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Method

Recruitment, exclusion criteria, and ethics

Participants were recruited as part of a large randomized-controlled trial investigating the effect of a psychological intervention on subclinical psychopathology (Dutch Trial Register number: NTR3808). Participants were recruited via posters in schools and public places, and via advertisements in local news magazines. Exclusion criteria for the group with no/low levels of psychopathology were a history of psychiatric diagnosis or treatment, or a current DSM-IV axis I disorder as screened with the MINI International Neuropsychiatric Interview (MINI) (1). For the subclinical symptom group, individuals with current psychiatric treatment or a significant need for care were excluded. For both groups, other exclusion criteria were left handedness, alcohol and substance dependence, current use of psychotropic drugs, a history of neurological disease, severe head trauma, organic brain disease, and MRI contra indications. The Maastricht University Medical Centre ethics committee approved the study (Human Ethics Reference number: NL41929.068.12). All participants provided written informed consent. Parental consent was additionally obtained for minors (age<18 years).

Assessment of Childhood Maltreatment

CM was measured with the childhood trauma questionnaire, short form (CTQ-SF) (2). The CTQ-SF consists of five subscales with five items, each rated on a 5-point Likert scale, concerning emotional abuse (EA), physical abuse (PA), sexual abuse (SA), emotional neglect EN) and physical neglect (3). Subscale scores range from 5 to 25, with the total varying between 25-125. The total score was used to categorize participants into no/low-CM or high-CM, above or below the median (4).

Assessment of subclinical symptom load

A subclinical symptom load was calculated, in which scores of anxiety, depression, and psychosis were combined. Anxiety symptomatology was measured with the trait subscale of the State-Trait Anxiety Inventory (STAI) (5), consisting of 20 items with a score varying between 20-80. The MADRS was used to assess depressive symptoms. Depression was measured with the MADRS is a 10-item semi-structured interview, with scores of items ranging from 0-6, resulting in a total score between 0 and 60 (6). To measure subclinical psychotic symptoms, the Structured Interview for Schizotypy – Revised (SIS-R) was administered (7). The SIS-R includes 20 schizotypal symptoms and 11 schizotypal signs rated on a 4-point scale. The mean score of 'positive' symptoms (referential thinking, suspiciousness, magical ideation, illusions, psychotic phenomena, and derealization/depersonalization) was used in the current study, as in line with previous studies (8, 9). The questionnaire scores showed high inter-correlations (all r's>.53, all p's<.0001). An exploratory factor analysis on

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the questionnaire scores confirmed that these scores loaded onto one factor (eigenvalue = 2.25, with factor loadings between 0.81 and 0.91), explaining 75.06% of the variance. To form a composite 'subclinical symptom load' score, the outcomes of the questionnaires were first transformed to z-scores, and these z-scores were subsequently averaged into one score.

Threat generalization task

A validated neuroimaging threat generalization task was used in the current study, which included generalization stimuli that parametrically differ in similarity from a threat stimulus, and which has been shown to induce generalization gradients in self-report ratings and in neural activations within a network of regions involved in threat and safety processing in healthy volunteers (10).

Stimuli: Stimuli were seven rings or rectangles of parametrically increasing sizes, and a triangle (Figure S1). The largest and smallest rings/rectangles served as the conditioned threat stimulus (CS^+) and safety stimulus (CS^-). For half of the participants, the largest ring/rectangle was the CS^+ and the smallest ring/rectangle the CS^-; for the other half this was reversed. The triangle served as a second CS^- (vCS^-), as a measure independent of perceptual generalization, as the shape differed from the other stimuli. The five intermediately sized rings/rectangles and were on a continuum of similarity to the CS+ and CS-, and served as generalization stimuli (GS1-GS5). GS1 was perceptually most similar to the CS^+, GS5 was the least similar to the CS^+. The unconditioned stimulus (US) was an electrical pulse of 200 msec delivered to the left inner ankle (Biopac Systems, Inc., USA). Prior to onset of the experiment, intensity of the US was calibrated so that each participant rated the US as 'highly uncomfortable but not painful'.

