Pain to remember: a single incidental association with pain leads to increased memory for neutral items one year later

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Abstract

Negative and positive experiences can exert a strong influence on later memory. Our emotional experiences are composed of many different elements – people, place, things – most of them neutral. Do negative experiences lead to enhanced long-term for these neutral elements as well? Demonstrating a lasting effect of negative experiences on memory is particularly important if memory for emotional events is to adaptively guide behavior days, weeks, or years later. We thus tested whether aversive experiences modulate very long-term memory for single events (episodic memory) in an fMRI experiment. Participants experienced episodes of high or low pain in conjunction with the presentation of incidental, trial-unique neutral object pictures. In a scanned surprise immediate memory test, we found no effect of pain on episodic memory strength. Critically, in a follow-up memory test one year later we found that pain significantly enhanced memory. Neurally, we found no significant predictors of immediate memory. However, for memory one year later, we found that greater insula activity and more unique distributed patterns of insular activity in the initial session correlated with memory for pain-paired objects. These results provide a novel demonstration of neural activity predicting memory one year later. Generally, our results suggest that pairing episodes with arousing negative stimuli may lead to very long-lasting memory enhancements.

Introduction

Experiences that lead to negative consequences, resulting in pain, fear, anger, and other aversive emotions, may remain in our memories longer than neutral experiences. A negative episode contains many separate elements that are often by themselves neutral. Prioritizing the content of emotionally arousing experiences in memory may reflect an adaptive function (Ochsner, 2000). Importantly, however, research to date on the effects of aversive experiences on episodic memory for neutral stimuli has not revealed a consistent enhancement of memory (Phelps et al., 1997; Maratos and Rugg, 2001; Smith et al., 2004a). To examine the effect of aversive experiences on very long-term memory, we investigated whether thermal heat pain, a highly arousal negative experience, affected memory for single experiences (episodic memory) one year later. Our approach utilized neutral items incidentally paired with aversive pain, allowing us to ask whether the original aversive experience modulates memory for these neutral items without the potential complications associated with using inherently aversive picture stimuli.

A critical consideration for the study of pain and memory is that the experience induced by heat pain, while inherently aversive, unpleasant, and physiologically arousing, is distinct from the experience of negative emotions such as fear, disgust, and unhappiness (Barrett et al., 2007; Buhle et al., 2013). However, one common feature shared between pain and negative emotions is that both can support avoidance learning, where through conditioning an agent learns to avoid stimuli associated with the aversive experience (e.g. Seymour et al., 2004; Delgado et al., 2009; Roy et al., 2014). Further, demonstrating the interplay between learning, pain, and negative emotions, knowledge of impending pain can induce fear (Vlaeyen and Linton, 2012) and pain has been used as an unconditioned stimulus in human fear conditioning studies (De Peuter et al., 2011). Overall, by learning and remembering what stimuli and environment are associated with pain or negative emotions, an agent can increase their health and well-being.

A rich literature has studied the effect of negative arousing experiences on memory, using stimuli such as well-characterized affective pictures (for review, see

Reisberg and Heuer, 2004; LaBar and Cabeza, 2006). In several studies, a benefit for remembering emotional stimuli has been found weeks or even a year later (Bradley et al., 1992; Cahill et al., 1996; Ochsner, 2000; Dolcos et al., 2005; Wagner et al., 2006; Weymar et al., 2011). However, the use of emotional stimuli in memory studies presents several difficulties. Emotional stimuli have been shown to attract increased attention during initial encoding that, along with increased semantic relatedness, may account for observed memory benefits (Talmi and McGarry, 2012; Talmi, 2013). As studies commonly employ both an immediate and a delayed memory test, the immediate memory test may further compound effects of attention that could further influence longer-term memory. Further, in memory tests, re-exposing participants to emotional stimuli can lead to new emotional processing, making neural effects at test difficult to interpret.

To avoid these concerns, researchers have utilized designs where neutral items are associated with aversive negative contexts during encoding and then memory is tested for the neutral items (Phelps et al., 1997; Maratos and Rugg, 2001; Smith et al., 2004a). When encoding is incidental (as in the majority of everyday experience), studies have reported null effects or even emotional memory impairments (Maratos and Rugg, 2001; Erk et al., 2003; Smith et al., 2004a; Smith et al., 2004b; Smith et al., 2006; Bingel et al., 2007; Forkmann et al., 2013; Zhang et al., 2015). Importantly, consolidation processes are known to play an important role in enhancing memory for emotional experiences, allowing for memory strengthening via neuromodulatory-induced plasticity (McGaugh, 2004; Yonelinas and Ritchey, 2015). Such consolidation processes operate over the course of hours after encoding, leading to changes in synaptic strength that could underlie observed changes in memory and behavior. Currently, however, only one behavioral study has reported increased day-later incidental memory for neutral stimuli associated with negative experiences (Schwarze et al., 2012), while several other fMRI and EEG and studies have reported null effects (Jaeger et al., 2009; Jaeger and Rugg, 2012; Schwarze et al., 2012).

Consolidation intervals longer than one day may reveal effects on emotion and memory that are not apparent in immediate or next-day memory tests. Longer consolidation may reveal differences, first, because intervals longer than one day allow

further time for memory for neutral stimuli to decay, leaving negative-paired stimuli relatively more preserved. Second, longer intervals may allow for systems consolidation, whereby memory traces originally stored in the hippocampus are in part shifted to other systems (Wang and Morris, 2010; Yonelinas and Ritchey, 2015). Importantly, no previous studies have examined the effects of extended consolidation on incidental memory for neutral stimuli. Demonstrating a lasting effect of emotion on memory is of particular interest if memory for aversive events is to adaptively guide behavior days, weeks, or years later. Further, previous studies have not shown whether neural activity during the initial experimental session predicts very long-term emotional memory (Dolcos et al., 2005).

We thus investigated whether very long-term memory for incidental neutral stimuli is modulated (increased or decreased) by a single aversive association, and also whether memory for pain-paired items related to activation during the initial session. In the initial fMRI session, neutral objects were presented once, incidentally paired with high or low pain (Fig. 1b). A scanned surprise memory test followed (Fig. 1c). One year later, participants returned to the lab for a follow-up memory test, allowing us to examine whether memory for the neutral objects was modulated by a single aversive experience one year before (Fig. 1d). When examining memory for aversive painful experiences, previous neuroimaging studies have found similar activity in the insula during pain and short-term remembered pain (Albanese et al., 2007; Fairhurst et al., 2012). Further, studies of post-traumatic stress disorder suggest a role for the insula in representing traumatic memories (Liberzon and Martis, 2006). Based on these findings and a hypothesized role of the anterior insula in processing the emotional and evaluative aspects of pain (Kurth et al., 2010; Wiech et al., 2014), we predicted that anterior insula activity during the initial experimental session would also be related to very long-term memory in particular for high pain-paired objects. Further, memory for affective experiences such as negative emotional pictures has been related to activity in the medial temporal lobe (MTL) including the amygdala (Murty et al., 2010). Multiple studies have also found that immediate hippocampal activity also relates to longer-term memory – i.e. greater than 24 hours – for neutral items (Uncapher and Rugg, 2005;

Carr et al., 2010; Sneve et al., 2015). We thus also examined whether MTL activity was related to memory for pain-paired experiences.

