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Emotional brain states carry over and enhance future memory formation

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Emotional arousal can produce lasting, vivid memories for emotional experiences, but little is known about whether emotion can prospectively enhance memory formation for temporally distant information. One mechanism that may support prospective memory enhancements is the carry-over of emotional brain states that influence subsequent neutral experiences. Here we found that neutral stimuli encountered by human subjects 9–33 min after exposure to emotionally arousing stimuli had greater levels of recollection during delayed memory testing compared to those studied before emotional and after neutral stimulus exposure. Moreover, multiple measures of emotion-related brain activity showed evidence of reinstatement during subsequent periods of neutral stimulus encoding. Both slow neural fluctuations (low-frequency connectivity) and transient, stimulus-evoked activity predictive of trial-by-trial memory formation present during emotional encoding were reinstated during subsequent neutral encoding. These results indicate that neural measures of an emotional experience can persist in time and bias how new, unrelated information is encoded and recollected.

We form lasting, vivid and detailed memories for only a subset of our experiences. Emotion is one key factor that influences the fate of our memories[1,2]. Compared to their neutral counterparts, emotional events and stimuli are more robustly remembered, with higher levels of confidence, vividness and detail[1–4]. The presence of emotion not only increases recollection of emotionally arousing experiences themselves but also has been shown to retroactively modulate recollection of neutral information preceding emotional arousal[5,6]. Despite such findings documenting the impact of emotion on memory for information before and during manipulations of emotional arousal, little work has examined whether emotion can prospectively enhance memory for subsequently encountered information minutes later. Moreover, it is unknown whether the persistence of emotional arousal is capable of impacting future brain states and modifying the neural structures that support memory formation for subsequently encountered stimuli. Here we assessed whether exposure to emotionally arousing stimuli prospectively enhances memory for subsequently encountered stimuli by biasing future states of brain activity.

A robust body of literature indicates that emotional arousal during an experience enhances the consolidation of memory for that particular event, resulting in more persistent, vivid and detailed emotional memories over time[1–4]. Mechanistically, emotional arousal has been linked with the release of norepinephrine and epinephrine, which, in concert with the amygdala, are thought to modulate hippocampal processes during both the encoding and subsequent consolidation of emotional experiences[1,7–9]. Supporting this notion, activity within and connectivity between the amygdala, hippocampus and medial temporal lobe cortex reliably predicts successful emotional memory formation and consolidation[10–13]. In addition to enhancing memory for emotional events themselves, emotional learning and exposure to emotional stimuli—or the induction of arousal pharmacologically or with shock—can retroactively enhance long-term retention of preceding neutral information[5,6,14–18] (but see refs. 15,19).

But what about unrelated neutral information that follows an emotional experience? Can extended periods of emotional arousal bias future brain states and, in doing so, prospectively modify the neural structures supporting memory formation for unrelated, neutral information? Prior work has examined the influence of emotion on memory for information encountered seconds after emotional arousal[6,15,19], and a recent study found that individual differences in arousal induced by a block of emotional stimuli were related to enhanced memory discrimination for similar visual images presented a few minutes later[20]. Previous studies have also examined the impact of stress on memory formation for both emotional and neutral stimuli. Memory for both emotional and neutral images is enhanced when encoding blocks are interleaved with a stress manipulation[21]. At the neural level, amygdala connectivity remains altered after stress induction[22–24], and stress induction can alter activation in brain regions related to memory formation for both emotional and neutral stimuli[21]. However, to our knowledge, no prior investigations have examined how extended periods of emotional arousal can prospectively bias future brain states and thereby modify the manner in which unrelated, neutral information is encoded into memory. Here, we tested whether arousal and brain states associated with an extended (~20 min) emotional experience can carry-over and bias the encoding of neutral stimuli encountered approximately 9 to 33 min later and thereby modulate how those stimuli are encoded into memory (**Fig. 1**).

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To this end, two groups of subjects underwent blood-oxygen-level-dependent (BOLD) functional MRI (fMRI) scanning during incidental encoding of extended (23 min) blocks of emotional and neutral complex scenes (emotional and neutral encoding conditions; **Fig. 1a**), returning 6 h later for a surprise memory test. One group of subjects first encountered an extended block of emotional stimuli followed by neutral stimuli ('E-N encoding order'), while a different group of subjects encountered neutral stimuli followed by emotional stimuli ('N-E encoding order'). A separate group of subjects incidentally encoded blocks of neutral stimuli under the same procedures as the fMRI subjects (but outside of the MRI scanner), and two other groups of subjects incidentally encoded blocks of emotional and neutral stimuli (E-N and N-E encoding orders) outside of the MRI scanner (**Fig. 1a**). We reasoned that the prospective influence of emotion, or a carry-over of an emotional state into subsequent neutral encoding, should be present when subjects encoded a long block of emotional stimuli first and neutral stimuli second (E-N encoding order) but not for the opposite encoding order (N-E encoding order) or when only neutral stimuli were encoded outside of the context of emotional stimuli (N-N encoding order). We first assessed the persistence of emotional arousal from emotional encoding into subsequent neutral encoding blocks by measuring skin conductance levels (SCL) as a proxy for sympathetic nervous system activation[25]. Behaviorally, we asked whether the carry-over of an emotional state would enhance the subjective recollection of subsequently encountered neutral stimuli (in the E-N encoding order relative to the other encoding orders). Finally, using fMRI, we examined whether emotion would prospectively bias future encoding-related brain activity in at least two ways (**Fig. 1b**): first, we asked whether BOLD activity patterns during emotional and neutral encoding were more similar when neutral stimuli were encoded after versus before emotional stimuli (E-N versus N-E encoding order) and second, whether brain regions supporting emotional memory (for example, amygdala and anterior hippocampus) were more active and showed greater levels of connectivity when neutral stimuli were encoded after versus before emotional stimuli (i.e., when a carry-over of emotional arousal may have been present).

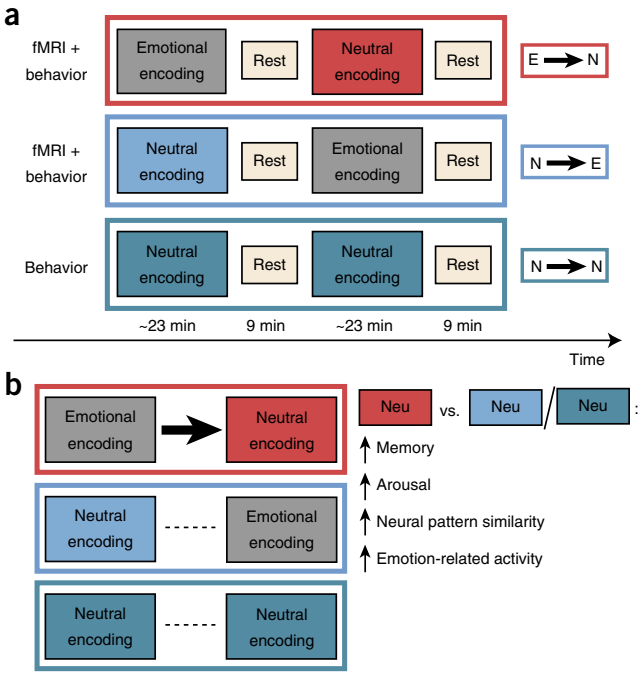

Figure 1 Experimental design and predictions. (**a**) Experimental design: separate groups of subjects performed blocks of incidental emotional and neutral encoding (E→N, subjects that performed emotional followed by neutral encoding; N→E, subjects that performed neutral followed by emotional encoding): two groups encoded stimuli during fMRI scanning (E→N and N→E encoding orders), while two other groups of subjects underwent E→N and N→E encoding blocks under behavioral testing conditions (**Supplementary Fig. 1**) and one other group encoded neutral stimuli outside of the context of emotional stimuli under behavioral testing conditions (N→N). Encoding blocks were separated by rest periods (cream boxes). (**b**) We predicted that arousal associated with emotional encoding would persist and carry over, prospectively biasing brain activity during subsequent neutral (neu) encoding and enhancing memory for neutral stimuli for the E→N encoding order. No emotional bias or carry-over of emotion should be present in the other encoding orders. A prospective influence of emotion on subsequent neutral encoding predicts that several factors listed should be differentially present during neutral encoding for the E→N vs. N→E encoding order (greater memory, arousal, emotion-related activity and neural pattern similarity between emotional and neutral encoding).

RESULTS

Skin conductance

If emotional arousal following an extended block of emotional encoding persists into neutral encoding, then overall SCL, which is related to sympathetic nervous system activation[25], should track this persistence and differ based on emotion and encoding order. To account for individual differences in baseline skin conductance, SCL was computed as the change relative to a baseline rest period obtained before the first encoding block (referred to as 'relative SCL'; Online Methods). When assessing relative SCL in the fMRI study, we found a significant interaction between emotion (emotional versus neutral encoding) and encoding order (**Fig. 2a**; $F_{1,42} = 8.72$, $P = 0.0051$; permutation test, $P = 0.0036$). Elevated SCL showed evidence of persistence from long-lasting blocks of emotional into subsequent neutral encoding (**Fig. 2a**). Specifically, SCL increased during emotional encoding compared to the preceding baseline rest scan ($t_{21} = 3.21$, corrected $P = 0.0167$; permutation test, corrected $P = 0.004$) and remained heightened during a block of subsequent neutral encoding (SCL relative to baseline rest scan, $t_{21} = 3.06$, corrected $P = 0.0239$; permutation test, corrected $P = 0.0096$). Thus SCL levels were equivalent between emotional and neutral encoding when neutral stimuli were encoded tens of minutes after emotional stimuli (E-N encoding order, $t_{21} = 0.51$, $P = 0.61$; permutation test, $P = 0.65$), despite the temporal separation of these encoding blocks. By contrast, SCL was significantly greater during emotional versus neutral encoding when neutral stimuli were encoded before emotional stimuli (N-E encoding order; **Fig. 2a**; $t_{21} = 3.19$, $P = 0.0044$; permutation test, $P = 0.0022$).