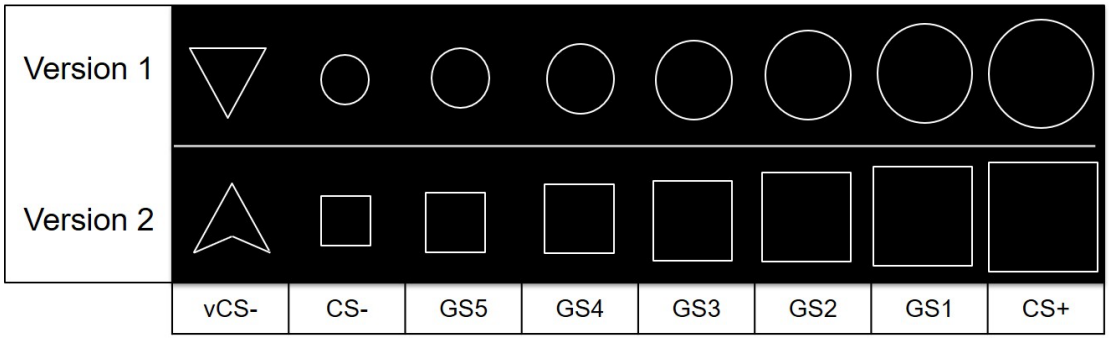

Figure S1. Conditioned stimuli (vCS^-, CS^-, CS^+) and generalization stimuli (GS1-5). As presented here, the largest circle/rectangle served as the CS+, the smallest circle/rectangle served as the CS- for half of the participants. For the other half of the participants, the smallest circle/rectangle served as the CS+, and the largest circle/rectangle as the CS-.

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Design: the task consisted of a pre-conditioning phase, a conditioning phase, and a generalization phase. During the pre-conditioning phase and generalization phase, all stimuli were shown. During the conditioning phase, only the CS+, CS-, and vCS- were shown. Stimuli were presented 12 times per task phase. The US co-terminated in 8/12 occurrences (66% reinforcement) with the CS^{+} during the conditioning phase, and in 6/12 occurrences (50% reinforcement) during the generalization phase. The sequence of stimuli was quasi-random; the same stimulus was not presented more than twice consecutively. In order to establish an even stimulus distribution each phase was divided into two blocks. The stimuli sequence was established by a genetic algorithm for optimizing experimental task designs (11). A fixation cross was shown at all times. Stimulus presentation was 4.4 seconds; the ISI was either 2.2 or 4.4 seconds.

Behavioral ratings: The task assessed both cognitive (i.e. US expectancy) and affective (i.e. fear) expressions of threat processing. During each task phase, participants were asked four times per stimulus type to rate US expectancy on a 4-point scale (1=no risk; 2=low risk; 3=moderate risk; 4=high risk of receiving a shock) when the color of the fixation cross changed from white to red (for 880 msec). After each task phase, participants rated their feelings of fear for each stimulus on a visual analogue scale, ranging from 0 (no fear) to 100 (high fear). Furthermore, ratings pertaining to valence (unpleasantness – pleasantness) and arousal (calm-aroused) were acquired after each task phase for each stimulus type.

Task instructions: Participants were instructed that they might learn to predict the shock when attending to the presented stimuli. Button box responses for the behavioral ratings were practiced before task onset.

Linear Departure Score

This score reflects to what degree the generalization gradient departs from linearity, and was calculated for each self-report measure (i.e. fear and US expectancy). The LDS was calculated by the following equation: average (GS1-5) – average (CS+, CS-). The latter part of the equation (average (CS+, CS-) reflects a theoretical midpoint of the generalization gradient; the first part reflects the average response to the GS that either falls above the midpoint (positive score), on the midpoint (zero), and below the midpoint negative score). The LDS therefore provides a single, continuous score of the degree of generalization, with a positive score reflecting a more gradual gradient (enhanced generalization), and a negative value a sharp gradient (lower generalization). LDSs were regressed on the symptom load.

Analyses

Three participants in the CM-absent group were excluded because of absence of subjective fear responses to the CS+ during the threat generalization phase.

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MRI acquisition, preprocessing, and first level modelling

Anatomical and Functional scans were acquired using a 3T Siemens Magnetom Prisma system (Siemens Healthcare, Erlangen, Germany) equipped with a 64-channel head/neck coil. T1-weighted Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) images with a voxel size of 1 mm x 1 mmx 1 mm were acquired (repetition time (TR)=2250 msec, echo time (TE)=2.21 msec, flip angle=9°, field of view (FOV)=256x256x192, sagittal slice orientation, GRAPPA=2) to serve as anatomical reference. Functional scans were acquired using a T2*- weighted echo-planar images (EPIs) sequence (TR=2450 msec, TE=28 msec, flip angle=75°, interleaved, FOV=216 mm, axial orientation, GRAPPA=3) with a voxel size of 3 mm x 3 mm x 3 mm. During the pre-conditioning and generalization phase, 303 volumes were acquired. During the acquisition phase, 114 volumes were acquired.