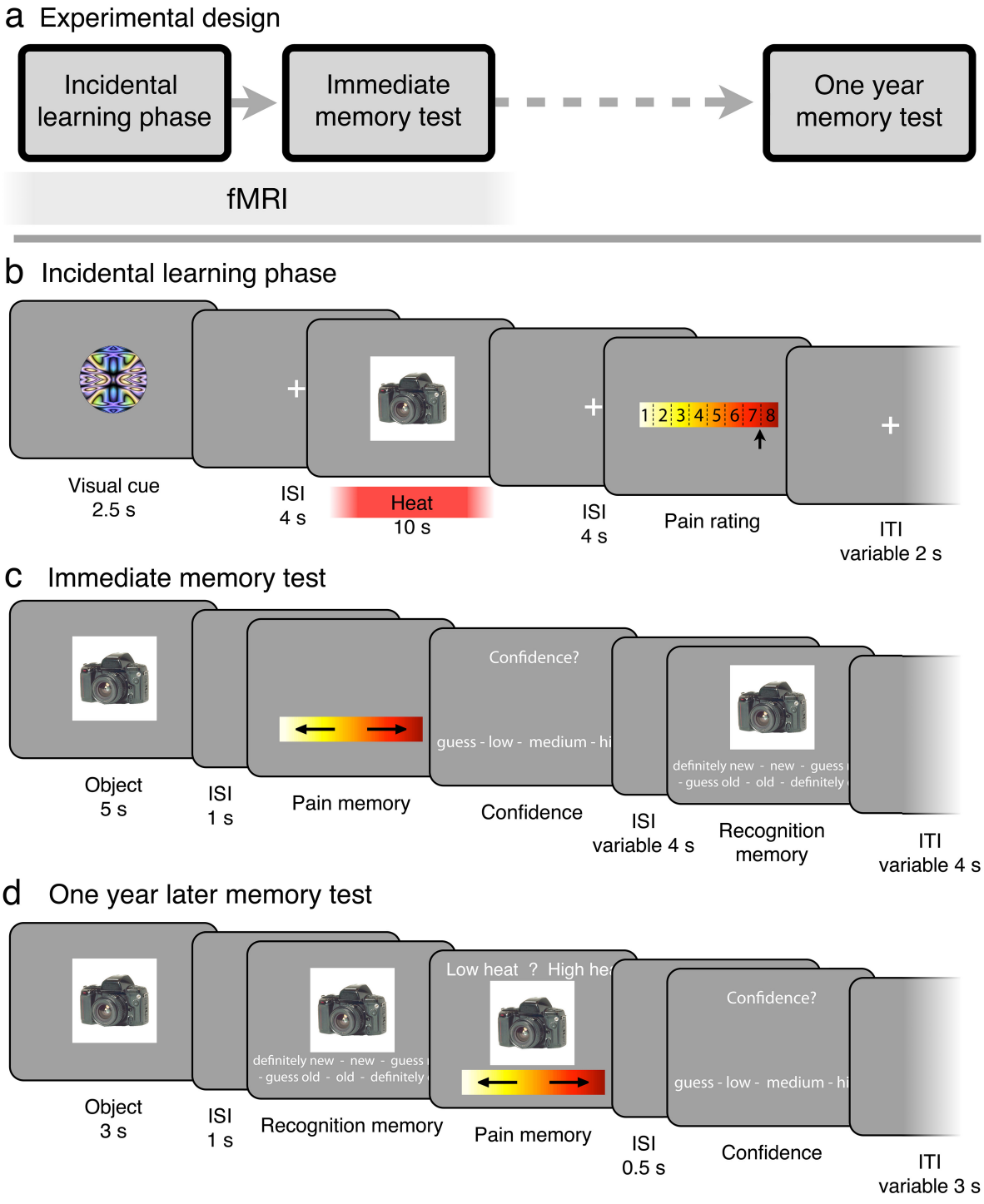

Fig. 1. Pain and incidental long-term memory experiment. (a) Experimental design: The incidental learning phase was followed by a surprise memory test phase during fMRI scanning, in which participants responded with whether objects had been paired with high or low pain and then rated their recognition strength. One year later, participants returned to the lab for a follow-up surprise recognition memory test. (b) In the incidental learning phase, participants experienced high or low heat pain while being exposed to an incidental trial-unique object pictures. Participants then rated their experienced level of pain. (c) Immediate memory test phase. After viewing an incidental object from the learning phase, participants responded with whether the object was paired with high or low pain and then rated their confidence in this response. Participants then rated their recognition strength on a 6-point new-to-old scale. (d) One year later memory test phase. After viewing an incidental object from the learning phase, participants rated their recognition strength on a 6-point new-to-old scale. If the object was rated “old”, participants then responded to a binary pain source memory question about whether the object had been paired with high or low pain in the incidental learning session one year prior, and then rated their confidence.

Materials and Methods

Participants: A total of 31 subjects participated in the experiment. Participants were right-handed fluent German speakers with no self-reported neurological or psychiatric disorders and normal or corrected-to-normal vision. Data from 2 participants were excluded due to technical problems with the thermode and 5 participants were unable to return for the one-year follow-up behavioral test, leaving 24 participants (12 female; mean age, 25.8 years; range, 20-33 years). In one participant, pain memory confidence ratings and memory recognition strength in the immediate test session were not recorded due to a technical error; this participant was excluded from analyses using immediate session data. The one-year later follow-up session was conducted on average 362.9 days after the initial scanning session (range: 316-469 days). The Ethics committee of the Medical Chamber Hamburg approved the study and all participants gave written consent.

The present experiment was designed to allow an investigation of two questions: first, behavioral and neural correlates of pain modulation of short and very long-term recognition memory and second, whether participants show accurate source memory for the level of pain incidentally paired with objects. Analyses and results in the current report focus on the modulation of recognition memory strength by pain in the immediate and one-year later memory tests and neural effects in the initial session that relate to memory performance. The separate, second question, regarding accuracy source memory accuracy for the pain incidentally paired with objects in the incidental learning phase will be reported separately.

Heat calibration. Before the incidental learning phase, heat levels were calibrated for each subject to achieve the same subjective high and low aversive pain experience across participants. Thermal stimulation was delivered via an MRI compatible 3 × 3 cm Peltier thermode (MSA; Somedic, Sweden), applied to the inner left forearm. During the visual presentation of a white square, heat was applied for 10 s. For pain rating, we used a 1-8 rating scale with 0.5-point increments, superimposed on a yellow-to-red gradient. An arrow cursor was moved from the initial mid-point starting location using

left and right key-presses and ratings were confirmed with the space bar. A rating of '8' corresponded to the highest level of heat pain a subject could endure multiple times. If the level of pain was intolerable, participants moved the rating past the '8' end of the scale, at which point a '9' appeared on the screen. Participants rated the pain associated with a pseudo-random list of 10 different temperatures ranging from 39.5 to 49.5ºC. A linear interpolation algorithm then selected a low temperature estimated to yield a '2' rating and a high temperature estimated to yield a '7.5' rating.

