Neural Processing Dysfunctions During Fear Learning but Not Reward-Related Processing Characterize Depressed Individuals With High Levels of Repetitive Negative Thinking

Supplement

Participants

A. Tulsa 1000 Study participation eligibility

Treatment-seeking individuals were considered eligible for Tulsa 1000 study if they fulfilled any of the following criteria: Patient Health Questionnaire (1) ≥ 10 and/or Overall Anxiety Severity and Impairment Scale (2) ≥ 8, and/or Drug Abuse Screening Test (3) score > 2, and/or Eating Disorder Screen (4) score ≥ 2. Healthy volunteers for comparison (HC) were individuals who did not screen positive for the inclusion measures. Exclusion criteria of the T-1000 study were 1) positive results on drug screening test; 2) disorders including lifetime bipolar, schizophrenia spectrum, antisocial personality, or obsessive-compulsive disorders; 3) active suicidal ideation; 4) moderate to severe traumatic brain injury; 5) severe/unstable medical concerns; 6) recent change in psychiatric medication dose; and 7) MRI contraindications.

B. Ruminative Responses Scale (RRS)

All participants completed self-report measures including the Rumination Response Scale (RRS, 5) and the World Health Organization Disability Assessment Schedule (WHODAS) for measuring RNT and functional disability respectively. RRS is a self-administered scale which asks the participant to rate how frequently he/she dwells in thoughts like “I won’t be able to do my job if I don’t snap out of this,” “I won’t be able to concentrate if I keep feeling this way,” or “What am I doing to deserve this” (5).

Experimental tasks

A. MID task

The Monetary Incentive Delay task (MID, 6) is an imaging experiment paradigm for investigating reward-related neural activity (6). The study design included valence (2: gain, loss) x magnitude (3: high, low, no) of the incentive resulting in 6 incentive conditions: high-gain (+$5), low-gain (+$1), no-gain (+$0), high-loss (-$5), low-loss (-$1), and no-loss (-$0). On each trial, participants were presented an object cue (2 s) on the screen and the shape of the object cue indicated the valence of an upcoming reward (circle: gain, square: loss), and the location of a horizontal line in the cue indicated reward magnitude (top: $5, middle: $1, bottom: $0). Following a jittered delay period (2.25 – 3 s), a triangle indicating a target prompted a response by pressing a button. Participants were instructed to respond as fast as they could, for obtaining potential gain or avoiding potential loss. The target duration was calibrated to each participant such that the participant would succeed on approximately 66% of trials. Feedback (2 s) which indicated the outcome (i.e., amount earned or lost) was presented after the target. Participants underwent the task in two runs of scanning with 90 trials for approximately 19 m. Participants received their earnings after the study.

B. Fear conditioning task

The study design was similar to the task used in (7). The unconditioned stimulus (US) was an 850 ms aversive scream. The conditioned stimuli (CS) were two abstract, geometric images, presented for 1.5 sec at a time. The CS images were assigned to either CS+ or CS-, counterbalanced across participants. The CS+ involved the pairing of a CS image with the US delayed 500 ms from the onset of image presentation,

whereas the CS- was never paired with the US. The task consisted of three phases: familiarization, acquisition, and extinction. First, the familiarization phase (2.5 m) included five presentations of each CS without the US, in order to familiarize with the CS images at the beginning of the task as well as to establish baseline ratings. The acquisition phase followed with two 8 m 40 s runs. Each acquisition run contained 15 CS- trials and 20 CS+ trials. While the CS- was never paired with the US during acquisition, the CS+ was either paired with the US (CS+ paired, 5 trials) or not paired with the US (CS+ unpaired, 15 trials), following (7). The CS+ unpaired was used to examine hemodynamic responses evoked by the CS+ without confounding effects of reacting to the US. The CS+ paired was only used to establish conditioning. The CS trials were presented in a pseudo-randomized order with the constraint of no more than two consecutive presentations of the same type of trial. The volume of the US was set to roughly 114dB and adjusted for each participant based on intensity ratings collected prior to the first run so that the final presentation volume ranged from ~108.5 to ~119.5dB. Finally, the extinction phase took place in a single 12 m 16 s run that included 25 presentations of the CS- and 25 presentations of the CS+ without the US. Additionally, a continuous performance task was performed between CS image presentations where arrows were presented on the screen for 2.5 s each (3-5 arrow trials followed each CS presentation). Participants were instructed to make a button press (left or right) in response to the direction an arrow was facing. This continuous arrow task served to keep participants' attention to the study. There was a 500 ms blank screen after each trial (arrow or CS). Participants also rated their valence, arousal, and anxiety levels to each CS four times during the task: after familiarization, after the first