We next directly tested whether relative SCL during neutral encoding was enhanced when neutral stimuli were encountered after emotional stimuli, compared to before emotional stimuli (N-E encoding order) or after neutral stimuli (N-N encoding order). Relative SCL during neutral encoding was marginally enhanced for E-N versus N-E ($t_{42} = 1.62$, $P = 0.056$, one-tailed t-test; one-tailed permutation test, $P = 0.053$) and N-N encoding orders (comparison with second block, $t_{43} = 1.32$, $P = 0.097$, one-tailed t-test; one-tailed permutation test, $P = 0.098$; comparison with first block, $t_{43} = 1.50$, $P = 0.071$, one-tailed t-test; one-tailed permutation test, $P = 0.073$). However, relative SCL during emotional encoding did not differ between encoding orders ($t_{42} = 0.82$, $P = 0.42$; permutation test, $P = 0.41$). These results provide evidence for enhanced SCL when neutral stimuli were encountered after emotional stimuli, supporting the notion that a block of emotional stimuli can induce arousal that persists tens of minutes later, into and during an extended block of subsequent neutral encoding.

Memory performance

Next, we compared memory for neutral stimuli as a function of encoding order, to assess whether a long block of emotionally arousing stimuli is capable of prospectively enhancing memory for neutral stimuli encountered 9 to 33 min later. Memory for stimuli encountered during the encoding sessions was assessed in a surprise memory test 6 h later using a remember (R)–know (K) procedure[26]. We first assessed whether emotional and neutral memory differed based on encoding order in the fMRI study (reported below), as well as in separate groups of behavioral participants that did not undergo fMRI scanning (**Supplementary Fig. 1**). As expected, overall memory accuracy (proportion of total R and K hits minus false alarms) was higher for emotional versus neutral stimuli (main effect of emotion: $F_{1,42} = 16.0$, $P = 0.00025$). However, memory accuracy differed as a function of encoding order (**Fig. 2b** and **Supplementary Fig. 1**; encoding order by emotion interaction: $F_{1,42} = 10.6$, $P = 0.0022$). As in the SCL data reported above, no reliable difference in emotional versus neutral memory was found in the fMRI study when neutral stimuli were encountered after emotional stimuli (E-N encoding order; **Fig. 2b**; $t_{21} = 0.66$, $P = 0.52$), during which emotional arousal showed evidence of persistence into subsequent neutral encoding (via enhanced SCL; **Fig. 2a**). However, a robust enhancement in emotional memory was found for the opposite N-E encoding order (**Fig. 2b**; $t_{21} = 4.37$, $P = 0.00027$), in which no carry-over of emotional arousal could be present. Our main question concerned whether exposure to extended blocks of emotionally arousing stimuli could prospectively enhance memory for neutral stimuli encountered tens of minutes later. Memory for neutral stimuli was significantly greater when they were encoded after versus before emotional stimuli and outside of the context of emotional stimuli (**Fig. 2b** and **Supplementary Fig. 1**): corrected recognition rates of 62.7% were found in the E-N encoding order compared to 53.5% in the N-E encoding order ($t_{42} = 2.06$, $P = 0.045$) and 52.0% and 46.5% in the N-N encoding order (first and second blocks of N-N encoding order; first block, $t_{43} = 2.41$, $P = 0.02$; second block, $t_{43} = 3.31$, $P = 0.002$; permutation test, $P = 0.0022$). In contrast to neutral stimuli, memory accuracy for emotional stimuli did not differ between E-N and N-E encoding orders (**Fig. 2b**; $t_{42} = -0.78$, $P = 0.44$). Note that the same pattern of results was observed in separate behavioral groups, with enhanced levels of memory for neutral stimuli when they were encountered after emotional stimuli (E-N encoding order) relative to the other encoding orders (**Supplementary Fig. 1**). These results demonstrate that emotion can prospectively enhance memory for neutral stimuli encountered tens of minutes later.

Given prior work indicating that emotion specifically enhances the subjective sense of recollection (for example, see ref. 3) we asked whether the memory benefit for neutral stimuli encountered after emotional encoding blocks showed the same specificity. Comparing emotional and neutral memory based on encoding order in the fMRI study, a significant triple interaction was found between R versus K responses, emotional versus neutral stimuli and encoding order ($F_{1,42} = 14.0$, $P = 0.0006$). As predicted, R responses differed for emotional versus neutral stimuli based on encoding order (emotion by encoding order interaction: $F_{1,42} = 20.7$, $P = 4.5 \times 10^{-5}$; **Fig. 2b** and **Supplementary Fig. 1**). Consistent with a carry-over or persistence of emotional arousal into subsequent neutral encoding, levels of emotional and neutral R responses were similar when neutral stimuli were encountered after emotional stimuli in the fMRI study (E-N encoding order; **Fig. 2b**; $t_{21} = 0.49$, $P = 0.63$). However, in the opposite N-E encoding order, R responses were significantly higher for emotional compared to neutral stimuli (**Fig. 2b**; $t_{21} = 5.99$, $P = 6 \times 10^{-6}$). Crucially, similar to the combined memory measure reported above, R responses were significantly greater for neutral stimuli when they were encountered after versus before long blocks of emotional stimuli (**Fig. 2b** and **Supplementary Fig. 1**; $t_{42} = 2.16$, $P = 0.037$) and versus neutral stimuli in the N-N encoding order (**Fig. 2b** and **Supplementary Fig. 1**; first block, $t_{43} = 2.20$, $P = 0.03$; second block, $t_{43} = 2.87$, $P = 0.006$). This enhancement in neutral R responses was found for both the fMRI study (reported here) and the behavioral participants (**Supplementary Fig. 1**). However, in contrast to neutral stimuli, R responses for emotional stimuli did not reliably differ across E-N and N-E encoding orders ($t_{21} = -1.38$, $P = 0.17$; **Fig. 2b** and **Supplementary Fig. 1**). In contrast to recollection-based R responses, K responses did not significantly differ for neutral versus emotional stimuli across encoding orders (**Fig. 2b** and **Supplementary Fig. 1**; emotional stimuli, $t_{42} = 0.97$, $P = 0.34$; neutral stimuli, $t_{42} = -0.48$, $P = 0.64$; emotion by encoding order interaction, $P = 0.09$).

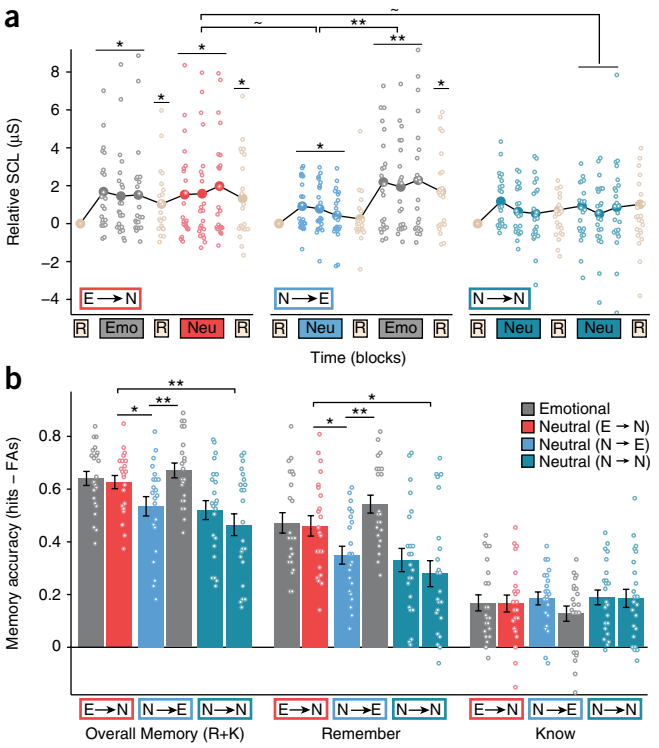

Figure 2 Skin conductance levels and behavioral results. (**a**) Mean galvanic SCLs are shown for each encoding order in the fMRI study ($n = 22$ for each N→E and E→N encoding order) and for the N→N encoding order for the behavioral study ($n = 23$), with each data point representing data from each subject. Larger (filled) data points indicate means across subjects. SCL is plotted relative to each subjects' baseline SCL during the first rest period. Each encoding block (emotional, emo; neutral, neu) has three measurements, corresponding to the mean relative SCL during three encoding scans (or 7.7 min epochs) for that block. Asterisks above data points denote significant differences in SCL from the first baseline rest scan (corrected for multiple comparisons in each encoding order). (**b**) Memory was assessed for stimuli seen during encoding using a remember (R)–know (K) procedure. Memory accuracy (hits minus false alarms) is shown for all hits (combined R and K responses, left bars), R responses (middle bars) and K responses (right bars) for the fMRI study (E→N and N→E encoding orders; $n = 22$ for each encoding order) and the N→N encoding order (behavioral study, $n = 23$). See **Supplementary Figure 1** for similar results for E→N and N→E encoding orders in the behavioral study. All error bars represent s.e.m. across subjects and individual dots represent data from each subject. ~$P = 0.053$, *$P < 0.05$, **$P < 0.005$.

