychophysiological threat independent of treatment (path b). Importantly, the a × b mediation effect reached significance, indicating that vmPFC activation critically mediated the effects of LT on extinction acceleration (Figure 4A; for details, see Supplemental Results). To test the robustness and to visualize the mediation effect, an independent vmPFC-focused mediation analysis was conducted, which confirmed the critical contribution of the vmPFC (Figure 4B, a × b effect, bootstrapped p = .016; each path is shown in Figure 4C).

Enhanced vmPFC-Amygdala Coupling

During early extinction, LT induced enhanced functional coupling between the vmPFC and the right amygdala [peak MNI x, y, z = 24, –3, –24; t_{57} = 3.557; $q < .05$; 1-tailed; FDR-small volume corrected in the amygdala, k = 13; location

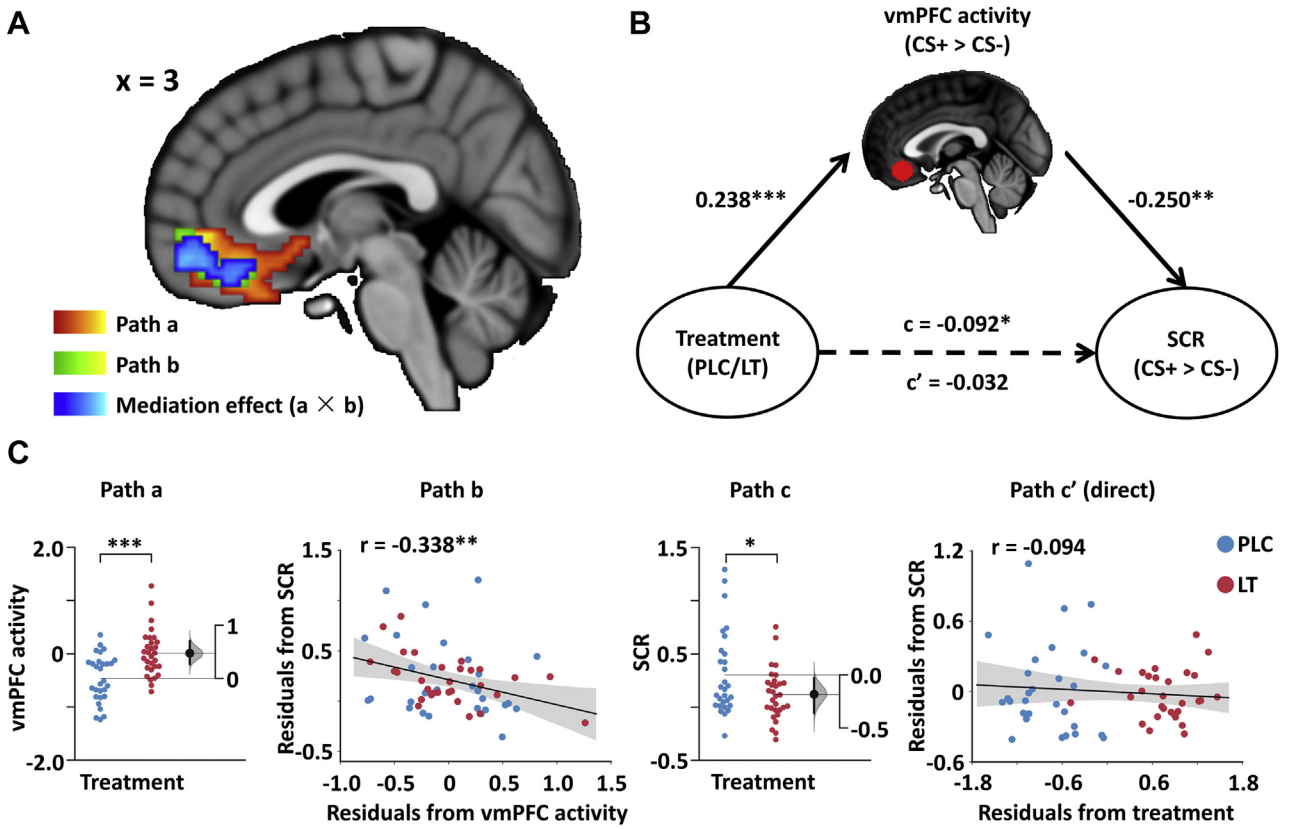

Figure 4. Ventromedial prefrontal cortex (vmPFC) activity mediated losartan (LT) treatment effect on accelerated extinction learning. **(A)** Sagittal slice showing regions whose activity increased response to the LT treatment in yellow (path a), regions whose activity significant negative correlated with psychophysiological threat responses while controlling for the treatment effect in green (path b), and regions whose activity showed significant mediation (a × b) effect in blue. All images were thresholded at $q < .05$, false discovery rate corrected within the vmPFC mask. **(B)** Mediation path diagram with the brain activity in the vmPFC region of interest. **(C)** Examples of each path in the mediation path diagram. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$. The filled curve indicates the null-hypothesis distribution of the difference of means (Δ), and the 95% confidence interval of Δ is illustrated by the black line. CS, conditioned stimulus; PLC, placebo; SCR, skin conductance response.

cyto-architectonically (44) mapped to the basolateral amygdala [BLA]; Figure 5A]. Subsequent examination of stimulus-specific connectivity estimates from the vmPFC-bilateral BLA pathway confirmed specific effects of LT on threat signal (CS+) processing ($t_{57} = 2.147$; $p = .036$; $d = 0.559$; Figure 5B).

DISCUSSION