run of acquisition, after the second run of acquisition, and after extinction. The task was comprised of 4 runs and took a total of approximately 35 to 40 minutes to complete. By splitting acquisition and extinction into two phases each, we aimed to compare the efficiency of contextual learning with time (early vs. late). This splitting approach in phases/blocks has been adopted in fear conditioning studies (e.g., 8).

Neuroimaging data acquisition and preprocessing

Two identical GE MR750 3T scanners in the same site equipped with 8 RF channel phased array coils were used to acquire both T1-weighted 3D high-resolution anatomical images (MP-RAGE pulse sequence, FOV 240 x 192mm, TR/TE = 5/2.012ms, 186 axial slices) and T2*-weighted echo-planar images (flip angle 78°, FOV 240 x 240mm, TR/TE = 2000/27ms, axial plane) per volume. Each volume comprised of 39 slices (2.9mm thick, 1.875 x 1.875mm voxels) were acquired in an interleaved sequence. Functional imaging data were collected in two runs, each with 281 volumes (~ 9m 22s). Preprocessing and statistical analyses of MRI data were performed using the Analysis of Functional NeuroImages software suite (AFNI, http://afni.nimh.nih.gov). The first 3 EPI volumes were discarded for signal stabilization. For preprocessing, imaging data were despiked, slice-time corrected to the first slice, co-registered to a T1-weighted anatomical image, and motion-corrected. Time points with large head movements were censored (ENORM > 0.3). Imaging data were also normalized to the standard MNI space with resampling of 2-mm isotropic voxels and smoothed with an isotropic 4-mm FWHM Gaussian kernel.

Supplemental Results

A. Participant characteristics

Participants did not differ across age, sex, ethnicity, education, employment, but did differ as expected on receiving psychotropic medications, measures of depression or anxiety, and levels of repetitive negative thinking (Table 1). Importantly, individuals with VH-RNT did not differ in anxiety and depression scores compared to H-RNT depressed participants. RRS scores were higher in individuals with VH-RNT than individuals with H-RNT, and both RNT groups showed higher RNT scores compared to HC. Disability indexed by WHODAS score was higher in individuals with VH-RNT than those with H-RNT, followed by HC.

B. Behavioral results - Monetary Incentive Delay task

Mean hits and mean RTs to incentive cues by valence, magnitude, and group are shown in Supplementary Table 1. An omnibus ANOVA on hit rates showed 3-way interactions between group x valence x magnitude $F[2,132] = 3.91$, $p = 0.02$, and 2-way interactions between valence and magnitude $F[1,132] = 7.15$, $p = 0.008$.[1] Follow-up tests showed that individuals with VH-RNT made fewer hits to $0-gain trials (61%) compared with $5-gain trials (67%), $F[1,216] = 5.24$, $p = 0.02$. However, no group effects were found in RTs. The RT data revealed only a main effect of reward magnitude with faster responses to $5 trials than $0 trials $F[1,132] = 152.13$, $p < .001$

[1] One participant was excluded from behavioral analysis due to incomplete data.

and the interaction effect between valence and magnitude $F[1,132] = 4.54$, $p = 0.03$ with faster responding to $5 gain trials. The amount earned did not differ by group.

