**SI includes:**

**Supplementary results**

**Figures S1-S3**

**Tables S1 & S2**

**Supplementary references**

**SUPPLEMENTARY RESULTS**

**Emotional memory reinstatement in additional post-hoc ROIs.**

Our whole-brain searchlight analysis revealed several cortical areas outside of the mPFC which exhibited significant CS+ > CS- reinstatement. We included additional post-hoc analyses in two of the ROIs identified in the searchlight. Firstly, we present an analysis of emotional reinstatement in the anterior insula. This analysis was motivated both by our searchlight results and based on this region’s consistent involvement in fear and fear extinction processes in humans 1. In healthy adults, we observed significant preferential CS+ reinstatement for items encoded during both fear conditioning (0.229, [0.177, 0.281], PFDR = 4.95e-17) and extinction (0.125, [0.073, 0.177], PFDR = 5.64e-6). There was no preferential CS+ reinstatement for items encoded during pre-conditioning (-0.012, [-0.065, 0.040], PFDR = 0.77). We observed the same pattern of results in individuals win PTSS; there was significantly more CS+ reinstatement for items encoded during fear conditioning (0.202, [0.149, 0.254], PFDR = 1.17e-13) and extinction (0.081, [0.027, 0.134], PFDR = 3.69e-3). Again, there was no preferential CS+ reinstatement for items encoded during pre-conditioning (-0.005, [-0.057, 0.048], PFDR = 0.86). The anterior insula exhibited more fear memory reinstatement compared to extinction memory reinstatement both in healthy adults (0.104, [0.030, 0.178], PFDR = 5.56e-3) and in individuals with PTSS (0.121, [0.047, 0.195], PFDR = 2.82e-3). We did not observe a main effect nor interactions with our *group* term in our model (all P≥ 0.33), and thus did not conduct additional follow up comparisons. In sum, the anterior insula reinstated CS+ representations of fear and extinction memories, while showing a strong preference for fear memories.

In addition, we also probed emotional memory reinstatement in the precuneus. This region is consistently recruited during episodic retrieval 2, and has also been identified to be recruited during fear conditioning in humans 1. In healthy adults, the precuneus exhibited trending preferential CS+ reinstatement for items encoded during fear conditioning (0.074, [0.001, 0.146], PFDR = 0.093), and significant preferential CS+ reinstatement for extinction memories (0.135, [0.063, 0.208], PFDR = 7.30e-4). There was no difference in CS reinstatement for items encoded during pre-conditioning (0.019, [-0.053, 0.091], PFDR = 0.60). In contrast to healthy adults, individuals with PTSS did not display greater CS+ > CS- reinstatement in the precuneus for items encoded during fear conditioning (0.049, [-0.023, 0.121], PFDR = 0.28), although there was preferential CS+ reinstatement for items encoded during extinction (0.165, [0.093, 0.238], PFDR = 5.00e-5). There was also no difference in CS reinstatement for items encoded during pre-conditioning in the PTSS group (-0.028, [-0.101, 0.044], PFDR = 0.54). In healthy adults, the precuneus did not differentiate reinstatement between fear conditioning and extinction (-0.062, [-0.164, 0.040], PFDR = 0.23). In PTSS, there was trending more extinction memory reinstatement compared to fear conditioning (0.11, [0.014, 0.219], PFDR = 0.053). We observed a significant *group* \* *encoding context* interaction (X2(2) = 28.6, P = 6.30e-7), which post-hoc contrasts revealed to be driven by more extinction related reinstatement in healthy adults compared to PTSS (collapsed across CS type; 0.15, [0.067, 0.241], PFDR = 1.57e-3). Thus, the precuneus exhibited significant CS+ preferential reinstatement for extinction memories in both groups.

Chart

Description automatically generated

**Figure S1. Emotional memory reinstatement in anterior insula and precuneus.** ROIs were defined coordinates from meta-analyses, see **Methods** for details. Error bars correspond to the 95% confidence interval of the CS+ - CS- difference in reinstatement. Both healthy adults and individuals with PTSS exhibited significant emotional memory reinstatement in the anterior insula, with both groups showing more fear memory reinstatement. In the precuneus both group exhibited significant emotional reinstatement for extinction memories only. \*\*\*P < 0.001, \*\*P < 0.01, \*P < 0.05, ~ P < 0.05 before FDR correction.