The present study demonstrated that LT treatment accelerated the attenuation of a previously acquired psychophysiological threat response, indicating its potential to facilitate threat extinction learning in humans. During early extinction, the acceleration was critically mediated by enhanced threat-signal-specific vmPFC activity in the context of stronger functional coupling of the vmPFC with the BLA. These findings were further paralleled by a pattern classification approach showing that LT treatment accelerated attenuation of the neural threat expression, particularly in the vmPFC. Overall, the present findings provide first evidence for an important contribution of the RA system to fear extinction in humans and the potential of LT to accelerate extinction through effects on the vmPFC and its inhibitory connections with the BLA.

In humans, successful extinction is accompanied by decreased psychophysiological threat reactivity and concomitantly increased vmPFC activation in response to the threat signal (37,40,45). In the present study, LT treatment reduced the psychophysiological threat responses and selectively enhanced vmPFC activation in response to the threat signal (CS+) during early extinction, indicating its potential to accelerate extinction learning in humans. Moreover, the acceleration effects were found not only during the initial extinction learning (i.e., run1), but also in the following "new" learning process (the instruction before run2 allowed us to treat the design as two factors—run [run1, run2] and phase [early and late within each run]—instead of treating phases 1 to 4 across the entire extinction as a single factor; see Supplemental Results). The findings resemble previously observed LT-enhanced extinction learning in rodents (19,20) and further confirm the important contribution of the vmPFC to successful extinction. Exploring the stimulus-specific effects of LT treatment on vmPFC activation revealed that reactivity to the safety signal (CS–) remained unaffected. Decoding the temporal pattern of LT effects further suggested that LT specifically attenuated vmPFC reactivity during early re-exposure toward the previously