C. Behavioral results - Fear conditioning task

The mean hit responses and RTs on valence, arousal, and anxiety ratings on CS images are displayed in Supplementary Table 2. Neither hits nor RTs of valence, arousal, and anxiety ratings on conditioning (CS+ vs. CS-) differed by the group during the familiarization phase (all *p*s > 0.22)[2]. No significant group differences were found in the accuracy (VH-RNT: 98.18% [*SD* = 8.80], H-RNT: 98.91% [*SD* = 1.96], HC: 99 12% [*SD* = 1.34]) on the continuous arrow task during familiarization. During the acquisition phase, in both the first half and second half of acquisition, no group difference was found (all *p*s > .11). During the extinction phase, an RT difference in the arrow task was shown ($F[2,117] = 3.07$, $p = 0.02$) such that individuals with VH-RNT made slower responses to the task compared to HC ($p = 0.03$).

C. Imaging results – Monetary Incentive Delay task

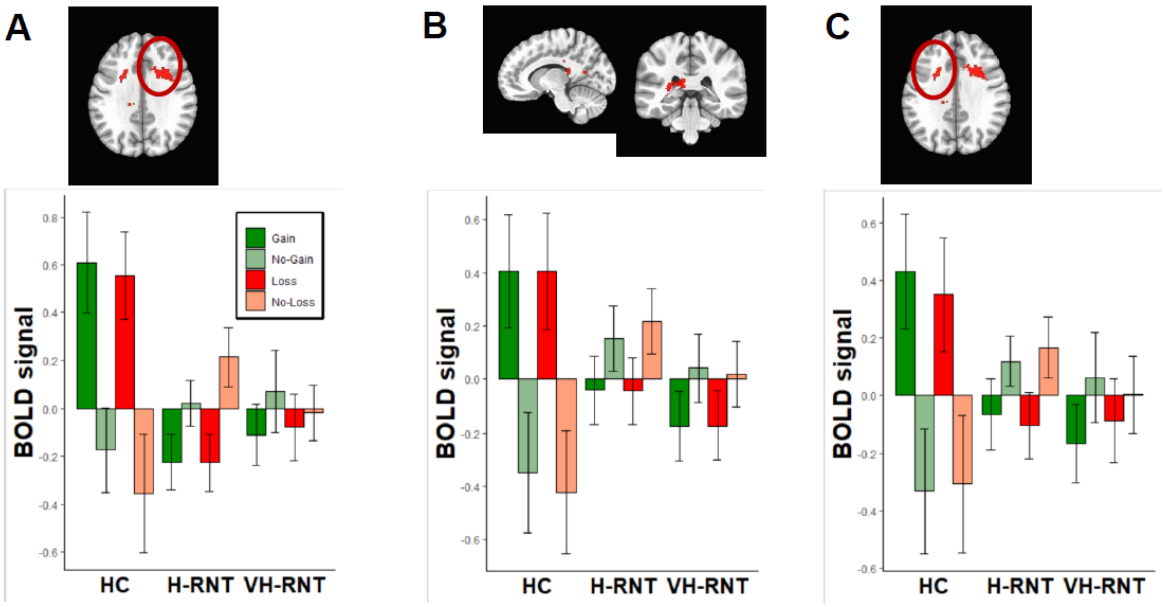
Group main effects were found in the right middle/inferior frontal gyrus (27,11, 33, 462 voxels, $F[2,133] = 27.91$), left parieto-occipital sulcus to the thalamus (-11,-35.17, 298 voxels, $F[2,133] = 19.91$), and left middle frontal regions (-27,7,33, 178 voxels, $F[2,133] = 20.60$). In these regions, overall anticipatory neural activity in VH-RNT and H-RNT was lower than activity in HC (VH-RNT: $\beta_1 = -1.93$, 95% CI: -2.57, -1.29, Cohen's $d = 1.32$; $\beta_2 = -1.99$, 95% CI: -2.68, -1.30, Cohen's $d = 1.26$; $\beta_3 = -1.74$, 95% CI: -2.36, -

[2] Fear conditioning ratings were available from 120 subjects.