**Interactions in MTL univariate predicting mPFC reinstatement location.**

In our analysis using univariate activity at the time of memory retrieval to predict the location of reinstatement in the mPFC, we observed several interactions with hippocampal subfield. For the pHC, there was a significant *pHC* \* *CS* *type* interaction(X2(1) = 11.2, P = 8.3e-4), such that the slope of pHC activity was significantly more negative for CS+ compared to CS- (CS slope diff. = -1.53e-3, [-2.43e-3, -6.34e-4], P = 8.24e-4). In the body of the hippocampus, there was a significant *HC body* \* *CS type* \* *encoding context* interaction (X2(1) = 5.46, P = 0.019). Post-hoc contrasts revealed that for items encoded during conditioning, the slope for the CS+ was significantly more negative than the CS- (CS slope diff. = -2.68e-3, [-4.21e-3, -1.15e-3], PFDR = 1.17e-3), while there was no difference in the slopes for extinction (CS slope diff. = -8.68e-5, [-1.63e-3, 1.46e-3], PFDR = 0.91). There were no significant interactions in the aHC, BLA, or CeM. In sum, MTL univariate activity predicted more reinstatement in the dACC. This effect was stronger for all CS+ items in the pHC compared to CS-, and was selective for conditioning CS+ items in the body of the hippocampus.

**Recognition memory does not influence reinstatement in the mPFC.**

We additionally tested if reinstatement in our *a priori* ROIs differed as a function of memory strength. Recognition memory was included as a categorical predictor (e.g., “high-confidence hit” or “miss”; *reinstatement ~ memory accuracy \* CS type \* encoding context \* group + (*1*|subject)*)*.* In the mPFC, there was no main effect of *memory accuracy* (X2(1) = 0.024, P = 0.89), and all interactions with this term were not significant (all Ps ≥ 0.14). A similar pattern emerged in the amygdala, with only a trending main effect of *memory accuracy* (X2(1) = 3.08, P = 0.08) and no significant interactions (all Ps ≥ 0.07). In the hippocampus, we again observed a trending main effect of *memory accuracy* (X2(1) = 3.47, P = 0.06), as well as several significant higher order interactions (*memory accuracy \* CS type \* encoding context*:X2(2) = 6.26, P = 0.044; *memory accuracy \* CS type \* subfield*: X2(2) = 6.69, P = 0.035; *memory accuracy \* encoding context \* group*: X2(2) = 11.8, P = 0.003).Thus, recognition memory did not influence reinstatement in the mPFC, and only slightly modulated reinstatement in the amygdala. Recognition memory did influence reinstatement in the hippocampus, which is consistent with this structure’s role in episodic retrieval. That recognition memory performance did not modulate neural reinstatement in the mPFC is consistent with previous work 3, which similarly describes a lack of association between memory performance and reinstatement along mPFC ROIs.

**No effect of US reinforcement on neural reinstatement.**

One possibility is that CS+s from fear conditioning exhibit more or less reinstatement based on whether or not they were paired with the US during fear conditioning. We tested this idea by comparing reinstatement between reinforced and unreinforced stimuli from fear conditioning in each group of ROIs. As we used a 50% reinforcement schedule, this analysis was sufficiently powered to compare between these trial types. For each group of ROIs, data was restricted to CS+ trials from conditioning, and US reinforcement was included as a categorical predictor (“CS+US” or “CS+”; *reinstatement* ~ *US reinforcement* \* *ROI* \* *group* + (1|*subject*)). There were no significant main effect or interactions with *US* *reinforcement* in the mPFC (all Ps ≥ 0.27), hippocampus (all Ps ≥ 0.62), or amygdala (all Ps ≥ 0.15). We have previously reported that in this same category conditioning US reinforcement did not influence recognition memory 4. In the previous study, as in the present study, there was a general enhancement in memory for CS+ items encoded in the fear conditioning context, regardless of if they were paired with the US. That US reinforcement did not influence neural reinstatement supports the idea that fear association readily generalizes to all relevant category exemplars encoded in the same temporal context.