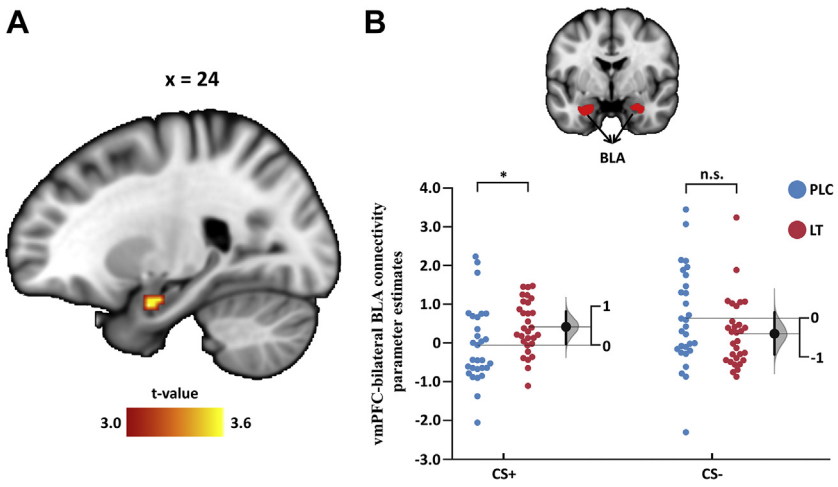

Figure 5. Losartan treatment effect on ventromedial prefrontal cortex (vmPFC)-amygdala functional coupling. **(A)** Sagittal slice showing that losartan (LT) increased functional connectivity between vmPFC and basolateral amygdala (BLA). The image was thresholded at $q < .05$, false discovery rate corrected within the amygdala mask. **(B)** Extracted generalized form of context-dependent psychophysiological interaction (gPPI) parameter estimates from the bilateral BLA showed that LT treatment specifically enhanced vmPFC-bilateral BLA functional connectivity during fear-associated stimulus presentation. The filled curve indicates the null hypothesis distribution of the difference of means (Δ), and the 95% confidence interval of Δ is illustrated by the black line. $^*p < .05$. CS, conditioned stimulus; n.s., not significant; PLC, placebo.

conditioned threat signal. Nonpharmacologic stimulation of the vmPFC homologous infralimbic cortex accelerates extinction learning in rodents (46–48), whereas inactivation or lesioning of this region critically impedes threat reduction during extinction (8,13). Compatible with the present findings, previous studies demonstrated that nonpharmacologic stimulation of the vmPFC can enhance early extinction learning in humans (49), although nonspecific effects on CS– reactivity have also been reported (50).

Converging evidence from different research focuses suggests that the vmPFC, or the homologous infralimbic cortex in rodents, critically contributes to the reduction of threat expression during extinction learning (3,8,12,13) and regulates amygdala output to inhibit the conditioned threat response (8,9). Consonant with the proposed contribution of the vmPFC to extinction learning, LT-attenuated psychophysiological threat responses during early extinction were accompanied by an attenuated neural threat expression, particularly in the anatomically defined vmPFC. Compatible with previous studies that showed critical contributions of the vmPFC to extinction enhancement (46–48) and associations between activity in this region and psychophysiological threat reactivity during extinction (51), an additional mediation analysis revealed that higher vmPFC activation was associated with stronger suppression of the psychophysiological threat response. Notably, LT-facilitated suppression of the psychophysiological threat response crucially involved enhanced vmPFC activation (for convergent mediation effects of vmPFC threat expression, see Supplemental Methods and Supplemental Results), further emphasizing the key role of this region in extinction enhancement.

On the network level, LT-accelerated threat reduction during early extinction was paralleled by stronger functional communication between the vmPFC and the amygdala, specifically the basolateral subregion. Previous lesion studies in humans demonstrated a critical role of the BLA in threat processing (52) and of the vmPFC in inhibiting amygdala threat responses by exerting top-down control over this region (53). Animal models have further confirmed the importance of pathway-specific neuroplastic changes in the vmPFC-amygdala circuitry during extinction memory formation (9–11) and suggest that vmPFC inputs to the amygdala instruct threat memory formation and/or gate the expression of conditioned threat (9) during early extinction (11). The present findings of CS+-specific increase in vmPFC-BLA functional connectivity following LT-treatment likely reflect an important modulatory role of angiotensin signaling on vmPFC regulation of the amygdala. Previously, animal models demonstrated that stimulation of vmPFC inputs to the amygdala promotes the formation of extinction memories (9). We suggest the notion that enhanced transmission in this pathway might reflect a core mechanism underlying angiotensin regulation of extinction learning. Angiotensin receptors are densely expressed in limbic and prefrontal regions critically engaged in extinction (16–18) and are considered to modulate learning-related neuroplasticity. LT is a selective competitive antagonist of the AT1 receptor, and it increases availability of angiotensin II–converted angiotensin IV—an agonist at the AT4 receptor subtype. The AT4 system is thought to play a role in neuroplasticity and learning and memory (16,17,33,54), a mechanism that is suggested to likely contribute to LT-induced extinction enhancement.