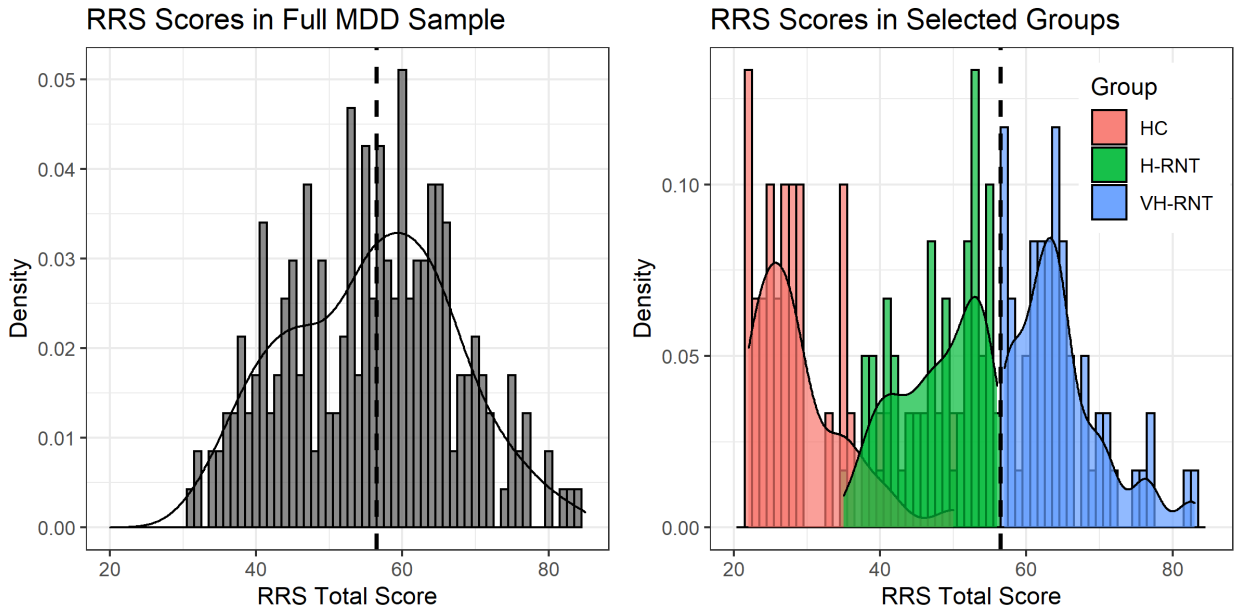
1.13, Cohen's *d* = 1.64; H-RNT: β_1 = -2.38, 95% CI: -3.02, -1.73, Cohen's *d* = 1.24; β_2 = -2.04, 95% CI: -2.74, -1.34, Cohen's *d* = 1.30; β_3 = -1.88, 95% CI: -2.50, -1.26, Cohen's *d* = 1.35)[3]. Follow-up tests showed that VH-RNT and H-RNT exhibited decreased activity to both gain cues and loss cues separately, R middle frontal- Gain *F*[2,133] = 13.96 (*β*: -0.96, 95% CI: -1.37, -0.55, Cohen's *d* = 1.03 for VH-RNT, *β*: -1.03, 95% CI: -1.44, -0.61, Cohen's *d* = 1.11 for H-RNT), Loss *F*[2,133] = 16.62 (*β*: -0.97, 95% CI: -1.43, -0.51, Cohen's *d* = 0.92 for VH-RNT, *β*: -1.35, 95% CI: -1.82, -0.89, Cohen's *d* = 1.29 for H-RNT); L parieto-occipital -Gain *F*[2,133] = 11.74 (*β*: -0.97, 95% CI: -1.40, -0.54, Cohen's *d* = 0.99 for VH-RNT; *β*: -0.95, 95% CI: -1.38, -0.51, Cohen's *d* = 0.97 for H-RNT), Loss *F*[2,133] = 12.49 (*β*: -1.02, 95% CI: -1.48, -0.56, Cohen's *d* = 0.97 for VH-RNT, *β*: -1.09, 95% CI: -1.55, -0.63, Cohen's *d* = 1.05 for H-RNT); L middle frontal -Gain *F*[2,133] = 15.19 (*β*: -0.99, 95% CI: -1.38, -0.61, Cohen's *d* = 1.13 for VH-RNT; *β*: -0.95, 95% CI: -1.34, -0.56, Cohen's *d* = 1.10 for H-RNT), Loss *F*[2,133] = 9.88 (*β*: -0.75, 95% CI: -1.17, -0.33, Cohen's *d* = 0.78 for VH-RNT, *β*: -0.93, 95% CI: -1.36, -0.51, Cohen's *d* = 0.98 for H-RNT). No difference between VH-RNT and H-RNT was found in any of the three areas.