Procedure: incidental learning phase. In the incidental learning phase, participants experienced high or low heat pain while being exposed to trial-unique incidental object pictures (Fig. 1b). Importantly, the encoding of the object pictures was incidental (not instructed), to more closely match the incidental nature of encoding in many real-world situations. Color pictures of objects were drawn from a database of images compiled via internet search; objects were largely composed of familiar non-food household items, set on white backgrounds. To maintain attention on the screen during object presentation, participants were instructed to respond to occasional flickers in image brightness. Pain was probabilistically cued to allow for the investigation of expectation effects, with a design adapted from Atlas et al. (2010). Across 4 blocks, 33 high heat trials and 34 low heat trials were presented (Fig. 1b). One additional low heat trial was presented at the beginning of the task, with the incidental object from this trial also shown at the beginning of the immediate and year-later memory tests; data from this initial trial in all phases were excluded from analysis.

On each trial a visual cue was presented for 2.5 s signaling likely high or low heat. After a 4 s ISI, the incidental object appeared. To allow for a better match between the appearance of the object and the onset of noticeable heat, heat onset started 0.75 s prior to object appearance (for a similar method, see Forkmann et al., 2013). The incidental object was presented for a total duration of 10 s, after which the temperature returned to baseline (33°C) over several seconds. After a 4 s ISI, the pain rating scale appeared. Participants used the left and right buttons to move a selection arrow from the initial cursor position (randomized between 4.5-5.5) to their experienced pain level and pressed the down button twice to make their selection; responses were self-paced.

After the subject entered their response, trials were followed by a variable 2 s mean (range: 0.5-6 s) inter-trial-interval (ITI).

To maintain attention on the screen during visual cue presentation, on a random 50% of trials the visual cue illumination flickered (decreased in illumination) once for 0.35 s. Flicker timing was randomly distributed throughout the first 1.5 s of visual cue presentation. Similarly, on a separately determined random 50% of trials the object picture flickered in illumination during heat stimulation. When either a visual cue or object flicker was detected, participants were instructed to press the down button.

Two pseudo-random orderings of incidental object pictures were used for counterbalancing object and heat associations. The assignment of abstract circles to high and low heat was also counterbalanced across participants, and after the first two blocks of the experiment, two new abstract circles were used as cues, with visual and verbal instruction about the new cues preceding the block. To investigate effects of anticipation and expectation violation, visual cues were probabilistically associated with the level of heat, correctly predicting high or low heat on 70% of trials (Atlas et al., 2010). On invalid trials, the alternative heat level was administered. Additionally, 6 trials included a probe of cue-related pain expectancy: after 2.5 s of cue presentation, a question appeared below the cue asking participants whether they expected low or high heat to follow. These probes were used to test the learning of the visual cue-pain associations. After the probe, trials continued as normal. During the three breaks between the four incidental learning phase blocks, the thermode was moved to a new location on the inner arm to avoid sensitization.

To maintain similar differences in subjective experience between the high and low heat conditions, temperatures were automatically adjusted throughout the task to maintain the targeted pain rating values. If the median of the previous 6 validly cued low heat trials fell below a rating of 1.5, the low temperature was increased by 0.2ºC; if the median rating was above 3, the low temperature was decreased by 0.2ºC. For the high temperature, if the median rating fell below 7.5, the high temperature was increased by 0.2ºC (if the temperature was below 50.5ºC). If a rating of “9” was given, indicating an intolerably high level of pain, the high temperature was decreased by 0.8ºC. Such on-line adjustments of administered temperature are not commonly employed in pain

research that focuses on effects of expectation or placebo (e.g. Atlas et al., 2010), as in these cases administered temperature needs to be constant across the task. However, our focus here was on the subjective response to pain, and thus on-line adjustment allowed us to maintain very similar subjective responses to the majority of high and low heat stimuli.

Procedure: immediate memory test phase. In the scanned surprise memory test following the incidental learning session, we assessed recognition strength and memory for the level of pain administered with the object and (Fig. 1c). As noted above, results related to pain source memory will be reported separately. Participants saw each of the 68 “old” objects from the incidental learning phase intermixed with 20 “new” objects (Fig. 1c). On each trial a single object was presented alone for 5 s. Next, after a 1 s ISI, an unmarked heat scale with superimposed left- and right-pointing arrows was shown. Participants pressed the left or right buttons to indicate whether they thought that the object had been associated with low heat pain or high heat pain in the incidental learning phase. For objects that participants definitely considered to be “new”, participants were told that they could pick either the high or low heat response at random. If they were not sure an object was new, participants were instructed to try to recall the level of heat it may have be paired with. All test phase responses were self-paced. Next, a confidence rating screen appeared with 4 levels of response: “guess”, “somewhat certain”, “certain”, and “very certain”. For stimuli participants believed were definitely new, participants were instructed to respond with a low confidence answer. After a variable ISI (mean: 4 s; range: 3-6.5 s), a 6-point memory recognition strength scale was presented (e.g. Schwarze et al., 2012). Participants indicated whether they thought the object was “new” (not previously seen) or “old” (seen during the learning task) with 6 levels of response: “certain new”, “somewhat certain new”, “guess new”, “guess old”, “somewhat certain old”, “certain old”. Participants used the left and right buttons to move from the randomly initially highlighted “guess new” or “guess old” response option to their selected response and then pressed the down button twice to make their selection. A variable ITI with a mean of 4 s (range: 2-8 s) followed. The order of the old pictures was pseudo-randomized from the incidental learning phase order,

and the old and new pictures were pseudo-randomly intermixed. The duration and distribution of ITIs (or "null events") was optimized for estimation of rapid event-related fMRI responses as calculated using Optseq software (http://surfer.nmr.mgh.harvard.edu/optseq/).

At the end of the experiment, participants completed a paper questionnaire querying their knowledge of the task instructions and their expectations (if any) regarding the incidental object pictures. Task instructions and on-screen text were presented in German for all parts of the experiment; on-screen text was translated into English for the methods description and task figures.

One year later memory test phase. Approximately one year after the initial fMRI experimental session, participants returned to the lab to complete a surprise behavioral memory test session (Fig. 1d). On each trial, objects were displayed alone for 3 s. Then, participants rated their recognition strength for the object on the 1-6 new-to-old scale. After a 1 s ISI, for objects rated as "old" participants then indicated whether they thought the object had been incidentally paired with pain in the incidental learning session. For objects rated "new" participants waited for a 6 s ISI. A variable 3 s mean ITI followed each trial. Participants saw each of the 68 old objects from the incidental learning phase one year prior intermixed with 32 new objects that had not been seen in the experiment before.

Data Acquisition. The experimental tasks were presented using Matlab (Mathworks, Natick, MA) and the Psychophysics Toolbox (Brainard, 1997). The task was projected onto a mirror above the subject's eyes. Responses were made using a 4-button interface with a "diamond" arrangement of buttons. Skin conductance was recorded from the hypothenar of the left hand. The signal was amplified using a CED 2502 amplifier and digitized at 200 Hz using a CED micro1401 and downsampled offline to 100 Hz (both by Cambridge Electronic Design, Cambridge, UK). The year-later behavioral session was completed on a laptop computer.

Whole-brain imaging was conducted on a Siemens Trio 3 Tesla system equipped with a 32-channel head coil (Siemens, Erlangen, Germany). Functional images were

collected using a gradient echo T2*-weighted echoplanar (EPI) sequence with blood oxygenation level-dependent (BOLD) contrast (TR = 2460 ms, TE = 26 ms, flip angle = 80, 2 x 2 x 2 mm voxel size; 40 axial slices with a 1 mm gap). Slices were tilted approximately 30° relative to the AC–PC line to improve signal-to-noise ratio in the orbitofrontal cortex (Deichmann et al., 2003). Head padding was used to minimize head motion; no subject's motion exceeded 3 mm in any direction from one volume acquisition to the next. For each functional scanning run, four discarded volumes were collected prior to the first trial to allow for magnetic field equilibration.