Functional magnetic resonance imaging data processing and analyses were carried out using FEAT (FMRI Expert Analysis Tool) of FSL (FMRIB's Software Library) version 5.0.6 (Smith et al., 2004). Pre-processing included non-brain removal (BET) (12), motion correction using MCFLIRT with the middle volume as reference (Jenkinson et al., 2002), high-pass temporal filtering with a cut-off of 100s, spatial smoothing with a Gaussian kernel of 6mm FWHM, pre-whitening (Woolrich et al., 2001), co-registration using FLIRT (Jenkinson et al., 2002), and normalization into Montreal Neurological Institute 152 stereotaxic space (MNI) using FNIRT for non-linear registration (Andersson et al., 2007). One participant of the CM-high group and one of the CM-low group were excluded because of excessive motion or motion-related artifacts.

First-level general linear models were computed for each participant. These models included eight explanatory variables (EVs) for the stimuli (CS+, vCS-, CS-, GS1, GS2, GS3, GS4, GS5), and covariates of no interest including shock onset, motion parameters, and motion outliers as measured with the FSL motion outliers program. Individual activation maps were created for all stimuli. Similar to the behavioral LDS, a contrast of parameter estimates was formed as main neural contrast as following: average (GS1-5) > average (CS-, CS+).

Manipulation check – generalization gradients in regions of interest

As a manipulation check, we first assessed whether neural activation related to threat and safety signaling, and generalization gradients in neural activation could be observed in regions of interest (vmPFC, dorsal ACC (dACC), insula, and hippocampus). The contrasts CS+>vCS- and vCS->CS+, with FEAT with mixed effects (FLAME1), a cluster significance level of $Z>3.1$ and $p<.05$ with Gaussian Random Field (GRF) correction for multiple comparisons were examined over the entire sample. The probabilistic Harvard-Oxford atlases (thresholded at 20%) were

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used for pre-threshold masking. Percent signal change from a 5mm sphere around the peak voxel in our ROIs was extracted for each stimulus. To confirm that the task induced generalization gradients in neural activation in the ROIs, repeated measures analyses in SPSS with stimulus type (7 levels) as within-subject factor, with post-hoc t-test and quadratic trend analysis when appropriate were conducted.

Results

Table S1. Overview of DSM-IV diagnoses per CM-group as assessed by the MINI International Neuropsychiatric interview

	no/low-CM		**high-CM**	
	n	*%*	*n*	*%*
Major depression	3	5.45	13	22.41
Dysthymia	0	0	10	17.24
Generalized anxiety disorder	7	12.72	22	37.93
Specific phobia	2	3.64	6	10.34
Panic disorder	0	0	1	1.72
Agoraphobia	0	0	1	1.72
Social phobia	1	1.81	9	15.52
Obsessive-compulsive disorder	0	0	2	3.45
Post-traumatic stress disorder	0	0	4	6.90
Alcohol abuse	1	1.81	0	0
Substance abuse	1	1.81	0	0
Psychotic disorder	1	1.81	1	1.72
Body dysmorphic disorder	0	0	6	10.34

Table S2. Generalization gradients in functional regions of interest.

fROI	**Peak voxel**			**F-test**	**Linear**	**Quadratic**
	x	y	z			
CS+ > vCS- Positive gradients						
dACC	2	16	40	F(4.85,533.80)=35.24, p<.000	F(1,110)=100.60, p<.000	F(1,110)=.46, p=.50
L insula	-30	20	-2	F(4.60,506.24)=56.39, p<.000	F(1,110)=166.91, p<.000	F(1,110)=26.25, p<.000
R insula	38	18	-4	F(4.46,490.36)=49.21, p<.000	F(1,110)=138.15, p<.000	F(1,110)=19.61, p<.000
vCS- > CS+ Negative gradients						
vmPFC	6	42	-18	F(2.82,309.72)=23.63, p<.000	F(1,110)=95.41, p<.000	F(1,110)=54.23, p<.000
L HC	-24	-18	-20	F(4.82,530.52)=34.11, p<.000	F(1,110)=119.54, p<.000	F(1,110)=21.82, p<.000
R HC	24	-16	-22	F(2.72,299.56)=18.24, p<.000	F(1,110)=61.49, p<.000	F(1,110)=40.00, p<.000