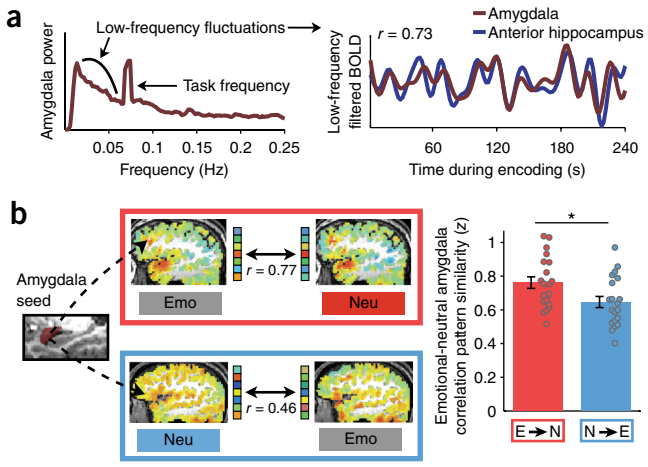

Figure 3 Low-frequency connectivity as a function of encoding order. (**a**) Low frequency connectivity analysis approach. Left: plot shows the power spectrum of amygdala BOLD encoding data for an example subject. A peak in the power spectrum can be seen for the task frequency, 0.0714 Hz. To examine LF connectivity, BOLD data were filtered below 0.06 Hz. Right: plot shows LF filtered time courses of amygdala and anterior hippocampus BOLD data from one subject during emotional encoding. (**b**) Similarity of multivoxel LF amygdala connectivity patterns between emotional and neutral encoding blocks as a function of encoding order. Example data from one subject from the E→N encoding order (top left) and one subject from the N→E encoding order (bottom left) are shown. An example amygdala ROI (used as the seed region) is shown in the left inset. Images show sagittal slices containing LF connectivity patterns with the amygdala. Vectors next to each image depict the analysis approach; the similarity of multivoxel connectivity patterns in each subject was computed between emotional and neutral encoding blocks. Right plot shows group data ($n = 20$ subjects in each encoding order) for the similarity of multivoxel LF amygdala correlation patterns between emotional and neutral encoding. Individual data points represent the similarity (correlation) for each subject. Error bars, s.e.m. $^*P < 0.05$

The above results indicate that subjective recollection of neutral stimuli was relatively greater when they were encountered 9 to 33 min after blocks of emotional stimuli, mirroring enhanced SCL found during neutral encoding. In contrast to differences in neutral memory and SCL during neutral encoding, levels of subjective recollection and SCL during emotional encoding did not consistently differ as a function of encoding order. This pattern of results was observed in two independent data sets (during fMRI scanning and in a separate behavioral study). Together, these findings are consistent with the notion that long-lasting blocks of emotional arousal can persist on an extended timescale and prospectively enhance the later recollection of neutral stimuli encountered at least 9 to 33 min later.

Low-frequency connectivity carry-over effects

Next, we sought to examine evidence for the hypothesis that exposure to long blocks of emotional stimuli created a specific, arousal-related brain state that could bias subsequent BOLD activity patterns during neutral encoding. Given the extended time period of the encoding sessions (~23 min) as well as the temporal gap between emotional and neutral encoding (~9 min), the carry-over of such an emotional brain state may have occurred at a relatively slow timescale (for example, on the order of minutes). Thus, we reasoned that this brain state might be manifest in low-frequency (LF) fluctuations in the BOLD signal, i.e., BOLD signal changes that were slower than trial-by-trial evoked activity. Such slow LF fluctuations have previously been shown to track different behavioral states[27–29]. To test this hypothesis, we examined whether patterns of LF connectivity that characterized emotional encoding blocks carried over or showed evidence of reinstatement during subsequent neutral encoding blocks (**Fig. 3a**).

Given the importance of the amygdala in mediating the influence of emotion on memory and cognition[30–32], we asked whether large-scale patterns of LF amygdala connectivity representative of an emotional brain state showed evidence of reinstatement during neutral encoding blocks. Such reinstatement would result in greater similarity between emotional and neutral encoding LF amygdala connectivity patterns when neutral stimuli are encountered after versus before emotional stimuli (E-N versus N-E encoding order). To this end, we computed LF connectivity across cortical and subcortical voxels using the amygdala as a seed region. Connectivity was computed separately for each encoding scan in each subject, resulting in distinct multivariate images or vectors representing LF amygdala connectivity patterns during emotional and neutral encoding (**Fig. 3b**). We then asked whether these global patterns of LF amygdala connectivity were more similar (i.e., exhibited higher levels of correlation) between emotional and neutral encoding blocks for the E-N encoding order (in which a carry-over of emotional arousal into subsequent neutral encoding was found) versus the N-E encoding order (in which no carry-over of emotional arousal should be present). LF amygdala connectivity patterns were significantly more correlated (i.e., more similar) during emotional and neutral encoding for the E-N versus the N-E encoding order (**Fig. 3b**; $t_{38} = 2.42$, $P = 0.02$). This result indicates that large-scale, multivoxel patterns of LF amygdala connectivity representative of emotional encoding can carry over and manifest tens of minutes later when unrelated neutral stimuli are later encountered.

At a more fine-grained level, we next examined specific interactions between the amygdala and anterior hippocampus, which are thought to underlie the memory benefit for emotional stimuli[9,12,32]. We targeted the anterior hippocampus since it is expected to show the strongest interactions with the amygdala, based on anatomy[33–36] and prior reports of functional correlations between amygdala and anterior hippocampal BOLD activity[37] and since anterior hippocampal activation is consistently predictive of successful emotional encoding[10]. As shown in **Figure 4**, levels of LF amygdala–anterior hippocampal connectivity also showed evidence of being reinstated or carrying over from emotional to neutral encoding blocks. First, connectivity during emotional versus neutral encoding differed as a function of encoding order (emotion by encoding order interaction, $F_{1,38} = 4.57$, $P = 0.039$). Mirroring our behavioral and SCL findings, similar levels of LF amygdala–anterior hippocampal connectivity were found during emotional and neutral encoding when neutral stimuli were encountered tens of minutes after emotional stimuli (E-N encoding order, $t_{19} = -0.82$, $P = 0.42$), but a significant enhancement in LF connectivity was found during emotional versus neutral encoding for the opposite encoding order (N-E encoding order, $t_{19} = 2.12$, $P = 0.048$). Moreover, LF amygdala–anterior hippocampal connectivity was significantly greater during neutral encoding blocks when neutral stimuli were encountered after versus before emotional stimuli (E-N versus N-E encoding orders; $t_{38} = 2.38$, $P = 0.023$). Yet no reliable difference in connectivity was found during emotional encoding blocks between encoding orders ($t_{38} = 0.97$, $P = 0.34$). This pattern of results suggests that LF amygdala–anterior hippocampal connectivity, like overall levels of skin conductance, showed evidence of carrying over from temporally extended blocks of emotional encoding into subsequent neutral encoding.

Lastly, we found similar evidence for a carry-over effect in levels of LF connectivity from emotional into neutral encoding within the ventral

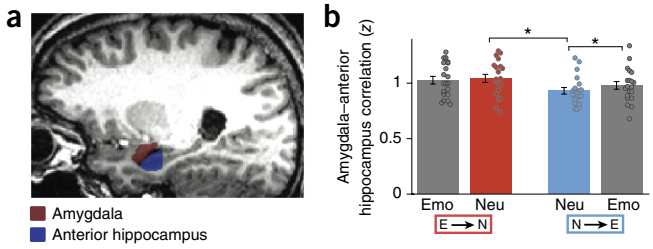

Figure 4 Low-frequency amygdala–anterior hippocampal connectivity as a function of encoding order. (**a**) Anatomically defined amygdala (red) and anterior hippocampus (blue) ROIs are shown for an example subject. (**b**) LF amygdala–anterior hippocampus connectivity as a function of encoding order across $n = 20$ subjects per encoding order. LF amygdala–anterior hippocampal connectivity is greater during neutral encoding for the E→N vs. N→E encoding order (red vs. blue bars). Individual data points represent connectivity for each subject. Error bars, s.e.m. $*P < 0.05$.

anterior insula (vAI) network (**Supplementary Fig. 2**). We additionally probed connectivity in this network as emotion-related activity has consistently been shown in the vAI (refs. 38–41), and greater levels of vAI network connectivity have been related to heightened arousal ratings of the emotional stimuli used in this experiment[38].

Taken together, these results provide evidence that emotional brain states, measured by correlations in LF BOLD fluctuations present during emotional encoding, can carry-over and become reinstated tens of minutes later when participants encountered unrelated, neutral information. Multiple signatures of emotion-related LF connectivity showed evidence of carrying over from emotional encoding into subsequent neutral encoding and were present in both local networks (for example, amygdala–anterior hippocampal connectivity) and in global pattern similarity across the brain (in multivoxel amygdala connectivity patterns).