[3] Beta coefficients were standardized (M=0, SD=1).

Supplemental Figure S1: Brain regions showing group main effects across gain and loss in BOLD signal response to reward (Gain: +$5, No-Gain: $0, Loss: -$5, No-Loss: $0) in individuals with repetitive negative thinking (HC: Healthy comparison, H-RNT: High RNT, VH-RNT: Very high RNT). Shown are right middle/inferior frontal (A), left parieto-occipital (B), and left middle frontal (C) regions. BOLD signals (arbitrary unit) were standardized (M=1 & SD=1).



Supplemental Figure S2: Frequency distribution of RRS scores in the original sample of MDD persons in the initial 500 participants of the T1000 study (left plot) and propensity-matched samples obtained thereof (right plot). The dashed line indicates the median split used to separate H-RNT from VH-RNT.



Supplementary Table S1. Mean hits (%) and RTs (s) by valence and magnitude of incentive on the MID Task (SD in parenthesis)

		HC	H-RNT	VH-RNT
Gain				
$5	Hit	62.62 (8.38)	67.00 (14.93)	67.00 (12.38)
	RT	.23 (.03)	.26 (.06)	.26 (.05)
$0	Hit	63.81 (10.65)	66.54 (11.16)	61.12 (10.28)
	RT	.26 (.03)	.29 (.06))	.29 (.06)
Loss				
$5	Hit	60.71 (8.18)	66.28 (14.31)	63.96 (12.80)
	RT	.24 (.03)	.26 (.06)	.26 (.06)
$0	Hit	60.71 (12.35)	67.31 (10.67)	65.15 (9.96)
	RT	.27 (.04)	.29 (.06)	.28 (.05)

Abbreviations: HC, Healthy comparison; H-RNT, High repetitive negative thinking; VH-RNT, Very High repetitive negative thinking

Supplementary Table S2. Mean hits (%) and RTs (s) on valence, arousal, and anxiety scales following conditioned stimuli in conditioning phrases by group (SD in parenthesis)