During the incidental learning phase, four functional runs of an average of 190 TRs (7 min and 48 s) were collected, each including 17 trials. During the memory test phase, four functional runs of an average of 196 TRs (8 min and 2 s) were collected, each including 22 trials. If a structural scan had not been collected for the subject at the center within the past 6 months, structural images were collected using a high-resolution T1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) pulse sequence (1 x 1 x 1 mm voxel size) between the incidental learning phase and the immediate memory test phase.

Behavioral Analysis. Our primary behavioral question was whether memory one year later was modulated by pain experience in the incidental learning session. First, we conducted simple a priori comparisons using t-tests between recognition memory accuracy (% of trials showing "old" responses) for objects incidentally paired with high pain vs. objects incidentally paired with low pain, with a significance threshold of $p < 0.05$ (two-tailed). Comparisons were conducted separately for immediate memory strength and year-later memory strength. We also verified in initial comparisons that "old" objects (paired with high or low pain) were recognized at a higher rate than "new" objects.

Multilevel regression models as implemented in R (R-project.org) were used to further investigate immediate and year-later recognition memory strength. In all regressions, subject was entered as a random effect. Regression analysis used the full range of the memory strength scale (1-6) instead of the binary hit vs. miss measure to increase precision. To determine unique predictors of our critical question about year-

later memory for high pain-paired objects, all multilevel regressions for year-later memory included immediate memory test phase responses (high vs. low pain, recognition memory strength) as control variables. Additional analysis examined only memory for objects on "match" trials where the cue validly predicted the level of heat pain.

Skin Conductance Response Analysis. A basic analysis of the skin conductance response (SCR) data was conducted to verify differential SCR responses to high vs. low pain stimuli. SCR data were recorded at 200 Hz and subsequently downsampled to 10 Hz. Single trial timecourses were extracted, and trials with recording artifacts were removed from analysis. Baseline signal was removed by extracting the value at the first timepoint in each trial from the remainder of the trial's data. Visual inspection of mean across-participants timecourses for high and low pain trials showed the largest difference between 15 and 20 s after trial onset. This corresponds to 8.5-13.5 s after pain onset, within an expected range given that the thermode takes multiple seconds to reach peak temperature. To analyze the effect of pain on trial-by-trial SCR responses, SCR data from that time window were averaged to yield one value per trial. Trial-by-trial SCR values were log-transformed and then entered into multilevel regression analyses along with other variables of interest such as administered pain level and pain rating. Multilevel regression was conducted in R, using regression methods as described above in the behavioral analysis. Exploratory analyses found no relationship between SCR and later memory.

fMRI univariate analyses. Preprocessing and data analysis was performed using Statistical Parametric Mapping software (SPM8; Wellcome Department of Imaging Neuroscience, Institute of Neurology, London, UK). Before preprocessing, individual slices with artifacts were replaced with the mean of the two surrounding timepoints using a script adapted from the ArtRepair toolbox (Mazaika et al., 2009); slices with artifacts were identified as having mean image intensity greater than or equal to 5% above the across-run slice mean. Images were slice-timing corrected, realigned to correct for subject motion, and then spatially normalized to the Montreal Neurological

Institute (MNI) coordinate space by estimating a warping to template space from each subject's anatomical image and applying the resulting transformation to the EPIs. Images were filtered with a 128 s high-pass filter and resampled to 2 mm cubic voxels. Images were then smoothed with a 6 mm FWHM Gaussian kernel.

fMRI model regressors were convolved with the canonical hemodynamic response function and entered into a general linear model (GLM) of each subject's fMRI data. The six scan-to-scan motion parameters produced during realignment were included as additional regressors in the GLM to account for residual effects of subject movement.

fMRI analyses focused on whether activity during the incidental learning phase or immediate memory test phase was correlated with immediate and one-year later measures of recognition memory strength for high greater than low pain-paired objects. The critical memory regressions included effects of immediate memory and year-later memory, separately for high and low pain-paired objects. All regressions utilized automatic orthogonalization in SPM, such that shared variance was assigned to the regressors entered earlier in the model. In our models below, immediate memory strength regressors are entered first, as our aim was to isolate effects of one year-later memory strength that are unrelated to immediate memory strength. This follows the similar approach was taken in the behavioral multilevel regressions. We utilized separate parametric modulators for high and low pain objects because this allows for the critical a priori contrast of memory for high vs. low pain-paired object memory at the second level, as well as supplemental contrasts for high and low pain-paired object memory separately. Further, the use of separate regressors for high and low pain-paired objects avoids making any assumptions about similarity in memory-related correlations across high and low pain-paired objects.

We first conducted "localizer" univariate analyses to identify main effects of pain in the incidental learning phase. The GLM included regressors for the cue (2.5 s duration), object and pain presentation (10 s duration), and the pain rating (variable duration). The cue regressor was accompanied by a modulatory regressor for high vs. low expected pain and the pain regressor was accompanied by a modulatory regressor for the pain rating given on that trial.

To examine the primary question about neural correlates of immediate and year-later recognition memory strength, we first examined activity during the incidental learning phase. We constructed two general linear models (GLMs): the first model ("object onset") focused on correlates of memory during the initial object presentation period, while the second model ("peak pain") focused on correlates of memory during the peak pain period of the trial. The object onset GLM included regressors for the cue period (0 s), pain onset (0 s), and pain rating onset (0 s). The cue period and the pain period regressors were accompanied by 4 parametric regressors entered in this order: immediate recognition memory strength for high pain-paired objects, immediate recognition memory strength for low pain-paired objects, one-year recognition memory strength for high pain-paired objects, and one-year recognition memory strength for low pain-paired objects. The peak pain GLM examined memory correlates once pain-related activation had reached a peak, as estimated using GLMs that systematically varied the onset of the pain rating regressor from in 1 s increments from 0 s to 8 s post-onset. We found peak responses in the anterior insula at 5 s post-onset, and thus we focused on this period. The peak pain GLM included regressors for the cue period (2.5 s), initial pain period (5 s), late pain period (5 s) and pain rating (2 s). The cue period and the pain period regressors were accompanied by the 4 parametric immediate and year-later memory regressors described above.

We next examined neural correlates of immediate and year-later memory during the immediate memory test phase. In the memory test phase, object pictures were presented alone for 5 s at the start of the trial and again during the memory recognition strength response. As the full trial concerned memory questions for the same object, and as cognitive and memory processes likely engaged some maintenance of the object even when the stimulus was not on the screen (between the initial presentation and the memory recognition rating), we modeled memory during the full trial duration. Trial durations varied based on individual response times. The test phase model thus included a regressor for the full trial, with 5 parametric regressors entered in this order: a control contrast of old vs. new objects, immediate recognition memory strength for high pain-paired objects, immediate recognition memory strength for low pain-paired

objects, one-year recognition memory strength for high pain-paired objects, and one-year recognition memory strength for low pain-paired objects.