Consistent with previous animal models demonstrating the potential of LT to enhance extinction in rodents (19,20), the present study successfully demonstrated the potential of a single low-dose administration of LT to facilitate extinction learning in humans. In the context of recent findings suggesting a direct association between extinction-related vmPFC functioning and exposure therapy success (55), the current results indicate that LT represents a highly promising candidate to augment the efficacy of exposure-based interventions in therapeutic settings. On the neural level, the effects of LT were mediated by circuits consistently involved in anxiety disorders, with exaggerated threat reactivity and deficient extinction being associated with decreased vmPFC activation and dysfunction in the vmPFC-BLA circuit (6,8,10,13). Importantly, dysregulation in this circuitry normalizes during the course of successful treatment (56), suggesting that they represent treatment-responsive—rather than stable—markers and consequently promising targets for therapeutic interventions. A previous human neuroimaging study reported that LT enhances early

amygdala discrimination of threat and safety signals in highly anxious individuals (18). Both vmPFC dependent extinction learning and amygdala threat discrimination are considered core therapeutic mechanisms of exposure-based interventions, suggesting that LT facilitates effects of exposure therapy via synergistic effects on both mechanisms.

The present study observed effects of LT on within-session extinction learning. Although accumulating evidence suggests an association between extinction learning and associated vmPFC activation with better therapeutic response to exposure-based interventions (55,57,58), extinction retention (e.g., extinction recall) deficits have been reported frequently in anxiety-related disorders (59–61). To further explore the clinical efficacy of LT to enhance extinction, future studies should examine effects of LT on extinction retention after longer time intervals. In addition, despite these initial promising results, subsequent studies need to 1) determine the generalization of the effects to female subjects [for instance, see also a recent study demonstrating that LT enhances fear extinction in female rats in an estradiol-dependent manner (62)] and 2) evaluate its potential to enhance exposure-based interventions in clinical trials. Moreover, pharmacologic blockade of the AT1 receptors in animal models has been shown to inhibit the hypothalamic-pituitary-adrenal stress response via both central and peripheral AT1 receptors (63). Thus, we cannot rule out that effects on peripheral AT1 receptors might have contributed to the observed effects.

Overall, the present results indicate an important regulatory role of the RA system in fear extinction learning in humans. This role is mediated by modulatory effects on vmPFC threat processing and its interaction with the amygdala. From a clinical perspective, adjunct LT treatment could represent an innovative strategy to enhance the efficacy of exposure-based interventions.

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FZ and BB designed the study, analyzed the data, and wrote the manuscript. FZ, YG, FX, JL, PF, CL, and WZ conducted the experiment. PF, TF, AG, RE, and KK revised the manuscript draft.

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Unthresholded group-level statistical maps are available on NeuroVault (https://neurovault.org/collections/4722/) and code that supports the findings of this study is available from the corresponding author upon reasonable request.

A preprint of the manuscript has been archived on the biorxiv.org repository (doi: https://doi.org/10.1101/512657).

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The present study was pre-registered at ClinicalTrials.gov (trial name: Losartan and Emotion Processing; registration number: NCT03396523; URL: https://clinicaltrials.gov/ct2/show/NCT03396523).

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