			Valence			Arousal			Anxiety		
			HC	H-RNT	VH-RNT	HC	H-RNT	VH-RNT	HC	H-RNT	VH-RNT
P1	CS-	Hit (%)	3.33 (0.70)	3.33 (0.90)	3.10 (0.78)	2.54 (0.72)	2.27 (0.91)	2.51 (1.01)	20.00 (23.31)	22.07 (22.96)	16.51 (18.99)
		RT (ms)	8.70 (2.52)	11.92 (15.27)	9.84 (3.29)	7.56 (2.49)	8.01 (6.82)	7.63 (2.46)	12.47 (4.30)	14.59 (6.32)	13.62 (6.12)
	CS+	Hit (%)	3.29 (0.62)	3.29 (0.92)	3.25 (0.98)	2.71 (1.04)	2.55 (1.14)	2.51 (0.99)	18.00 (21.75	21.67 (23.72)	16.22 (19.81)
		RT (ms)	7.42 (1.69)	8.90 (5.14)	9.16 (2.58)	6.86 (2.32)	7.46 (3.45)	7.61 (3.83)	18.00 (21.75)	9.93 (3.99)	10.23 (5.23)
P2	CS-	Hit (%)	3.08 (1.02)	3.13 (0.94)	3.06 (0.99)	2.58 (1.06)	2.47 (1.04)	2.71 (1.04)	24.33 (27.39)	27.64 (24.47)	19.84 (22.03)
		RT (ms)	7.87 (2.20)	9.05 (4.82)	9.17 (2.83)	6.44 (2.11)	6.84 (3.79)	6.55 (2.61)	8.93 (1.89)	10.99 (3.22)	10.91 (5.41)
	CS+	Hit (%)	2.50 (0.93)	2.47 (0.99)	2.64 (0.98)	3.08 (1.21)	2.91 (1.18)	3.32 (1.20)	32.46 (31.45)	37.20 (31.29)	32.52 (29.10)

			6.88 (2.18)	7.18 (3.30)	7.31 (2.50)	5.81 (1.91)	5.81 (2.88)	6.61 (4.13)	7.27 (2.42)	8.28 (4.31)	7.52 (3.41)
P3	CS-	Hit (%)	2.96 (0.62)	2.98 (0.84)	3.00 (0.96)	2.63 (1.01)	2.62 (1.01)	2.49 (1.05)	21,13 (23.00)	28.02 (27.04)	18.49 (22.56)
		RT (ms)	6.74 (2.16)	7.35 (4.23)	7.48 (2.76)	5.89 (2.64)	5.49 (2.79)	5.60 (2.42)	7.90 (2.80)	10.60 (5.23)	8.78 (2.72)
	CS+	Hit (%)	2.58 (0.88)	2.40 (0.99)	2.35 (0.96)	2.96 (1.23)	3.09 (1.26)	3.08 (1.28)	30.25 (29.31)	42.56 (33.66)	34.35 (32.38)
		RT	6.80 (2.50)	5.62 (2.75)	6.51 (4.05)	5.30 (2.29)	5.13 (2.84)	5.14 (2.24)	6.12 (2.31)	7.02 (3.07)	7.16 (3.01)
P4	CS-	Hit (%)	3.08 (0.78)	2.93 (0.82)	3.08 (0.82)	2.38 (0.88)	2.48 (1.11)	2.63 (1.06)	18.54 (22.82)	23.30 (24.65)	16.57 (21.10)
		RT (ms)	6.55 (1.73)	6.91 (3.27)	6.76 (2.69)	5.50 (2.60)	5.30 (3.30)	4.79 (2.38)	7.88 (4.54)	8.59 (3.77)	7.72 (2.87)
	CS+	Hit (%)	2.92 (0.83)	2.48 (0.82)	2.75 (0.77)	2.77 (1.05)	2.77 (1.05)	2.73 (0.98)	26.46 (29.17)	30.68 (26.51)	25.53 (25.33)
		RT (ms)	5.35 (2.54)	5.57 (3.45)	5.50 (3.06)	4.06 (2.48)	4.65 (3.02)	4.53 (2.74)	5.47 (2.72)	5.78 (2.34)	6.00 (4.30)

Abbreviations: HC (Healthy comparison); H-RNT (High repetitive negative thinking); VH-RNT (Very High repetitive negative thinking); P1 (Phase 1)- familiarization; P2 (Phase 2) – first acquisition 1; P3 (Phase 3) – second acquisition 2; P4 (Phase 4) – extinction; CS- (conditioned stimulus without the unconditioned stimulus); CS+ (conditioned stimulus with the unconditioned stimulus)

Supplemental References

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