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dACC=dorsal anterior cingulate cortex; HC=hippocampus; vmPFC = ventromedial prefrontal cortex, L=left; R=right

Valence and arousal ratings

Both CM groups showed generalization gradients in valence and arousal ratings, as lower valence and increased arousal were reported for stimuli that became more similar to the CS+ (all p's<.000) (Figure S2). For the valence ratings, a group x stimulus interaction was observed (F(3.30, 357.12), p=.03. Bonferroni-corrected post-hoc tests showed that high-CM group only differed from the no/low-CM group at the end of the spectrums (CS+ compared to CS-; p=.01). Quadratic trend analyses of the valence gradient did not reveal group differences (1,108)=1.51, p=.22). Similarly, the arousal scores showed group differences (F(3.15, 340.21)=3.88, p=.008), with Bonferroni-corrected follow-up tests showing differences mainly at the end of the spectrum (CS+ vs CS-; p=.002). In addition, no differences in quadratic trends in the gradient across groups were found (F(1, 108)=.24, p=.62).

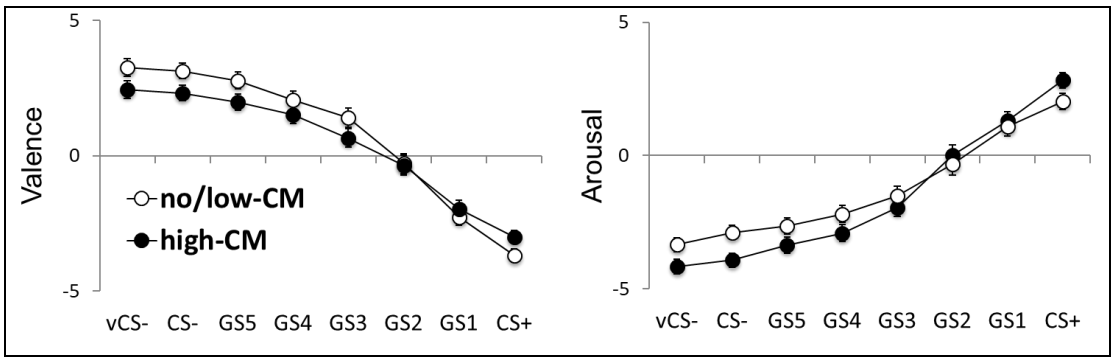

Figure S2. Valence (left) and arousal ratings (right side) in the no/low-CM and high-CM groups.

US expectancy: CM exposure and subclinical symptom load

A more positive association between the US expectancy generalization index (i.e. US expectancy LDS) and the subclinical symptom load was found in the high CM-group compared to the no/low-CM group, as indicated by a significant interaction between CM-group and US Expectancy LDS on symptom load (β=.58, p<.0001) (Figure S3). Further within-group correlation analyses revealed that only in the high-CM group, a higher US expectancy LDS was related to a higher symptom load (r=.40, p=.003). In the no-low-CM group, a negative association between US expectancy LDS and symptom load was found (r=-.33, p=.02). Follow-up analysis with a median-split symptom load score * quadratic trend analysis on the US expectancy gradient also indicated, in the high-CM group, that individuals with a low symptom load score showed a stronger quadratic trend in the US expectancy generalization gradient than individuals with a high symptom load (F(1,52)=5.89), p=.02). There was no difference in the quadratic trend of the US expectancy generalization gradient in the

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low CM-group between individuals with a low or high symptom load (F(1, 49)=2.52, p=.28).