For univariate analyses, linear contrasts of univariate SPMs were taken to a group-level (random-effects) analysis. The critical contrast of interest was for memory effects specific to high vs. low pain-paired objects. Such a contrast reveals effects related to our a priori interest in regions whose activity predicts year-later memory for high pain but not low pain-paired objects. We also report results of secondary interest: the main effects of memory for high pain-paired objects, low pain-paired objects, and memory across high and low pain-paired objects. Further, as in the behavioral analysis, we attempted to examine responses in the subset of "match" trials where pain level was validly predicted by the cue. Before this analysis, we verified that an effect would be detectable given the lower number of trials in the "match" analysis (~23 vs. ~33 high pain-paired object trials in the main analysis). Permutation analyses, which left out random subsets of 10 match trials in separate GLMs, revealed that a lower number of trials, independent from the question of whether match trials were different from mismatch trials, greatly decreased the ability to detect the effect that is present in the full dataset. Given this lack of power, we do not report results from fMRI analyses limited to match trials.

fMRI multivariate analyses. We used representational similarity analysis (RSA) to examine patterns of activity evoked by stimuli that were remembered vs. forgotten one year later during the initial fMRI session (Kriegeskorte et al., 2008). For these analyses, we modeled the non-smoothed fMRI data in a GLM with separate regressors for each trial (88 total), in addition to six motion nuisance regressors and regressors for block effects. In the test phase, similar to the univariate GLM described above, the individual trial regressor duration covered the full memory trial, including the initial presentation period and the recognition strength response. These GLMs provided beta values for each voxel for each trial, which we extracted within regions of interest.

We separately computed representational similarity for the main types of trials, where pain level (high, low) interacted with memory (hit, miss) yielded four types of trials for the year-later memory analysis. Extracted beta values within the anterior insula ROI

were converted into vectors. For each trial within each participant, we computed correlations of the vector beta values with the vector of beta values from other trials of the same type. For example, the pattern for a high pain-paired object remembered trial was correlated separately with all other high pain-paired object trial beta values. Correlations between patterns of beta values in ROIs were computed using Pearson's r. The correlations of a single trial pattern with all other same-type trial patterns were averaged, producing one mean correlation value for that trial with other trials of the same type. After repeating this procedure for all trials, we averaged these correlation values to derive one correlation value for each of the four trial types per participant. Resulting r values were Fisher-transformed to z-scores before statistical comparison. Differences between these mean correlations (indexing representational similarity) were then compared using paired t-tests.

Our primary RSA analysis focused on differences in representational similarity for high pain-paired objects related to memory one year later. Given the selective univariate memory-related effect in the insula in the test phase, our RSA analyses focused on test phase activity. Previous studies have shown that higher within-item similarity across repetitions or across encoding to retrieval are related to better memory (Xue et al., 2010; Ritchey et al., 2013). Given the relationship between within-item similarity and memory, we expected that successful memory may be related to more distinct processing of individual objects, leading to higher across-item dissimilarity. A parallel control analysis was conducted using immediate recognition strength. As there were few instances of forgotten objects in the immediate test (old objects rated as new), recognition values were instead binned based on above- and below-mean immediate recognition memory strength.

We also examined representational similarity across learning and test phase presentations (Ritchey et al., 2013). However, initial control analyses indicated that within-object similarity was strongly affected by the application of thermal heat pain: learning-test correlations in an object-responsive region of the visual cortex were highly significant when the initial learning phase was modeled with a 0 s duration regressor at the onset of pain, but these within-item correlations were eliminated when the full pain

period was modeled. Given the absence of within-object similarity effects, we did not conduct further memory-related analyses across the learning and test phases.

Regions of interest. We report results corrected for family-wise error (FWE) due to multiple comparisons (Friston et al., 1993). We conduct this correction at the peak level within small volume ROIs for which we had an a priori hypothesis (after an initial thresholding of $p < 0.005$ uncorrected) or at the whole-brain cluster level, with a cluster threshold of 10 voxels. With the exception of pain-related activation (Table 1), we found no significant results outside of our a priori regions of interest.

We focused on two a priori ROIs motivated by two separate hypotheses. Given the anterior insula's role in processing the affective qualities of pain (Kurth et al., 2010; Wiech et al., 2014), we predicted that the insula may relate to the modulation of memory by pain. For this pain-hypothesis motivated anterior insula ROI, we first created a bilateral anterior insula mask (Brooks et al., 2002; Wiech et al., 2014) covering the insular cortex anterior to $y = 9$, as well as up to 4 millimeters lateral or superior to the insular cortex to account for signal blurring and anatomical variability. This mask was further restricted by the main effect of pain taken from the incidental learning phase localizer GLM defined above, thresholded at $p < 0.0001$ uncorrected. Separately, we focused on the medial temporal lobe (MTL) because of its role in episodic memory (Eichenbaum and Cohen, 2001). For the memory-hypothesis motivated MTL ROI, we included the hippocampus, parahippocampal cortex, and amygdala, based on the AAL atlas (Tzourio-Mazoyer et al., 2002). All voxel locations are reported in MNI coordinates, and results are displayed overlaid on the average of all participants' normalized high-resolution structural images.

Results

Pain ratings and skin conductance response during pain administration. Pain ratings given after each trial reliably differentiated high and low heat (high, 7.34 ± 0.06 (mean ± SEM); low, 2.34 ± 0.12; scale range: 1-8). The temperature on high heat trials was on average 49.4 ± 0.3°C and on low heat trials was on average 42.2 ± 0.3°C. Note that temperature was adjusted throughout the experiment to maintain a difference in ratings. Next, we examined skin-conductance responses (SCR) during pain. High vs. low pain significantly predicted trial-by-trial peak SCR response (multilevel regression; coef. = 0.067 ± 0.00, $t_{(22)}$ = 19.49, $p < 0.0001$). Conversely, SCR responses were a significant predictor of trial-by-trial variability pain ratings (coef. = 15.27 ± 0.81, $t_{(22)}$ = 18.82, $p < 0.0001$). These results indicate that high pain was consistently rated as aversive, that high and low levels of pain were clearly discriminable, and that high pain induced strong increases in physiological arousal.

A visual pain-predictive cue preceded the onset of heat and the incidental object. On 30% of trials, the pain expectation created by the predictive cue was violated. Expectation violation tended to increase pain ratings for low pain trials where the expectation was for high pain (high expectation and low pain vs. validly cued low pain: $t_{(23)}$ = 1.90, $p = 0.07$; low expectation and high pain vs. validly cued high pain: $t_{(23)}$ = 0.67, $p = 0.51$). Finally, on the 6 trials where visual cue-pain association knowledge was assessed, the pain level associated with cues was correctly identified on 89.9 ± 4.8% of probes. These results indicate that expectation had little effect on pain experience and that participants were able to easily remember the predictive cues.