Event-related subsequent memory carry-over effects

In addition to asking whether correlations in LF fluctuations in the BOLD signal showed evidence of carry-over from blocks of emotional to subsequent neutral encoding, we also examined whether emotional encoding prospectively influenced transient, stimulus-evoked or event-related BOLD activity during subsequent exposure to neutral stimuli. Given our data suggesting that emotion prospectively enhanced behavioral signatures of memory for subsequently encountered neutral stimuli, we asked whether the neural structures supporting memory formation for neutral stimuli were modulated on a trial-by-trial basis by the prior induction of an emotional state. Specifically, we compared stimulus-evoked activity related to subsequent recollection-based memory (i.e., greater activation for stimuli later labeled as R versus K) during emotional and neutral encoding blocks based on the order of encoding blocks, using both univariate and multivariate approaches. As a function of encoding order, we compared (i) the similarity of global, multivoxel patterns supporting later recollection between emotional and neutral encoding and (ii) univariate activation predicting subsequent recollection between emotional and neutral encoding.

First, we asked whether global, multivoxel patterns that characterize recollection-based emotional memory formation were reinstated and similarly supported the later recollection of neutral stimuli encountered after emotional stimuli. Such a reinstatement would result in greater similarity between multivoxel patterns supporting later recollection during emotional and neutral encoding when neutral stimuli are encountered after versus before emotional stimuli. To test this prediction, we measured activation patterns related to successful recollection-based memory formation (differences in activation estimates for trials later labeled as R versus K) separately for emotional and neutral stimuli in each participant (**Fig. 5a**). This resulted in multivoxel activity patterns across cortical and subcortical voxels that were characteristic of recollection-based memory formation, which we then compared between emotional and neutral stimuli as a function of encoding order. Enhanced levels of similarity, or correlation, were found between multivoxel patterns supporting recollection-based memory for emotional and neutral stimuli when neutral stimuli were encountered after versus before emotional stimuli (in the E-N versus N-E encoding order; **Fig. 5b**; $t_{40} = 3.00$, $P = 0.0046$). A nonparametric permutation test was performed to ensure that this difference in multivoxel pattern similarity was not driven by differences in R versus K bin sizes between encoding orders ($P = 0.00083$; **Supplementary Fig. 3**). This result demonstrates that spatially broad activation patterns related to recollection-based memory formation were more similar between emotional and neutral stimuli when neutral stimuli were encountered after versus before emotional encoding blocks. This result suggests that patterns of brain activity supporting successful recollection-based memory formation of neutral

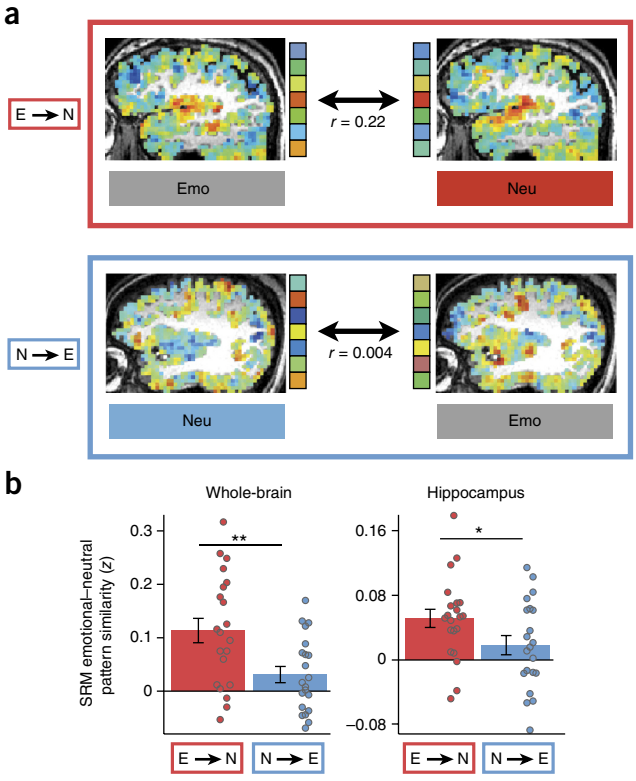

Figure 5 Similarity of multivoxel subsequent recollection-based memory differences between emotional and neutral encoding as a function of encoding order. (**a**) Example data are shown from one subject of the E→N encoding order (top) and one subject of the N→E encoding order (bottom). Sagittal slices depict subsequent recollection patterns (R – K activity estimates) across cortical and subcortical voxels. (**b**) Group data for the similarity of multivoxel encoding patterns (activity patterns supporting subsequent recollection memory (SRM)) between emotional and neutral encoding based on encoding order ($n = 21$ subjects per encoding order). Greater similarity of patterns supporting subsequent emotional and neutral recollection was found for the E→N vs. N→E encoding orders for both global patterns across cortical and subcortical voxels (whole-brain, left bar plot) and within the hippocampus (right bar plot). Individual data points represent similarity (correlation) for each subject. Error bars, s.e.m. $*P < 0.05$, $**P < 0.005$.

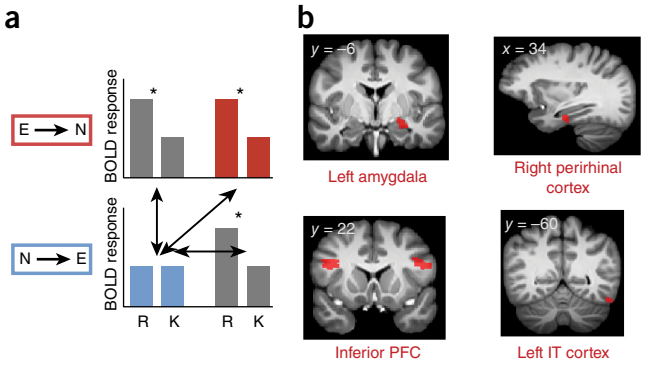

Figure 6 Subsequent recollection-based memory carry-over effects. (**a**) Schematic of response patterns required for a subsequent recollection carry-over effect, or reinstatement of emotional subsequent recollection effects during later neutral encoding (six-way conjunction analysis). Regions showed significant subsequent recollection effects or significantly greater activity estimates for R vs. K trials during emotional encoding (both encoding orders) and neutral encoding when neutral stimuli were encountered after emotional stimuli (E→N encoding order, red bars). Regions also showed significantly greater R – K differences between the three conditions with arrows. Individual contrasts were thresholded at $^{*}P < 0.05$. (**b**) Brain regions showing emotional subsequent recollection effects that carry over during later neutral encoding, isolated from the conjunction analysis shown in **a**. Six regions emerged from this analysis (shown in red): left amygdala ($P = 0.013$), right perirhinal cortex ($P = 0.004$), left inferior temporal (IT) cortex ($P = 0.0088$) and three inferior prefrontal cortex (PFC) regions (two shown; left $P = 0.0006$, right $P = 0$). Results are FWE-corrected at $P < 0.05$, and the resulting map was smoothed for visualization. Statistical map is displayed on the group template anatomical image and can be found in MNI space on neurovault.org (http://neurovault.org/collections/JOYXPMRX/).

stimuli were prospectively biased by the presence of prior emotional stimuli tens of minutes earlier, with activation patterns supporting later neutral stimulus recollection being more similar to emotional encoding patterns when neutral stimuli were preceded by emotional stimuli.

Next, we examined whether similar effects were found within the hippocampus, as emotion is thought to enhance recollection-based memory formation via hippocampal mechanisms[1,7–9]. Hippocampal activity patterns showed evidence of carry-over effects or reinstatement of activity patterns representative of emotional encoding during subsequent neutral encoding blocks, both in multivoxel patterns predictive of later recollection (**Fig. 5b**; $t_{40} = 2.02$, $P = 0.049$) and in the anterior versus posterior localization of hippocampal voxels contributing to subsequent recollection[36,37] (**Supplementary Fig. 4** and Online Methods). These findings indicate that patterns of hippocampal activity supporting recollection-based memory formation of neutral stimuli were also biased by the presence of prior emotion, specifically by enhancing the contribution of the anterior hippocampus to memory formation.

Lastly, we asked whether individual brain regions supporting successful recollection-based memory formation were more similar between emotional and neutral stimuli when neutral stimuli were encountered after emotional stimuli than when neutral stimuli were encountered before emotional stimuli. To this end, we identified regions that showed significant subsequent recollection effects (R > K BOLD responses) for emotional stimuli as well as greater R > K activity when neutral stimuli were encountered after versus before emotional stimuli (E-N versus N-E encoding order). This carry-over recollection-based memory effect was operationalized as brain regions that showed a conjunction between six contrasts, depicted in **Figure 6a** (Online Methods; note that this analysis also controls for differences in R and K bin sizes based on encoding order). Six regions emerged from this whole brain conjunction analysis (**Fig. 6b**; family-wise error (FWE)-corrected, $P < 0.05$): the left amygdala, the right perirhinal cortex, the right posterior inferior temporal gyrus and three regions in bilateral inferior prefrontal cortex. Similar regions have previously been shown to predict successful memory formation for emotional versus neutral stimuli[10], and a formal decoding analysis performed on the uncorrected conjunction map revealed the highest similarity with terms "emotional stimuli," "affect" and "salient" (using neurosynth.org[42], http://neurosynth.org/decode/?neurovault=JOYXPMRX-26012). Here we found that regions associated with emotional processing and memory formation were predictive of subsequent neutral memory when neutral stimuli were encountered after, but not before, extended blocks of emotional stimuli. The engagement of these additional brain regions subserving neutral memory formation after emotional arousal supports the idea that emotion- and encoding-related mechanisms were reinstated and thus impacted memory formation 9 to 33 min later.