**Analysis of false alarm rate between groups**

In addition to shock expectancy, SCR, and memory hit rate we also compared false alarm rate during the recognition memory test between healthy adults and individuals with PTSS. A mixed ANOVA of high confidence false alarm rate showed a trending effect of *group* (F­1, 46 = 4.01, P = 0.051), such that individuals with PTSS did record marginally more high confidence false alarms compared to healthy adults. There was no main effect of CS type (P = 0.13), and no significant CS type \* group interaction (P = 0.68). Even though there exists this slight bias in behavioral responding to novel lures, an mixed ANOVA of high confidence corrected recognition (hit – false alarm rate) reveals no significant group differences in actual memory performance (main effect of *group*: F1, 46 = 0.05, P = 0.83, all interactions with *group* Ps ≥ 0.39). Note that this analysis differs slightly from the group comparison published in Hennings et al., 2021, as participants were not excluded in the current analyses for low memory performance. Thus, this trending difference in responding to novel lures during the recognition memory test is unlikely to influence our neural results, which focus solely on previously encoded probe items.

**Alternative approaches to assess encoding-retrieval similarity**

*Set level reinstatement.* The primary purpose of our experimental design was to leverage episodic item-to-item comparisons to reveal the subtle reinstatement of associative memories. To that end, we deliberately aligned our analytical approach with the corpus of extant episodic memory encoding-retrieval similarity studies. Our main analyses thus leverage same item-to-item representations between encoding and retrieval to reveal the temporal context dependent organization of emotional memories in the mPFC. However, we acknowledge that this is not the only possible way to investigate the organization of these memories. Another valid approach is to probe the “set” level encoding-retrieval similarity in a given ROI. For example, for a single CS+ encoded during fear conditioning, we can take the correlation of that encoding pattern with the retrieval patterns of all other CS+s encoded in the same temporal context. These set level similarity values can be analyzed the same way as item level similarity, by comparing CS+ - CS- values by temporal context in our mPFC ROIs. For completeness, we report this complementary analysis below. Based on our previous work using a trial-unique category conditioning design 5,6, we predicted a strong degree of neural similarity for all CS+ items encoded within the same temporal context, and for this analysis to closely match the item-focused analyses.

In healthy adults, the dACC exhibited greater set level reinstatement for CS+ items (compared to CS- items) that were encoded during fear conditioning (0.179, [0.149, 0.208], PFDR = 2.34e-31). This greater set level CS+ reinstatement was stronger for fear memories than for extinction memories (0.135, [0.093, 0.177], PFDR = 1.04e-9) in the dACC. Unlike our item level analysis, the dACC did show greater set level CS+ reinstatement for extinction memories (0.044, [0.014, 0.074], PFDR = 6.02e-3), but again did not for pre-conditioning memories (0.009, [-0.020, 0.039], PFDR = 0.58). In the vmPFC, there was greater set level CS+ reinstatement for both fear memories (0.085, [0.058, 0.115], PFDR = 4.74e-8) and extinction memories (0.104, [0.074, 0.133], PFDR = 2.33e-11). There was no greater CS+ reinstatement for pre-conditioning memories (-0.012, [-0.041, 0.018], PFDR = 0.52). As with the item level analysis, there was a significant double dissociation in the set level reinstatement of fear and extinction memories between these two regions (significant *CS type \* encoding context \* ROI* interaction; X2(1) = 16.2, P = 5.71e-5). That is, there was stronger set level reinstatement of fear memories in the dACC relative to the vmPFC (0.94, [0.052, 0.135], PFDR = 2.29e-5), and stronger set level reinstatement of extinction memories in the vmPFC relative to the dACC (0.060, [0.018, 0.101], PFDR = 6.74e-3). As with our item level analyses, these results again show that in healthy adults these mPFC regions exhibit a double dissociation in the reinstatement of emotional memories, based on the temporal context in which they were encoded.