Pain predicts memory strength for objects one year later. In the immediate surprise memory test phase, on each trial, participants first viewed an object picture for 5 s. Participants then indicated if the object had been associated with high or low heat pain and then rated their confidence. Next, participants rated their recognition strength on a 1-to-6 new-to-old scale (Fig. 1c), which we converted to a binary correct vs. incorrect recognition accuracy measure. In our analysis, we first verified that participants reliably discriminated objects seen during incidental learning from new objects (high pain vs.

new, $t_{(22)}$ = 16.69, p < 0.0001; low pain vs. new, $t_{(22)}$ = 17.09, p < 0.0001; immediate memory rating data missing for one participant; Fig. 2a). Regarding the central question of whether pain modulated memory, we found no effect of pain on immediate recognition memory (high heat objects: 81.6 ± 2.3%; low heat objects: 81.7 ± 2.2%; new objects false alarm rate: 13.7 ± 3.1; high vs. low, $t_{(22)}$ = 0.07, p = 0.94; Fig. 2a). Immediate memory recognition was not affected by whether the pain level was validly signaled by the predictive visual cue than when it was unexpected (p-values > 0.29). When excluding objects on cue-to-pain mismatch trials from the memory analysis, we also found no difference in memory due to pain ($t_{(22)}$ = 0.54, p = 0.59). The immediate memory measure was also not related to pain ratings or administered temperature (multilevel regression analysis on memory ratings; pain ratings: p = 0.88; heat temperature: p = 0.82). These results support the previously reported absence of a memory enhancement for emotion-associated neutral stimuli when tested immediately (e.g. Maratos et al., 2001; Schwarze et al., 2012). Further, the null effect of pain on immediate memory, as well as previous reports supporting an interruptive effect of pain on memory and cognitive processing (Bingel et al., 2007; Talmi and McGarry, 2012), suggest that pain did not increase attention to incidental objects in the incidental learning phase. However, it is possible that the order of the memory questions in the immediate test phase, where pain source memory preceded recognition memory, may have affected the ability to detect any differences in immediate memory due to pain.

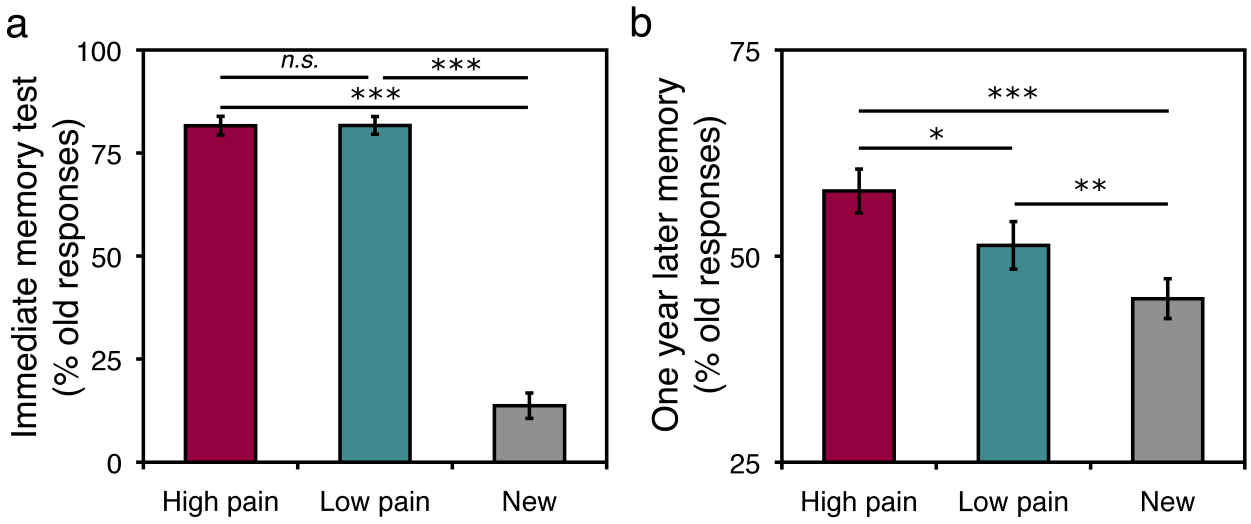

Fig. 2. A single association with pain enhances memory for objects one year later. (a) Recognition memory rate (percent "old" responses) in the immediate memory test for high pain-paired objects, low pain-paired objects, and new objects revealed no difference due to pain experience. (b) After one year, pain-paired objects showed significantly higher recognition rate than low pain-paired objects. (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.)

One year after the initial fMRI session, participants returned to the lab for a behavioral surprise memory test session (Fig. 1d). This allowed us to answer the critical question of whether a single incidental pairing with pain affected memory for neutral items one year later. We first verified that memory for old objects was significantly higher than memory for new objects. High and low pain-paired objects showed significant levels of recognition accuracy one year later, although the low pain-paired object memory effect does not survive correction for multiple comparisons (high pain vs. new, $t_{(23)} = 6.10$, $p < 0.001$; low pain vs. new, $t_{(23)} = 2.34$, $p = 0.028$; Fig. 2b). Next, turning to the primary question of whether pain modulation of memory, we indeed found that recognition accuracy for high pain-paired objects was significantly higher than recognition accuracy for low pain-paired objects and new objects (high heat objects, 57.9 ± 2.7%; low heat objects, 51.3 ± 2.9%; new objects false alarm rate, 44.7 ± 2.5%;

high vs. low, $t_{(23)}$ = 2.43, p = 0.024; Fig. 2b). Overall, memory recognition accuracy for high pain-paired objects was higher when the high pain was validly signaled by the cue than when it was unexpected (high pain objects match effect, $t_{(23)}$ = 2.21, p = 0.037; low pain objects match effect, $t_{(23)}$ = 0.99, p = 0.31). When excluding objects from the cue-to-pain mismatch trials from the analysis, we again found a significant difference in year-later memory due to pain ($t_{(23)}$ = 2.37, p = 0.026). Critically, while the numerical increase in memory due to pain is not a large difference, the significance of the effect indicates that the influence of pain on year-later memory was reliable across participants.

We conducted multilevel regression analyses to further investigate the effect of pain on memory strength. When including immediate test phase behavioral responses as control variables, we found that high vs. low pain remained a significant predictor of year-later memory (regression on memory ratings; coef. 0.14 ± 0.06; $t_{(20)}$ = 2.26, p = 0.024; when excluding mismatch trials, p = 0.017). Further, the year-later enhancement was not related to object-by-object variability in initial memory strength (initial memory strength: coef. 0.01 ± 0.02; $t_{(20)}$ = 0.64, p = 0.52), demonstrating that the year-later maintenance of memory was not driven by memory strength differences already present in the initial test session. Pain value memory (“high pain” vs. “low pain” responses) showed a trending positive effect on year-later memory (coef. 0.12 ± 0.06; $t_{(20)}$ = 1.91, p = 0.056). Additionally, pain ratings for the trial-by-trial pain experienced during individual objects positively predicted year-later memory (coef. 0.027 ± 0.011; $t_{(20)}$ = 2.34, p = 0.020; excluding mismatch trials, p = 0.023). The administered temperature of heat stimulation was also a significant predictor of year-later memory (coef. 0.018 ± 0.006; $t_{(20)}$ = 3.00, p = 0.0027; excluding mismatch trials, p = 0.0021). Notably, even within high pain-paired objects, temperature remained a significant positive predictor of year-later memory (coef. 0.02 ± 0.01; $t_{(20)}$ = 2.04, p = 0.042; excluding mismatch trials, p = 0.064). The effect of heat temperature on memory suggests that the nociceptive and arousal-related responses due to variations in temperature are a robust predictor of the strength of consolidated memory after one year.

Responses in the insula predict memory for pain-related objects one year later. In the fMRI analysis, prior to memory analyses we verified the main effect of high vs. low pain. we found that trial-by-trial pain ratings positively correlated with activation in a wide system of regions previously implicated in pain processing (Apkarian et al., 2005) including the anterior and posterior insula, cingulate, thalamus, and secondary somatosensory cortex (all $p < 0.05$ whole-brain FWE corrected; Fig. 3 and Table 1).