DISCUSSION

It is widely acknowledged that emotion can modulate what and how we remember. Although prior work has shown that emotion can retroactively influence memory for preceding neutral experiences[5,6,14,15], less is known about how emotional experiences can linger and prospectively enhance memory formation for neutral information encountered many minutes later. We found that exposure to extended blocks of emotion-evoking stimuli induced a lasting emotional state that enhanced participants' later recollection of neutral images encountered 9 to 33 min later, suggesting that the impact of emotion carried over into and biased subsequent stimulus processing and encoding. We directly queried the persistence or carry-over of an emotional brain state into subsequent neutral encoding blocks by analyzing global multivoxel patterns across the brain and activity in emotion- and arousal-related brain regions. First, we found that LF fluctuations of the BOLD signal previously shown to track behavioral or neural states[27–29] carried over from emotional encoding into subsequent neutral encoding blocks. These included global multi voxel amygdala–whole-brain connectivity patterns, as well as correlated activity in targeted circuits (amygdala–anterior hippocampus and ventral anterior insula network). Second, we found that brain regions exhibiting successful encoding effects for neutral stimuli were also modulated by preceding emotional experiences. Thus, both large-scale patterns and overall levels of BOLD activity supporting recollection-based memory formation were reinstated during neutral encoding after emotional encoding blocks. Together, these findings provide evidence that the induction of a relatively lasting emotional state was associated with brain states that could later be reinstated, biasing the way future neutral events were encoded and potentially imbuing neutral experiences with emotional properties that enhance their recollection.

Previous studies of emotion's influence on memory have demonstrated that the neurohormonal changes underlying physiological arousal are critical for mediating emotion-enhanced memory[7,8], specifically subjective recollection[43], although these might not be the only contributing factors. In the present study, we induced an emotional state through the use of stimuli previously rated as subjectively negative and arousing (compared to stimuli rated as neutral). We then used SCL, a noninvasive assessment of autonomic arousal, as our primary measure of emotion[25]. Notably, increased levels of emotional arousal induced by the exposure to emotional stimuli

persisted in time and likely influenced the encoding, and perhaps consolidation, of subsequently encountered neutral stimuli, resulting in increased levels of subjective recollection when the neutral memory was assessed 6 h later. This enhancement in recollection of neutral stimuli was observed across two independent data sets. Crucially, mirroring our behavioral findings, SCL was distinctly higher when neutral stimuli were encountered 9 to 33 min after emotional stimuli versus before emotional stimuli as well as after neutral stimuli. For these reasons, together with prior evidence linking emotion-related neurohormonal changes with subjective recollection[43], we believe the subsequent influence of emotion on memory for future neutral events may be mediated by noradrenergic activation triggered by the emotional images.

It is important to note that our finding of enhanced memory for neutral stimuli encoded after emotional stimuli is similar to, but distinct from, prior demonstrations that emotional arousal or adrenergic agonists administered after the encoding of neutral stimuli retroactively enhances later memory for neutral information[5,16,18,44,45]. Post-learning arousal is proposed to heighten memory consolidation of preceding neutral information[5,16,45]. Here we show that pre-encoding emotional arousal that persists during exposure to neutral stimuli can prospectively influence both their initial encoding and their recollection 6 h later. Notably, BOLD activity during neutral encoding tens of minutes after emotional encoding in many respects resembled BOLD activity when participants viewed and processed emotional stimuli. Correspondingly, neutral stimuli encoded during heightened arousal showed the same mnemonic profile as emotional stimuli (enhanced recollection) relative to neutral stimuli encoded before and outside the context of emotional arousal. Taken together, these findings paint a picture of emotion as capable of biasing neutral memory via multiple mechanisms: retroactively enhancing the consolidation of previously encoded information, as well as prospectively biasing future brain states and mechanisms underlying the encoding of new experiences into memory.

Our findings that LF connectivity (amygdala–hippocampus and ventral anterior insula network correlations), subsequent recollection effects in individual brain regions (amygdala, perirhinal cortex, inferior prefrontal cortex, inferior temporal cortex) and patterns of hippocampal recollection effects characteristic of emotional encoding carried over into subsequent neutral encoding blocks are consistent with prior work showing that emotion is related to BOLD activity and connectivity in similar regions[10,11,37,38,41,46,47] (assessed using a reverse analysis in neurosynth.org[42]). Notably, however, the present data extend these findings in several ways. First, consistent with a carry-over hypothesis, brain regions related to emotional processing and memory formation were not only engaged when participants viewed emotional stimuli but also during the subsequent encoding of neutral stimuli. This result suggests that emotion can bias BOLD activity over an extended time period (at least 9 to 33 min later). Second, differences in LF connectivity during emotional and neutral encoding in the N-E encoding order suggested that emotion modulated not only trial-by-trial evoked brain activity but also lower-frequency background BOLD activity. This modulation of background BOLD activity by emotion complements previous findings showing that background BOLD activity reflects specific types of information processing, such as distinct memory states[29] and the processing of distinct stimulus classes[27,28].

Although the present study found evidence that an extended emotional experience could bias future brain states and memory encoding, we note that it is unclear which specific aspects of our experimental design were critical for this effect to emerge. In our design, encoding blocks lasted for 23 min; thus, it is unclear how much time is necessary to induce a state of emotional arousal that will persist and bias future behavior. Moreover, it is also unclear how long emotional arousal may potentially last and whether this is related to the duration of the initial arousal induction. It is also noteworthy that participants performed the same task when they encountered emotional and neutral stimuli in our study (rating of visual complexity), so it is unknown whether this similarity in task context was necessary for eliciting a carry-over of an emotional state into later neutral encoding. Lastly, it is also unknown how expectations or explicit strategies developed by participants may have facilitated the reinstatement of emotional arousal or brain states during neutral encoding. Future work is needed to understand how these different factors contributed to the present findings.

The present results add to our understanding of the many ways emotion can influence memory for unrelated neutral events. When examining memories for events themselves, there is evidence that more neutral details of an emotional event may be less well remembered[48,49], suggesting a trade-off in memory[49,50]. However, the influence of emotion on memory can also extend over time, between events. Not only can emotional arousal following a neutral event influence the storage of that event but, to the extent that arousal persists, it can also influence memory for future neutral events that are temporally and semantically distinct. Our results suggest that this prospective memory enhancement may be due to a carry-over of the brain states that underlie arousal and its influence on memory.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

A.T., U.R., E.A.P. and L.D. designed the experiment and wrote the paper. A.T. and U.R. collected and analyzed the data.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Subjects. *fMRI Study.* Fifty right-handed native English speakers with normal or corrected-to-normal vision participated in the study. Six subjects were excluded, two due to a high rate of spike artifacts in the fMRI data and four due to very poor memory performance (miss or false alarm rates > two s.d. above the mean of all subjects). The average age of the remaining 44 subjects was 24.1 years (range: 19–34) and included 23 male and 21 female participants. Informed consent was obtained from all participants in a manner approved by the institutional review board at New York University. No a priori sample size calculations were performed, but the sample size was chosen based on common practices in the field[43]. Subjects were assigned to groups (E-N and N-E encoding orders) in alternating fashion as the data was collected. Age and gender were approximately matched across encoding orders (E-N: 11 female, 11 male, mean age = 24.9; N-E: 10 female, 12 male, mean age = 23.4).

Behavioral groups. Eighty-two participants with normal or corrected-to-normal vision participated in the behavioral studies. The E-N and N-E encoding order groups were collected simultaneously, with participants assigned to groups in alternating fashion as the data was collected (n = 55 participants total). Most of the participants were recruited at New York University (31/55, 16 in E-N encoding order, 15 in N-E encoding order) while the rest of the participants were recruited at the University of Geneva (24/55 participants, 12 in each encoding order). The N-N encoding order was run as a separate study and consisted of 27 participants, all recruited at New York University. In total, 11 participants were excluded: one who did not follow the instructions (N-N group) and ten due to very poor memory performance (miss or false alarm rates > two s.d. above the mean of all subjects; three N-N participants, four E-N participants and three N-E participants). Of the remaining 71 participants, 46 were female (mean age: 24.5 years, range: 18–33), resulting in a total of 23 participants for the N-N encoding order and 24 participants for each of the N-E and E-N encoding orders. Age and gender were approximately matched across the N-N, E-N and N-E encoding orders (N-N: 18 female, mean age = 24.96; E-N: 13 female, mean age = 24; N-E: 15 female, mean age = 24.5). Informed consent was obtained from all participants in a manner approved by the institutional review boards at New York University and the University of Geneva.

Procedure. *fMRI study (E-N and N-E encoding orders).* Before entering the scanner, subjects were informed about the scanning procedures and performed a brief practice session of the image complexity task to be performed during the encoding sessions as well as a one-back task used for localizer scans. Prior to scanning, electrodes were placed on the index and middle fingers of the subject's right hand to measure galvanic SCL.

During the scanning session, subjects performed incidental encoding blocks (emotional and neutral encoding) interleaved with rest scans (**Fig. 1a**). The order of the encoding blocks was counterbalanced across subjects, such that half of the subjects performed emotional followed by neutral encoding (referred to as the E-N encoding order) and the other half of the subjects performed neutral before emotional encoding (referred to as the N-E encoding order). Additional scans were collected after the last rest scan but were not used in the present analyses (localizer scans in which subjects viewed scenes, objects and other stimulus categories). A high-resolution anatomical scan was collected after all functional scans. After the scanning session, subjects returned to the lab to perform a surprise memory test for stimuli encountered during emotional and neutral encoding, approximately 6 h after the last encoding session. Subjects were not informed before the scanning session that their memory would be tested for stimuli encountered during the encoding sessions.