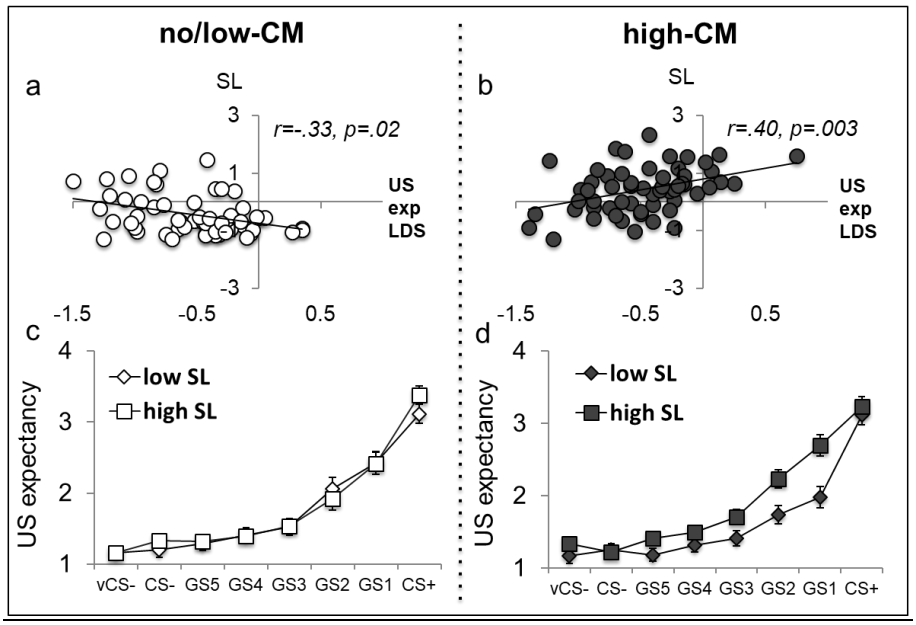

Figure S3. Generalization in US expectancy (US exp) moderates the link between childhood maltreatment and later psychopathology. a. correlation between the US expectancy (US exp) linear departure score (LDS) and symptom load (SL) in the no/low-CM group. b. correlation between the US expectancy (US exp) linear departure score (LDS) and symptom load (SL) in the high-CM group. c. visualization of the generalization gradient in US expectancy in the no/low-CM group for participants with a low symptom load (SL) versus a high symptom load (median split). d. visualization of the generalization gradient in US expectancy in the high-CM group for participants with a low symptom load (SL) versus a high symptom load (median split). Errors bars represent standard error of the mean.

Whole-brain interaction analysis

The whole-brain interaction analysis revealed that, compared to the no/low-CM group, the high-CM group showed a more negative association between the subclinical symptom load and activation in a cluster encompassing the left hippocampus, parahippocampus, and temporal fusiform gyrus ([-30 -42 -12], k=138, Z=4.48, p=.01). Correlation analyses with the extracted average parameter estimate of the LDS-contrast in this cluster revealed that, in the high-CM group, a negative association was found between subclinical symptom load and activation in this cluster (r=-.49, p<.001), while in the no/low-CM group, no significant associations could be observed (r=.24, p=.09) (Figure S4). A follow-up analyses on the shape of the neural

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generalization gradient in this cluster per symptom load level and level of CM (median-split symptom load score * quadratic trend analysis within each CM-group) revealed that individual with high levels of CM and a low symptom load showed an increased quadratic trend in the neural generalization gradient compared to individuals with high symptom load (F(1,52)=9.71, p=.003). No differences in the quadratic trend of the gradient were observed in the low-CM group between individuals with a low or a high symptom load (F(1,49)=1.24, p=.27).