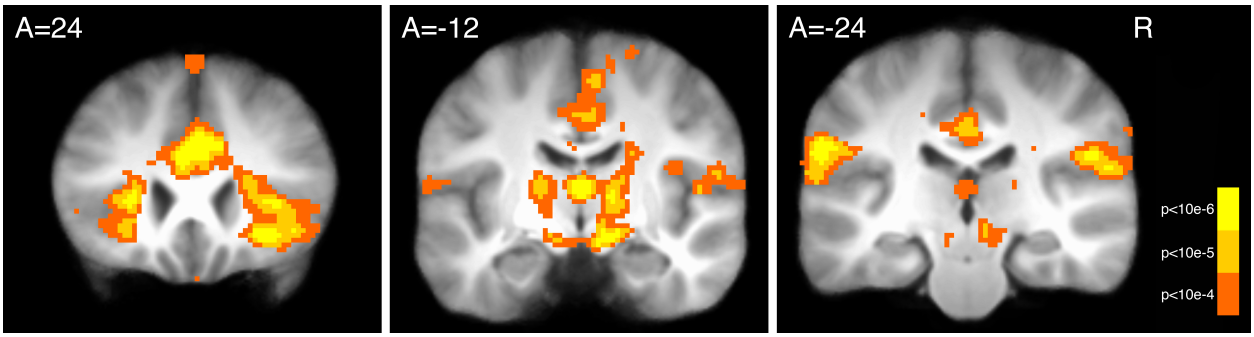

Fig. 3. Pain-correlated responses during the incidental learning phase. Brain activation was positively correlated with trial-by-trial pain ratings in the anterior insula, cingulate (left panel), thalamus, midbrain (middle panel), and secondary somatosensory cortex (right panel). See also Table 1. (Images thresholded at $p < .0001$ uncorrected for display; A = Anterior, R = Right.)

We then turned to our primary question, asking whether neural activity during the initial fMRI session correlated with year-later memory for pain-paired objects. In the incidental learning phase, we found no significant activation related to immediate memory for high pain vs. low pain-paired objects or high and low pain objects separately. At an uncorrected level, we observed activitiy in left hippocampus correlated with overall immediate test memory strength across high and low pain-paired objects (Right, Anterior, Superior: -24, -10, -22; $Z = 2.88$, $p = 0.002$, unc.). For memory one year later, during the incidental learning phase we also found no relationship between activity

and memory for high pain vs. low pain-paired objects, high and low pain objects separately, or across high and low objects, either at the onset of objects or during the peak pain period.

Next, we examined correlates of memory in the immediate memory test session, where objects were presented in the absence of pain. We found no correlates of immediate recognition memory strength specific to high- pain-paired objects, to high or low pain-paired objects separately, or immediate memory strength overall. Critically, for one year-later recognition memory strength, we found a correlation specific to high pain-paired objects in the right anterior insula (34, 24, 4; $Z = 3.84$, $p = 0.030$ SVC; Fig. 4). The peak of the insula cluster is within a region of insula activation correlated with trial-by-trial pain ratings in the incidental learning session (peak: 32, 14, 8; $Z = 6.36$, $p < 0.001$ whole-brain FWE; Table 1). This effect was driven by a correlation between insula activity and memory for high pain-paired objects (high objects: 36, 28, 2; $Z = 3.51$, $p = 0.084$ SVC); we found no positive or negative correlation with insula activity and memory for low pain-paired objects even at a liberal threshold of $p < 0.01$ uncorrected. We also found no significant correlates of memory across high and low pain-paired objects overall. As described in the Methods, with regards to memory on the subset of match trials (where the visual cue correctly predicted pain), our current study is underpowered to examine functional measures such as insula activity on smaller subsets of trials.

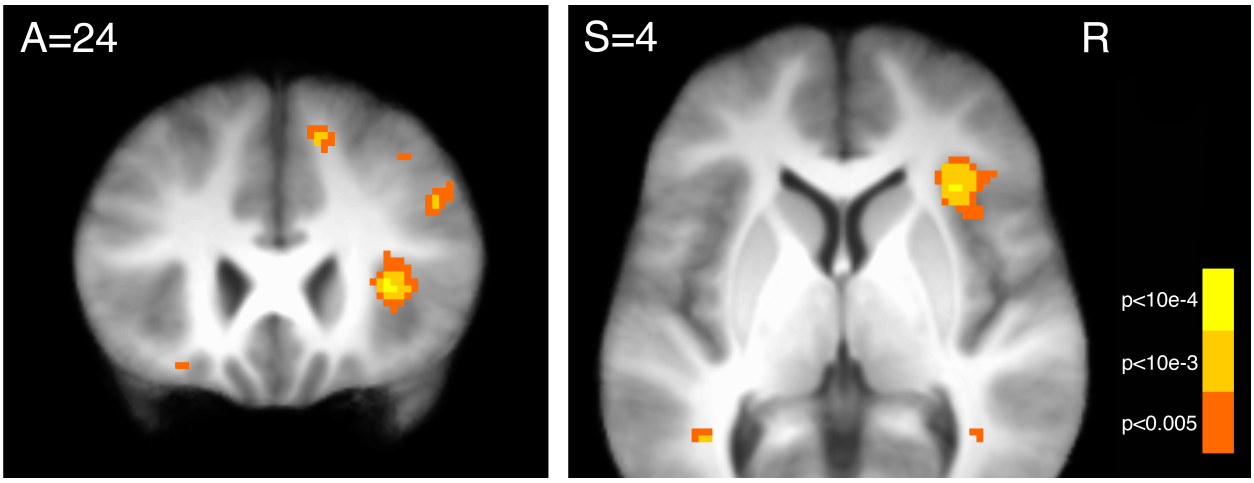

Fig. 4. Insula activity during the immediate memory session selectively correlates with memory strength for high pain-paired objects one year later. A contrast of one-year later memory strength for high pain-paired objects vs. memory strength for low pain-paired objects was significantly correlated with activation in the right anterior insula. (Images displayed at $p < 0.005$, uncorrected for display.)

Next, we examined memory for pain-paired objects using representational similarity analysis (RSA; Kriegeskorte et al., 2008). Previous studies have shown an association between higher within-item similarity and memory (Xue et al., 2010; Ritchey et al., 2013). Building on this within-item similarity memory effect, we predicted that higher across-item distinctness (or dissimilarity) may be related to better year-later memory. In the anterior insula, we indeed found a significant difference in representational similarity, such that patterns for objects remembered one year later were less similar to patterns for other remembered objects than patterns evoked by subsequently forgotten objects (high pain remembered 0.082 ± 0.008; high pain forgotten 0.095 ± 0.009; $t_{(23)} = 2.50$, $p = 0.020$). The pattern similarity effect was selective to memory for high pain-paired objects and showed a significantly stronger effect than similarity across low pain objects (high vs. low memory effect comparison, $t_{(23)} = 2.08$, $p = 0.048$; low pain memory effect, $t_{(23)} = 0.34$, $p = 0.73$). Moreover, the representational similarity difference was not driven by overall insula activity (such as

the preceding univariate result), as demonstrated in a regression controlling for trial-by-trial activation in the bilateral anterior insula ROI (similarity regression coef. -1.98 ± 0.081; $t_{(22)}$ = -2.45, p = 0.015; insula coef. 0.18 ± 0.09; $t_{(22)}$ = 1.90, p = 0.058). We found no year-later memory-related differences in the MTL, and when conducting the same analysis using immediate recognition memory strength, we found no difference in the anterior insula ($t_{(23)}$ = 0.80, p = 0.44) or MTL.