By necessity, data collection and analysis were not performed blind to the conditions of the experiments. However, subjective outcome assessments were not performed.

Behavioral groups (N-N, N-E and E-N encoding orders). The same experimental procedures used in the fMRI study were used in the behavioral groups, except that all procedures took place outside of the MRI scanner and participants in the N-N encoding order incidentally encoded two blocks of neutral stimuli instead of blocks of emotional and neutral stimuli. Subjects were exposed to an auditory stimulus of white noise during encoding and during rest periods in between encoding blocks to resemble the constant noise during fMRI scanning. A surprise memory test for neutral stimuli was administered with the same delay (6 h) as the fMRI study.

Encoding sessions. All fMRI participants and E-N and N-E behavioral groups incidentally encoded both emotional and neutral stimuli in separate blocks (emotional and neutral encoding). In the N-N behavioral group, subjects incidentally encoded two separate blocks of neutral stimuli. During encoding, subjects rated the visual complexity of complex scenes. Each block consisted of three 7.7-min scans or epochs and contained a total of 99 trials (33 trials in each scan). The order of stimulus presentation was randomized for each subject. Each trial lasted for 14 s and included a 1-s fixation cue, presentation of a complex scene for 2 s, a response window of 1 s, followed by the performance of a baseline 'arrows' task for 10 s in between trials[51]. Subjects were instructed to view each image for as long as it remained on the screen and examine the visual complexity of each image. The response window contained the words "Complexity?" and "High Medium Low." Subjects pressed their ring, middle or index finger of their left hand to indicate complexity ratings of high, medium or low, respectively. During the arrows task, a series of arrows were presented pointing either left, up, or right on the screen. Subjects were instructed to push their ring finger when the arrow pointed to the left, their middle finger when the arrow pointed up and their index finger when the arrow pointed to the right.

Memory test. Approximately 6 h after the end of the last encoding session, all subjects completed two separate memory tests (one for each encoding session) outside of the scanner. The order of the memory tests matched the order of their presentation during encoding, to roughly equate the study–test intervals across the two encoding sessions. Each trial consisted of the presentation of a complex scene, and subjects performed a remember (R), know (K) or new (N) judgment.

Subjects were trained on R, K and N decisions before the start of the memory test[26]. After reading detailed instructions, participants explained the meaning of R and K judgments in their own words. During the practice trials, subjects indicated why they judged a scene as remembered or known out loud to the experimenter. The recognition test was administered once the participants had correctly understood the instructions, i.e., they judged a scene as remembered when it brought back to mind a specific detail from the episodic context in which the scene had been experienced, such as a sensory detail, a thought or a feeling.

Memory tests were self-paced and contained 198 trials each. Half of the stimuli (99) in the memory test were novel distractor scenes and half were those presented during the encoding session. For the fMRI study and the E-N and N-E behavioral groups, the stimulus sets presented at encoding and test were counterbalanced across encoding orders: for half of the subjects stimulus set 1 was used during the encoding session and stimulus set 2 was used as distractors in the memory test; the other half of the subjects viewed stimulus set 2 during encoding set 1 as distractors in the memory test. For the N-N behavioral group, four neutral stimulus sets were created and counterbalanced across subjects with respect to the first versus second stimulus set during encoding and with respect to serving as encoding or distractor sets during memory testing. Stimulus sets for both the fMRI and behavioral studies were matched for image complexity and the presence of people, animals, inanimate objects and scenes.

Stimuli. The complex scenes used in all encoding sessions spanned 465 × 620 pixels.

fMRI study and E-N and N-E behavioral groups. The complex scenes used in the encoding sessions were drawn from the International Affective Picture Set (IAPS)[52] as well as a few in-house stimuli (**Supplementary Table 1**). Stimuli were classified as emotional or neutral based on the normative ratings provided for emotional arousal (emotional scenes: M = 5.65, s.d. = 0.82; neutral scenes: M = 3.92, s.d. = 0.96) and valence (emotional scenes: M = 2.83, s.d. = 0.92; neutral scenes: M = 5.94, s.d. = 1.00) assessed with the Self-Assessment Manikin (SAM) scale (1 = calm, 9 = excited; 1 = unhappy, 9 = happy)[52]. Stimuli used for emotional and neutral encoding as well as for the encoding versus distractor sets in the memory test were matched for image complexity and the presence of people, animals, inanimate objects and scenes.

N-N behavioral group. In addition to the 198 neutral stimuli used in the fMRI study, another 198 neutral complex scenes were added to the stimulus set to construct two blocks of stimuli for neutral encoding for the N-N encoding order. Stimuli were taken from the IAPS[52], the Necki Affective Picture System[53] and our own images. As in the fMRI study, stimuli were selected based on normative ratings provided for emotional arousal (M = 3.98, s.d. = 0.79) and valence (M = 5.99,

s.d. = 0.95) assessed with the SAM scale described above. Note that arousal and valence ratings for neutral stimuli were well-matched across the E-N/N-E encoding orders and the N-N behavioral group. The stimuli were classified into four sets of 99 neutral photos, which were matched for image complexity, the presence of people/animals, inanimate objects and scenes and which did not differ between emotional arousal and valence (all *P*-values > 0.85).

Rest scans. Each rest scan lasted for 9 min. Subjects were instructed to close their eyes and simply rest and think about anything that they wanted but to try to remain awake[54,55]. We used only the fMRI data from the first rest scan in the current analyses, to isolate regions in the ventral anterior insula (vAI) network[38].

Behavioral analyses. Memory for emotional and neutral stimuli was assessed by examining proportions of hits and false alarms for each encoding order (E-N, N-E and N-N), collapsed across R and K responses (overall memory accuracy), as well as separately for R and K responses. To analyze memory as a function of emotion and encoding order (fMRI study and behavioral groups), mixed-effects ANOVAs were performed on corrected memory accuracy rates (proportion of hits minus false alarms) with a within-subjects factor of emotion (emotional and neutral stimuli) and a between-subjects factor of encoding order (E-N and N-E encoding orders). Separate ANOVAs were performed for all hits and false alarms (combined R and K responses) and for R and K responses separately. Follow-up *t*-tests were performed to investigate differences in memory between emotional and neutral stimuli within encoding orders (paired *t*-tests) as well as between encoding orders (unpaired *t*-tests). To assess whether neutral memory was enhanced when they were encoded after emotional versus after neutral stimuli (E-N versus N-N encoding orders), we performed planned comparisons between corrected neutral memory accuracy (combined R and K responses and R responses alone) in the E-N encoding order versus both blocks in the N-N encoding order (behavioral study) using an unpaired *t*-test. All memory data were determined to be normally distributed (using a Lilliefors test; see "Statistics" below) with the exception of overall memory accuracy (combined R and K responses) for the second block of neutral stimuli in the N-N encoding order. We thus additionally performed nonparametric permutation tests when statistically evaluating this data (when comparing neutral memory in the N-N encoding order to the E-N encoding order, for both the fMRI and behavioral studies). To do so, we computed the true difference in memory across each of these comparisons. We then derived a null distribution of the difference in memory across these conditions by performing 10,000 null simulations in which the group labels (E-N or N-N encoding order labels) were randomly shuffled and the difference in memory for each null permutation of the data was computed. The true difference in memory was compared to this null distribution to estimate a *P*-value for these comparisons (see "Results" and **Supplementary Fig. 1**).

Skin conductance level analyses. In order to examine differential levels of arousal based on encoding order, mean skin conductance levels (SCL) were analyzed, which have been related to sympathetic nervous system activation (see ref. 25 for a review). Specifically, mean SCL was computed in each subject for each rest and encoding scan (fMRI study) or each rest and encoding block (N-N behavioral study). Relative SCL changes were then used as our dependent measure of interest, which were computed for each encoding and rest block by subtracting mean SCL measured during the pre-encoding baseline rest period from mean SCL during each encoding and rest block (for both the fMRI and N-N behavioral group). This procedure of computing SCL changes relative to a baseline period controlled for individual differences in SCL and allowed us to compare SCL across different groups of subjects (E-N encoding order versus other encoding orders). To compare relative SCL during emotional versus neutral encoding as a function of encoding order (in the fMRI study), a mixed-effects two-way ANOVA was performed with a within-subjects factor of emotion (emotional and neutral encoding blocks) and a between-subjects factor of encoding order (E-N/N-E). Follow-up *t*-tests were used to examine elevated SCL relative to baseline rest before encoding (*t*-test in relative SCL versus zero, corrected for multiple comparisons across encoding and rest blocks) and differences in relative SCL during neutral encoding in the E-N versus N-E and N-N encoding orders (unpaired *t*-tests). One-tailed *t*-tests were used to assess differences in relative SCL during neutral encoding as a function of encoding order, as we specifically hypothesized that the presence of prior emotional encoding would enhance SCL during neutral encoding compared to the opposite encoding order (i.e., neutral encoding SCL would be higher for the E-N versus N-E and N-N encoding orders). However, relative SCL data distributions were found to be not normally distributed for several blocks of interest. We thus also performed nonparametric permutation tests for all statistical tests involving SCL. The same methods were applied as in the permutation tests as described for the memory analyses (see "Behavioral analyses"). Null simulations were performed in which the data labels were randomly permuted and the comparison of interest was computed. The true difference of interest was then compared to the null distribution generated for that particular test. To assess the significance of the encoding order by emotion interaction observed in SCL, we computed the effect of interest as the difference in emotional versus neutral encoding SCL across encoding orders (E-N emotional encoding SCL – E-N neutral encoding SCL – (N-E emotional encoding SCL – N-E neutral encoding SCL)) and compared this true difference to a null distribution derived from permuting E-N and N-E encoding order labels for each participant.