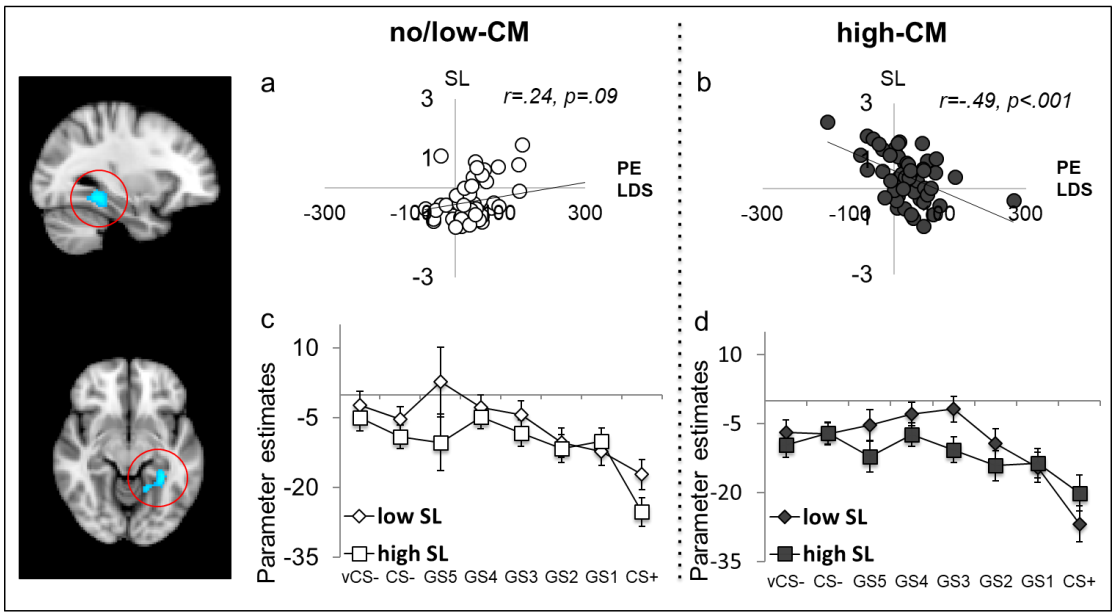

Figure S4. The link between childhood maltreatment and psychopathology is moderated by threat generalization-related activation in a cluster encompassing the left hippocampus/parahippocampal gyrus/temporal fysifom gyrus. a. correlation between the average parameter estimate (PE) of the LDS-contrast and symptom load (SL) in the no/low-CM group. b. correlation between the average parameter estimate (PE) of the LDS-contrast and symptom load (SL) in the high-CM group. c. visualization of the hippocampal generalization gradient in the no/low-CM group for participants with a low symptom load (SL) versus a high symptom load (median split). d. visualization of the hippocampal generalization gradient in the high-CM group for participants with a low symptom load (SL) versus a high symptom load (median split). Errors bars represent standard error of the mean.

References

1. Overbeek I, Schruers K, Griez E. Mini international neuropsychiatric interview: Nederlandse versie 5.0. 0. DSM-IV [Dutch version] Maastricht, The Netherlands: Universiteit Maastricht. 1999.

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2. Bernstein DP, Fink L, Handelsman L, Foote J, Lovejoy M, Wenzel K, et al. Initial reliability and validity of a new retrospective measure of child abuse and neglect. The American journal of psychiatry. 1994;151(8):1132.
3. Sakai ST, Stepniewska I, Qi HX, Kaas JH. Pallidal and cerebellar afferents to pre-supplementary motor area thalamocortical neurons in the owl monkey: a multiple labeling study. Journal of Comparative Neurology. 2000;417(2):164-80.
4. Aas M, Kauppi K, Brandt C, Tesli M, Kaufmann T, Steen NE, et al. Childhood trauma is associated with increased brain responses to emotionally negative as compared with positive faces in patients with psychotic disorders. Psychological medicine. 2017;47(4):669-79.
5. Spielberger CD, Gorsuch RL, Lushene RE. Manual for the state-trait anxiety inventory. 1970.
6. Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. The British journal of psychiatry. 1979;134(4):382-9.
7. Vollema MG, Ormel J. The reliability of the Structured Interview for Schizotypy–Revised. Schizophrenia Bulletin. 2000;26(3):619.
8. Lataster T, Verweij K, Viechtbauer W. Effect of illness expression and liability on familial associations of clinical and subclinical psychosis phenotypes. Acta Psychiatrica Scandinavica. 2014;129(1):44-53.
9. van Winkel R, Investigators G. Further evidence that cannabis moderates familial correlation of psychosis-related experiences. PloS one. 2015;10(9):e0137625.
10. Lange I, Goossens L, Michielse S, Bakker J, Lissek S, Papalini S, et al. Behavioral pattern separation and its link to the neural mechanisms of fear generalization. Social Cognitive and Affective Neuroscience. 2017;12(11):1720-9.
11. Wager TD, Nichols TE. Optimization of experimental design in fMRI: a general framework using a genetic algorithm. Neuroimage. 2003;18(2):293-309.
12. Smith SM. BET: brain extraction tool. FMRIB TR00SMS2b, Oxford Centre for Functional Magnetic Resonance Imaging of the Brain), Department of Clinical Neurology, Oxford University, John Radcliffe Hospital, Headington, UK. 2000.