Discussion

We found that single episodes incidentally associated with painful experiences were not differentially remembered immediately but showed significantly enhanced memory one year later. In addition to an overall effect of pain on memory, we found that the degree of administered heat predicted year-later memory for high pain-paired objects. We also demonstrate a novel neural correlate predictive of very long-term memory: activity in the anterior insula predicted the strength of memory for pain-paired objects one year later. Further, multivariate patterns of activation in the anterior insula were additionally related to year-later memory for pain-paired objects.

Neurally, our results suggest a mechanism by which single aversive experiences modulate very long-term memory. We found that activity in the anterior insula during the immediate memory test positively correlated with memory strength for pain-paired objects one year later. Paralleling the lack of an immediate behavioral effect of pain on memory, activity in the insula was unrelated to immediate memory strength for high pain-paired objects. However, using univariate and multivariate measures, we found that insula activity was significantly related to memory one year later for high pain-paired objects. During the incidental learning phase, the same anterior insula region was strongly correlated with pain ratings and administered temperature. The anterior insula has been associated with many processes in the fMRI literature, but in the context of pain, it is hypothesized to play a particular role in the emotional and evaluative aspects of pain (Kurth et al., 2010; Wiech et al., 2014). Interestingly, insula activation in post-traumatic stress disorder has also been associated with recollecting traumatic memories (Liberzon and Martis, 2006). The current results suggest that the anterior insula may be related to long-term memory for aversive experiences in healthy human participants. It is possible that the insula activity we observed in the test phase reflects already-engaged memory consolidation processes which lead to very long-term memory benefits for pain-paired objects.

It is important to note that pain and negative emotions are distinct (Barrett et al., 2007). However, pain does share some important properties with negative emotions, in that both involve physiological arousal, support avoidance learning (Seymour et al.,

2004; Delgado et al., 2009; Wiech and Tracey, 2013), and have been shown to involve some similar neural substrates (Buhle et al., 2013). Previous fMRI studies in humans have emphasized the role of MTL-amygdala activity and connectivity in modulating emotional memory over time (Ritchey et al., 2008; Murty et al., 2010; Qin et al., 2012). In the consolidation of emotional memory, the amygdala may play an important role in consolidation by triggering the release of neuromodulators such as norepinephrine and corticosteroids (McGaugh, 2013). In our study, heat pain itself elicited activation in the dorsal amygdala / sublenticular extended amygdala. However, we did not find any correlates of immediate or year-later memory in the amygdala. This null effects should be interpreted with caution. It is possible that for aversive somatosensory stimulation, consolidation is related to interaction of the MTL with different regions such as the insula. Finally, while previous reports have supported a role for the hippocampus in longer-term memory for neutral items (Uncapher and Rugg, 2005; Carr et al., 2010; Palombo and Madan, 2015; Sneve et al., 2015), we did not find any significant memory-related effects in the MTL.

Remarkably, the memory enhancement that we observed was for pictures of everyday objects. With only one pairing with an aversive heat pain stimulus, these objects showed better memory that lasted at least one year, even though participants were likely exposed to many of these objects in the real world in the intervening time. However, the use of everyday objects likely also contributed to the relatively high false alarm rate observed in the year-later memory test. Speculatively, the arousal-related memory enhancement we observed may be even more robust for more unique experiences or intentionally studied information.

As the majority of research on the modulation of memory has utilized emotional pictures, it has remained largely unknown whether and how inherently neutral stimuli may be enhanced by association with an emotional experience (Phelps et al., 1997; Maratos and Rugg, 2001; Smith et al., 2004a; Anderson et al., 2006). When considering inherently emotional stimuli, an agent does not need memory to act to quickly avoid these stimuli in the future: for example, a large snarling dog remains aversive, and it would be simple to avoid such a threat, even without memory (Phelps et al., 1997; Maratos and Rugg, 2001). Thus, it is important to demonstrate that long-term memory is

enhanced for the stimuli that are not so easy to subsequently discriminate and act upon. Increased memory for neutral stimuli associated with aversive arousing experiences would allow for adaptive processing such as increased attentional orienting, which could facilitate more rapid adaptive responding if these stimuli are encountered again.

In conclusion, we demonstrate that a single aversive experience increases memory for neutral items one year later. Importantly, our neural results establish a novel connection between brain activity during the initial experimental session and memory one year later, such that increased insula activity predicted later memory strength. The long-term memory enhancement of neutral elements from emotional experiences may have implications for memory function in chronic pain (Oosterman et al., 2011), as well as the understanding and treatment of mood disorders and post-traumatic stress disorder (Hamilton and Gotlib, 2008; Shin and Liberzon, 2010). While our results concern arousing negative experiences, it is possible that a similar memory enhancement would be found for stimuli associated with positive affective experiences, where enhancements of very long-term memory may have important implications for education and learning.

Author Contributions: G.E. Wimmer and C. Büchel designed the experiment; GEW collected and analyzed data; GEW and CB wrote and revised the manuscript.

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Region	Right	Anterior	Superior	Z-score	Voxels	p-value
Right anterior insula	32	14	8	6.36	1222	< .001
Right inferior frontal gyrus / precentral gyrus	48	4	8	5.99		
	52	10	2	5.86		
Cingulate	0	12	34	6.03	537	< .001
	2	22	30	5.85		
	-4	22	22	5.22		
Left anterior insula	-36	10	10	5.9	757	< .001
Left inferior frontal gyrus	-50	18	-8	5.44		
	-56	6	0	5.43		
Left postcentral gyrus / secondary somatosensory cortex	-60	-24	26	5.58	120	< .001
Left cerebellum	-44	-50	-42	5.42	106	< .001
	-44	-60	-32	4.97		
Thalamus	-2	-6	4	5.38	156	< .001
	0	-14	8	5.19		
Left thalamus	-14	-6	10	4.76		
Right subthalamic nucleus	12	-18	-6	5.33	100	< .001
Right midbrain	12	-10	-10	5.33		
Left cerebellum	-24	-50	-46	5.27	72	< .001
Right postcentral gyrus / secondary somatosensory cortex	52	-28	24	5.2	78	< .001
	60	-26	18	4.88		
Cingulate	0	-22	34	5.15	25	.004
Right middle frontal gyrus	40	44	24	5.03	47	< .001
	36	38	20	4.97		
	38	52	18	4.74		
Left middle frontal gyrus	-30	48	20	5.03	54	< .001
	-32	40	24	4.86		
Left cerebellum	-26	-64	-22	5	52	< .001
	-24	-74	-20	4.84		
Right thalamus	14	-14	6	4.91	48	< .001
	12	-2	10	4.9		
Right sublenticular extended amygdala / dorsal amygdala	22	6	-18	4.63	71	< .001*
Left sublenticular extended amygdala / dorsal amygdala	-20	0	-14	4.4	56	< .001*

Table 1. Neural correlates of pain ratings during pain administration in the incidental learning phase. All p-values are whole-brain FWE-corrected, except where * indicates SVC p-values.