MRI data acquisition. Scanning was performed using a 3T Siemens Allegra MRI system with a whole-head coil. Functional (BOLD) data were collected using a gradient-echo planar pulse (EPI) sequence (repetition time (TR) = 2 s, echo time = 30 ms; field of view = 192 mm; 31 slices oriented perpendicular to the long axis of the hippocampus; 3 × 3 × 3 mm voxel size; 0.6 mm interslice gap; flip angle = 80°). Functional scans contained 231 or 270 volumes or TRs for the encoding and rest scans, respectively, after discarding the first four volumes to allow for T1 equilibration. High-resolution T1-weighted (magnetization-prepared rapid-acquisition gradient echo) images were acquired after the last functional scan.

MRI preprocessing. The imaging data were preprocessed using SPM5 (Wellcome Department of Cognitive Neuroscience, University College London, London, UK). The BOLD data were first corrected for differences in slice timing acquisition followed by motion correction across all runs and the removal of low frequency trends (< 0.009 Hz) from each scan. Each subject's functional data were then co-registered to their own T1-weighted anatomical image. For the definition and analysis of anatomically defined regions of interest (ROIs; for example, amygdala) as well as the analysis of whole-brain activity and connectivity patterns, the functional data were analyzed in a subject-specific space and spatially smoothed with a 6-mm full-width at half-maximum (FWHM) isotropic Gaussian kernel. For group-level analyses, a group-level anatomical template was created using advanced normalization tools (ANTs)[56] based on the T1-weighted anatomical images of all 44 subjects. Statistical maps from native subject spaces were then transformed into this template space for group analyses.

Noise corrections were applied to the BOLD data from all encoding and rest scans based on methods from Behzadi *et al.* (aCompCor)[57]. A nuisance ROI was generated from white matter (WM) and cerebrospinal fluid (CSF) probabilistic maps (see "Anatomical masks," below). The WM and CSF maps were converted to functional resolution and thresholded at probability values of 0.98 or 0.99 for the WM maps and at 0.97, 0.98 or 0.99 for the CSF maps. After thresholding, the WM mask was eroded by two voxels to avoid contaminating the WM signal with GM signal[57]. We used an iterative procedure for defining the probability threshold for each subject, such that the highest threshold was used with the stipulation that the resulting CSF or eroded WM mask contained a minimum of 10 voxels at functional resolution. On each iteration (for a given probability threshold) we additionally excluded voxels from the nuisance ROI if their time-course during any one encoding scan was modestly correlated ($P < 0.2$) with a model of task activity[57] (with task activity modeled as a 4-s boxcar corresponding to fixation, stimulus presentation and the response window, convolved with the canonical hemodynamic response function, HRF). The resulting voxels from the thresholded WM and CSF maps were then combined to form one nuisance ROI for each subject.

In order to extract dominant signals accounting for substantial variance in the BOLD signal from the nuisance ROI, principal components analysis (PCA) was performed on the BOLD data from the nuisance ROI. PCA was performed on the *z*-scored BOLD data (note that LF trends were already removed from the data), separately for each encoding scan and rest scan. We then performed simulations to determine the number of principal components (PCs) to extract from the nuisance ROI data for each scan. A null distribution of the expected eigenvalues was generated separately for the encoding and rest data for each subject by performing PCA on normally distributed data of equal rank to the encoding and rest

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nuisance ROI data[57]. PCs from the true nuisance ROI data were then selected for a given encoding or rest scan if their associated eigenvalues exceeded the 99% confidence interval of the eigenvalues from the simulations. However, if different numbers of PCs were chosen across any of the encoding and rest scans for each subject, then the minimum number of PCs selected across all scans was used for all scans. This procedure ensured that removal of any nuisance signals was consistent across all encoding and rest scans, to avoid differentially biasing the data in any particular scan. After selecting the number of PCs for the encoding and rest data for the nuisance ROI, the associated temporal projection of each selected PC was removed from the whole-brain BOLD data, in conjunction with the six motion parameters estimated during processing, using linear regression in a voxelwise fashion. TRs surrounding time periods of sudden motion (see below for definition) were not included in the estimation of beta coefficients in this regression[58,59].

Additional measures were taken to address the influence of motion on BOLD data and connectivity estimates, which was not completely addressed by the nuisance regression approach taken above[60,61]. All encoding and rest BOLD data were temporally censored or 'scrubbed', such that TRs surrounding time periods of sudden motion were removed from the analyzed data[61]. To accomplish this, we calculated frame-wise displacement (FD) of head motion based on the motion parameters estimated during preprocessing[61], and TRs including and surrounding (1 TR before, 2 TRs after) FD values greater than 0.5 mm were flagged to be scrubbed.

The scrubbing procedure was first performed at multiple levels: first, in the estimation of beta coefficients when regressing nuisance signals from the BOLD data[58,59]. For the encoding data analyzed via the general linear model (GLM; analyses shown in **Figs. 5** and **6** and **Supplementary Figs. 2** and **3**), the flagged time points were removed from the BOLD data and the design matrices. For LF connectivity analyses, the flagged data points were removed after the time-series were low-pass filtered but before the estimation of correlation values. After this scrubbing procedure was performed, a substantial number of time points were missing for a small subset of subjects. Subjects were excluded from LF connectivity analyses if less than 5 min of data were remaining in any given scan[60]. For the encoding data, this included $n = 2$ subjects for emotional encoding and $n = 1$ subject for neutral encoding; for the data from the first rest scan, this included $n = 1$ subject. Since a major goal was to compare connectivity between emotional and neutral encoding as a function of encoding order (see below), we excluded $n = 4$ total subjects from LF connectivity analyses (2 from each encoding order) to obtain equal bin sizes across E-N and N-E encoding orders (one additional subject from the E-N encoding order that had the most censored time points was excluded).

Anatomical ROI definition. FSL's FIRST segmentation was used to define the amygdala and hippocampus, based on each subject's high-resolution T1-weighted scan. All ROIs were manually inspected and edited when appropriate to ensure proper definitions according to Pruessner *et al.*[62]. The hippocampus was then divided into anterior, middle and posterior portions by splitting the coronal sections into thirds along the anterior–posterior axis.

Anatomical masks. SPM5 was used to create white matter (WM), cerebrospinal fluid (CSF) and gray matter (GM) probabilistic maps based on each subject's T1-weighted anatomical image. To create a mask of voxels in the cerebral cortex, each subject's anatomical image was parcellated using FreeSurfer, and all voxels labeled in any structure in the cerebral cortex were combined to create a mask of the cortex[63]. To create a mask of voxels in subcortical structures, FSL's FIRST segmentation was used and all voxels in any subcortical structure were combined to form one subcortical mask.

Ventral anterior insula network analyses. The BOLD data from the first rest scan was used to define regions in the ventral anterior insula (vAI) network[38]. To accomplish this, the rest data were low-pass filtered (< 0.1 Hz) after the removal of nuisance signals. MNI coordinates from a previously reported study were used to define a seed region in the right vAI (ref. 38). To extract the time-course of the vAI from these coordinates, we co-registered our group template brain to the MNI template brain, computed the location of the MNI coordinates in our group template space and then extracted the time course of the vAI in native subject space using the inverse normalization procedure in ANTs. The correlation between this vAI seed and all voxels in the brain were computed in native space, after discarding time points flagged from the scrubbing procedure (see "MRI preprocessing"). The resulting correlation values were Fisher z-transformed, transformed into our group template space and averaged across subjects. This average correlation map was thresholded at $z > 0.35$ to define four regions in the vAI network (bilateral vAI, medial prefrontal cortex and posterior cingulate cortex, as in ref. 38; **Supplementary Fig. 2**). To compute connectivity within this network during encoding blocks, the group level ROIs comprising the vAI network were inverse-normalized into native subject space, and the average of the pairwise correlations between these ROIs was computed for each encoding scan.

Low-frequency encoding connectivity analyses. To examine low-frequency (LF) connectivity during encoding blocks, the BOLD data were low-pass filtered (< 0.06 Hz) below the task frequency of 0.0714 Hz (**Fig. 3a**). Before computing connectivity, time points flagged from the scrubbing procedure (see "MRI preprocessing") were removed. Since each encoding block was comprised of three scans, the scrubbed data were first z-scored within each scan and then the data was concatenated across scans for each encoding block. Pearson correlation coefficients were then computed between the concatenated BOLD time-courses in each encoding block and correlation coefficients were Fisher z-transformed.