Our analysis of set level reinstatement in PTSS again closely mirrored our results from the item level analyses. Individuals with PTSS exhibited greater CS+ set level reinstatement in the dACC for items encoded during conditioning (0.173, [0.143, 0.202], PFDR = 1.84e-29), and this reinstatement was stronger in the dACC relative to the vmPFC (0.144, [0.103, 0.186], PFDR = 5.39e-11). The dACC again did not exhibit selective reinstatement for pre-conditioning memories (0.003, [-0.026, 0.033], PFDR = 0.80). Individuals with PTSS thus did not exhibit any deficits in fear related processing, as in our item level analysis. As in healthy adults, both the dACC (0.080, [0.050, 0.110], PFDR = 3.27e-7) and vmPFC (0.051, [0.021, 0.081], PFDR = 1.45e-3) exhibited greater set level CS+ reinstatement for items encoded during extinction. However, there was no difference in extinction memory reinstatement between these two ROIs (-0.029, [-0.071, 0.013], PFDR = 0.18). As in the item level analysis, there was unexpected selectively for the CS- items encoded during pre-conditioning in the vmPFC (-0.042, [-0.072, -0.013], PFDR = 7.39e-3). Unlike the healthy adults, there was no preferential reinstatement in the vmPFC for fear conditioning memories (0.028, [-0.001, 0.058], PFDR = 0.83). Again, the significant double dissociation we observed in set level reinstatement of healthy adults was not present in the PTSS group (no significant *CS type \* encoding context \* ROI* interaction X2(1) = 0.882, P = 0.35). Thus, as in our item level analyses, these results show that individuals with PTSS exhibit normal reinstatement for fear memories but exhibit dysregulated organization of extinction memories.

We again directly tested if the observed pattern of set level reinstatement significantly differed between groups by comparing vmPFC – dACC difference in restatement for each phase between healthy adults and individuals with PTSS. The groups did not in their patterns of set level fear memory reinstatement (-0.051, [-0.110, 0.008], PFDR = 0.092); however as expected there was a significant difference between healthy adults and individuals with PTSS in their patterns of set level extinction memory reinstatement across the mPFC (0.089, [0.030, 0.148], PFDR = 6.89e-3).

**Diagram, schematic

Description automatically generated**

**Figure S2. Dissociable reinstatement of emotional memories at the set level.** All error bars correspond to the 95% confidence interval of the CS+ ‒ CS- difference. \*\*\*P < 0.001, \*\*P < 0.01, \*P < 0.05, FDR corrected. *Top.* As in our item-to-item analysis healthy adults exhibited a significant double dissociation of set level emotional reinstatement in the mPFC, such that reinstatement for conditioning was higher in the dACC, and extinction reinstatement was higher in the vmPFC. *Bottom*. In adults with PTSS, there was no difference in extinction reinstatement between the two mPFC ROIs, suggesting that extinction memory organization is dysregulated in this group.

*Cross phase encoding-retrieval similarity.* A possible alternative explanation for our main results is that there exists a general pattern of activity for all CS+ trials, regardless of when (conditioning or extinction) the particular item was encoded. Our interpretation of our mPFC results was to suggest the dACC and vmPFC separately and selectively organize CS+s as a function of the temporal context at encoding. But a reasonable question is to ask whether this double dissociation is truly item specific, or instead can be uncovered using any CS+ to CS+ comparison. To test this alternative hypothesis, we analyzed the mean cross phase encoding-retrieval similarity in each ROI. For example, we correlated the encoding pattern of a single CS+ from conditioning to the retrieval patterns of all CS+ from extinction. We calculated this similarity for all encoding trials during conditioning and extinction using the corresponding retrieval trials (i.e., extinction and conditioning). As with our other analyses this procedure was done within category, and the CS+ - CS- difference (based on encoding phase) was taken in our two mPFC regions of interest.

In healthy adults, we found significant greater CS+ cross phase similarity in both the dACC and vmPFC for both conditioning and extinction (dACC conditioning: 0.098, [0.077, 0.119], PFDR = 6.15e-20; vmPFC conditioning: 0.089, [0.068, 0.109], PFDR = 1.30e-16; dACC extinction: 0.058, [0.037, 0.078], PFDR = 7.28e-08; vmPFC extinction: 0.044, [0.023, 0.065], PFDR = 3.93e-5). Crucially however, there was no difference in cross phase similarity between the dACC and vmPFC for either conditioning (0.001, [-0.020, 0.039], PFDR = 0.52) or extinction (-0.014, [-0.043, 0.016], PFDR = 0.48). Unlike in our item-to-item analysis, there was no significant double dissociation between the dACC and vmPFC (no significant *CS type \* encoding context \* ROI* interaction: X2(1) = 0.036, P = 0.85). These results suggest two things: firstly, and unsurprisingly, they suggest that there are shared neural responses between CS+ items that are encoded during conditioning and extinction across the mPFC. Secondly, this analysis supports our main hypothesis that the double dissociation we observed in the item-to-item analysis arises a function of the temporal context in which each item encoded, not simply CS type.