First, we compared the global similarity (correlation) of multivoxel LF amygdala connectivity patterns between emotional and neutral encoding blocks as a function of encoding order (**Fig. 3b**). Multivoxel LF connectivity (correlation) patterns with the amygdala were computed using the bilateral amygdala LF filtered time-course. The Fisher z-transformed correlation value (measuring connectivity with the amygdala) was then extracted for all voxels that were labeled as gray matter (value of 0.3 or greater using the probabilistic GM mask created in SPM5) and fell within a mask of cortical or subcortical structures (see "Anatomical masks"). Voxels that fell in the amygdala ROI were excluded from this analysis, although the exclusion of these voxels did not modify the results. To measure the similarity of multivoxel LF amygdala connectivity patterns between emotional and neutral encoding blocks, the Pearson correlation was computed between the LF amygdala connectivity patterns during emotional and neutral encoding across these cortical and subcortical voxels. These correlation values (representing similarity of amygdala connectivity patterns between emotional and neutral encoding blocks) were Fisher z-transformed, and the difference between multivoxel pattern similarities for E-N versus N-E encoding orders was assessed using an unpaired t-test (**Fig. 3b**).

To compare levels of LF connectivity between ROIs as a function of encoding order (**Fig. 4** and **Supplementary Fig. 2**), two-way mixed-effects ANOVAs were performed on the z-transformed correlation values in each encoding block using a within-subjects factor of emotion (emotional/neutral) and a between-subjects factor of encoding order (E-N/N-E). Additional follow-up t-tests were performed to assess differences based on emotion within encoding order as well as differences between neutral encoding connectivity as a function of encoding order. Paired t-tests were used to evaluate differences within an encoding order as a function of emotion (between emotional and neutral encoding blocks), and unpaired t-tests were used to evaluate differences across encoding orders between condition types (i.e., neutral encoding connectivity as a function of encoding order).

Subsequent memory analyses. GLMs were used to measure BOLD activation associated with different levels of subsequent memory, specifically activity that tracked future levels of recollection-based memory. To do so, separate regressors were included for: (i) subsequent R, K and missed (M) trials (trials labeled 'new' at test), modeled as 2-s boxcars; (ii) stimulus-preceding fixation periods (modeled as 1-s boxcars); and (iii) response periods (modeled as 1-s boxcars), such that the arrows task served as an implicit baseline. Each regressor was convolved with the canonical HRF. GLMs were estimated for emotional and neutral encoding blocks in native subject specific space (**Fig. 5**). For group level analyses, the resulting contrasts (R – K trials) were normalized into our group template space (**Fig. 6**).

Two subjects were excluded from all subsequent recollection analyses: one subject in the E-N encoding order had less than two K responses for emotional stimuli and another subject from the N-E encoding order with the fewest number of R or K responses whose exclusion served to match the number of subjects from both encoding orders.

To assess multivoxel patterns of subsequent recollection effects across the brain and whether subsequent recollection patterns were more similar between emotional and neutral encoding for the E-N versus N-E encoding order, subsequent recollection effects were computed as the difference in activity estimates (beta coefficients) for subsequent R – K trials. This R – K effect was then extracted for all voxels that were labeled as gray matter (value of 0.3 or greater in a probabilistic GM mask) and fell within a mask of cortical or subcortical structures (see "Anatomical masks"). The Pearson correlation was then computed for each subject between the emotional and neutral subsequent recollection patterns across these voxels. Resulting correlation values were Fisher *z*-transformed and the difference between the similarities for the E-N versus N-E encoding order was assessed using an unpaired *t*-test (**Fig. 5**).

To ensure that differences in multivoxel subsequent recollection patterns between emotional and neutral encoding based on encoding order were not potentially driven by differences in R and K bin sizes across encoding orders (as an interaction between R versus K responses, emotion and encoding order was observed), a nonparametric permutation test was used that controls for different bin sizes across comparisons. Simulations were performed (n = 1,000) to generate a null distribution of the expected effect size of interest (difference in similarity between emotional and neutral R – K patterns between the E-N and N-E encoding orders) that would arise by chance based on the properties of the data while shuffling the conditions of interest (activity related to true subsequent recollection differences). For each simulation, a new design matrix was created for each subject by randomly shuffling the subsequent memory labels. The GLM was then recomputed, the resulting subsequent recollection effects (R – K activity estimates) were recorded and the similarity between the null multivoxel emotional and neutral subsequent recollection patterns was computed for each simulation in each subject. The true difference was then compared to the null distribution of differences to assess statistical significance (**Supplementary Fig. 3**).

To examine the presence of univariate carry-over on subsequent recollection effects (**Fig. 6**), i.e., subsequent recollection effects that were present during emotional encoding and persisted into subsequent neutral encoding for the E-N encoding order, we performed a series of conjunctive tests. Based on the R versus K activity estimates, a carry-over effect on subsequent recollection was operationalized as a conjunction of the following effects (depicted in **Fig. 6a**):

- Emotional encoding R > K for E-N encoding order;
- Emotional encoding R > K for N-E encoding order;
- Neutral encoding R > K for E-N encoding order;
- Neutral encoding R – K for E-N encoding order > neutral encoding R – K for N-E encoding order;
- Emotional encoding R – K for E-N encoding order > neutral encoding R – K for N-E encoding order;
- Emotional encoding R – K for N-E encoding order > neutral encoding R – K for N-E encoding order.

In other words, regions emerging from this contrast had to demonstrate significant emotional subsequent recollection effects for both encoding orders, a significant subsequent recollection effect during neutral encoding for the E-N encoding order, with the stipulation that all of these subsequent recollection effects had to be greater than the subsequent recollection effect when this emotional carry-over effect should not be present (during neutral encoding when it occurred before emotional encoding). All individual tests were thresholded at $P < 0.05$ using a one-tailed *t*-test, as the directionality of all differences was uniquely specified, and the conjunction of the above contrasts was performed. Null simulations described above were performed to determine the voxel extent that resulted in a whole-brain family-wise error (FWE) rate of $P < 0.05$ for this conjunction analysis[64]. These null simulations were performed in normalized template space rather than native subject space (used for null simulations for subsequent recollection pattern analyses). For each null simulation, a new design matrix was created by shuffling the subsequent memory labels of each subject, GLMs were recomputed for each encoding order, the above contrasts were performed and the cluster sizes resulting from the above conjunction analysis were recorded. This approach generated a null distribution of cluster sizes associated with a specific contrast (in this case a conjunction analysis). The cluster size (73 voxels) was chosen as the smallest cluster for which the probability of observing this cluster size was less than 0.05 across 1,000 simulations.

We also assessed whether emotion-related activity predictive of subsequent recollection was localized within the anterior versus posterior hippocampus, based on prior notions that the amygdala should predominantly influence the anterior hippocampus during emotional memory formation[33,36,37]. We then examined whether such an anterior versus posterior bias was present during neutral encoding when neutral stimuli were encountered after extended blocks of emotional stimuli (E-N encoding order). To do so, we simply measured the anterior versus posterior position all voxels showing subsequent recollection-based encoding effects (all voxels showing R > K activity estimates) in each subject on a scale of −1 (fully posterior) to +1 (fully anterior). This resulted in an anterior versus posterior bias score for all subjects, separately for emotional and neutral encoding blocks. This measure was found to not be normally distributed (using the Lilliefors test) for emotional encoding in the N-E encoding order. We thus computed both parametric and nonparametric permutation tests when assessing differences in the anterior–posterior hippocampal subsequent recollection memory bias score as a function of encoding order. Permutation tests were performed in the same manner as described for memory performance and SCL (see "Behavioral analyses" and "Skin conductance level analyses").

Statistics. To examine the impact of encoding order on SCL, memory accuracy and neural measures, the data were first analyzed using mixed-effects ANOVAs including within-subjects factors of emotion and between-subjects factors of encoding order. Follow-up *t*-tests were used to examine differences between emotional and neutral encoding within each encoding order (paired *t*-tests) and between encoding orders (unpaired *t*-tests). All of the *t*-tests performed were two-tailed, with the exception of the SCL data (as described in "Skin conductance level analyses" and noted in the "Skin conductance" subsection of "Results"), as well as when examining carry-over subsequent recollection effects at the whole-brain level, since the direction of the subsequent memory effects were uniquely specified (**Fig. 6**; described in "Subsequent memory analyses"). Multiple comparison corrections were performed for the SCL data (comparing relative SCL across multiple scans versus zero in each encoding order in **Figure 2a**; described in "Skin conductance level analyses") as well as for the whole-brain analysis in **Figure 6** (described in "Subsequent memory analyses"). The normality of data distributions was tested using the Lilliefors test. All data were found to be normally distributed (did not reach significance to reject the null hypothesis that the data were derived from a normal distribution) with the exception of mean SCL during several blocks, overall memory accuracy (R + K hits) for the second block of the N-N encoding behavioral group and the anterior–posterior bias scores of hippocampal subsequent recollection memory for one of the four encoding blocks (emotional encoding in N-E encoding order). Thus, tests of statistical significance involving these data were performed using both parametric and nonparametric permutation tests (both statistical tests were reported and resulted in the same interpretation for all data). Note that nonparametric permutation tests were also used for subsequent memory analyses (see "Subsequent memory analyses").

Data and code availability. The data that support the findings of this study are available on reasonable request from the corresponding author (L.D.). The data are not publicly available because they contain information that could compromise research participant privacy/consent. The statistical map associated with the whole brain conjunction analysis in **Figure 6** is available (in MNI space rather than group template space) on neurovault.org (http://neurovault.org/collections/JOYXPMRX/). Standard software packages (SPM5 and FSL) were used for processing the MRI data in addition to custom Matlab scripts. Custom-written code is available upon reasonable request to the corresponding author (L.D.).

A **Supplementary Methods Checklist** is available.

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