In individuals with PTSS, we also observed significant greater CS+ cross phase encoding retrieval similarity (dACC conditioning: 0.127, [0.106, 0.147], PFDR = 3.51e-32; vmPFC conditioning: 0.039, [0.018, 0.059], PFDR = 2.83e-4; dACC extinction: 0.079, [0.058, 0.010], PFDR = 2.05e-13; vmPFC extinction: 0.035, [0.014, 0.056], PFDR = 9.73e-4), again indicating shared neural responses for these items. However, unlike healthy adults, this group displayed greater cross phase similarity in the dACC (compared to the vmPFC) for both conditioning (0.088, [0.059, 0.117], PFDR = 1.63e-8) and extinction (0.044, [0.015, 0.073], PFDR = 6.84e-3). There was a significant *CS type \* encoding context \* ROI* interaction in this group (X2(1) = 4.56, P = 0.033), such that the dACC – vmPFC difference was stronger in conditioning compared to extinction. These results further support that in individuals with PTSS, the vmPFC is dysregulated during the encoding and retrieval of fear and extinction.

Diagram, schematic

Description automatically generated

**Figure S3. Cross phase encoding-retrieval similarity.** All error bars correspond to the 95% confidence interval of the CS+ ‒ CS- difference. \*\*\*P < 0.001, \*\*P < 0.01, \*P < 0.05, FDR corrected. *Top.* In healthy adults cross phase similarity does not show a double dissociation between the vmPFC and dACC, suggesting that temporal context of encoding was a key factor in our primary results. *Bottom*. In adults with PTSS, there was significantly more cross phase similarity in the dACC for both conditioning and extinction, again suggesting general vmPFC dysfunction.

**Searchlight Clusters**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Group | Encoding context | Label (hemisphere) | MNI coor. peak | Size in voxels (3mm3) |
| Healthy | Conditioning | Inferior frontal gyrus (R) | 33, 9, 27 | 741 |
|  |  | Superior frontal gyrus (L) | -6. 18, 51 | 608 |
|  |  | Middle frontal gyrus (L) | -39, 33, 15 | 414 |
|  |  | Angular gyrus (L) | 33, -57, 36 | 308 |
|  |  | Insula (L) | -27, 24, -6 | 280 |
|  |  | Inferior frontal gyrus (L) | -45, 3, 21 | 178 |
|  |  | Precuneus (L) | -9, -66, 42 | 175 |
|  |  | Inferior parietal lobule (R) | 30, -54, 42 | 117 |
|  |  | Cerebellar tonsil (R) | 36, -63, -45 | 40 |
|  |  | Cerebellar tonsil (L) | -33, -60, -33 | 32 |
|  |  | Medial frontal gyrus (L) | -15, 48, -3 | 25 |
|  |  | Precuneus (R) | 12, -75, 42 | 25 |
|  |  | Middle temporal gyrus (L) | -57, -51, -6 | 22 |
|  |  |  |  |  |
|  | Extinction | Medial frontal gyrus (L) | -3, 51, 0 | 191 |
|  |  | Precuneus (L) | -6, -63, 27 | 113 |
|  |  | Angular gyrus (L) | -39, -75, 36 | 69 |
|  |  | Angular gyrus (R) | 42, -69, 30 | 44 |
|  |  | Middle temporal gyrus (R) | 63, 0, -24 | 26 |
|  |  |  |  |  |
| PTSS | Conditioning | Superior frontal gyrus (R) | -6, 18, 51 | 326 |
|  |  | Insula (L) | -33, 24, 9 | 214 |
|  |  | Insula (R) | 30, 24, -6 | 201 |
|  |  | Precuneus (L) | -18, -66, 48 | 119 |
|  |  | Supramarginal gyrus (L) | -54, -48, 27 | 62 |
|  |  | Culmen / Parahippocampal gyrus (L) | -30, -51, -24 | 61 |
|  |  | Inferior frontal gyrus (R) | 45, 6, 24 | 52 |
|  |  | Middle frontal gyrus (L) | -51, -3, 39 | 52 |
|  |  | Fusiform gyrus (L) | -57, -63, -12 | 29 |
|  |  | Precentral gyrus (L) | -42, -3, 51 | 23 |
|  |  | Middle frontal gyrus (L) | -42, 24, 24 | 22 |
|  |  | Superior temporal gyrus (L) | -51, -54, 15 | 20 |
|  |  |  |  |  |
|  | Extinction | Cuneus (L) | -6, -75, 30 | 42 |
|  |  | Insula (R) | 36, 30, 3 | 34 |
|  |  | Insula (L) | -39, 27, 0 | 25 |

**Table S1.** Whole-brain searchlight results. Clusters correspond to significant CS+ ‒ CS- reinstatement. Coordinates refer to the peak voxel in each cluster. Anatomical labels were derived from the Talairach-Tournoux Atlas.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Group | Encoding Context | CS Type | Low confidence | High confidence |
| Healthy | Pre-conditioning | CS+ | 0.75 (0.17) | 0.5 (0.22) |
|  |  | CS- | 0.72 (0.17) | 0.45 (0.22) |
|  | Conditioning | CS+ | 0.84 (0.17) | 0.59 (0.27) |
|  |  | CS- | 0.65 (0.22) | 0.38 (0.26) |
|  | Extinction | CS+ | 0.7 (0.2) | 0.41 (0.29) |
|  |  | CS- | 0.59 (0.19) | 0.31 (0.21) |
| PTSS | Pre-conditioning | CS+ | 0.78 (0.14) | 0.59 (0.18) |
|  |  | CS- | 0.73 (0.19) | 0.5 (0.21) |
|  | Conditioning | CS+ | 0.83 (0.15) | 0.62 (0.25) |
|  |  | CS- | 0.68 (0.16) | 0.45 (0.26) |
|  | Extinction | CS+ | 0.69 (0.17) | 0.45 (0.2) |
|  |  | CS- | 0.63 (0.19) | 0.37 (0.22) |

**Table S2.** Recognition memory performance does not differ by group.Mean (standard deviation) low and high confidence hit rates for each group, encoding context, and CS type. Note that values differ slightly from Hennings et al., 2021, as participants were not excluded in the present analyses for low memory performance as they were in Hennings et al., 2021.

**SUPPLEMENTARY REFERENCES**

1. Fullana, M.A., Harrison, B.J., Soriano-Mas, C., Vervliet, B., Cardoner, N., Àvila-Parcet, A., and Radua, J. (2016). Neural signatures of human fear conditioning: An updated and extended meta-analysis of fMRI studies. Molecular Psychiatry *21*, 500–508.

2. Kim, H. (2019). Neural correlates of explicit and implicit memory at encoding and retrieval: A unified framework and meta-analysis of functional neuroimaging studies. Biological Psychology *145*, 96–111.

3. Ritchey, M., Wing, E.A., LaBar, K.S., and Cabeza, R. (2013). Neural Similarity Between Encoding and Retrieval is Related to Memory Via Hippocampal Interactions. Cerebral Cortex *23*, 2818–2828.

4. Dunsmoor, J.E., Kroes, M.C.W., Moscatelli, C.M., Evans, M.D., Davachi, L., and Phelps, E.A. (2018). Event segmentation protects emotional memories from competing experiences encoded close in time. Nature Human Behaviour *2*, 291–299.

5. Dunsmoor, J.E., Kragel, P.A., Martin, A., and La Bar, K.S. (2014). Aversive learning modulates cortical representations of object categories. Cerebral Cortex *24*, 2859–2872.

6. Morey, R.A., Haswell, C.C., Stjepanović, D., Dunsmoor, J.E., and LaBar, K.S. (2020). Neural correlates of conceptual-level fear generalization in posttraumatic stress disorder. Neuropsychopharmacol. *45*, 1380–1389.