Representational similarity analysis offers a preview of the noradrenergic modulation of long-term fear memory at the time of encoding

Visser, R.M.; Kunze, A.E.; Westhoff, B.; Scholte, H.S.; Kindt, M.

DOI
10.1016/j.psyneuen.2015.01.021

Publication date
2015

Document Version
Final published version

Published in
Psychoneuroendocrinology

Psychoneuroendocrinology (2015) 55, 8—20

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/psyneuen

Representational similarity analysis offers a preview of the noradrenergic modulation of long-term fear memory at the time of encoding

Renée M. Visser[a,b], Anna E. Kunze[a,b], Bianca Westhoff[a], H. Steven Scholte[a,b], Merel Kindt[a,b,*]

[a] Department of Psychology, University of Amsterdam, 1018 XA Amsterdam, The Netherlands
[b] Amsterdam Brain and Cognition (ABC), University of Amsterdam, 1018 VZ Amsterdam, The Netherlands

Received 11 October 2014; received in revised form 7 January 2015; accepted 7 January 2015

KEYWORDS
Fear conditioning;
fMRI;
Multi-voxel pattern analysis;
Noradrenergic activation;
Representational similarity analysis;
Salivary α-amylase

Summary Neuroimaging research on emotional memory has greatly advanced our understanding of the pathogenesis of anxiety disorders. While the behavioral expression of fear at the time of encoding does not predict whether an aversive experience will evolve into long-term fear memory, the application of multi-voxel pattern analysis (MVPA) for the analysis of BOLD-MRI data has recently provided a unique marker for memory formation. Here, we aimed to further investigate the utility of this marker by modulating the strength of fear memory with an α2-adrenoceptor antagonist (yohimbine HCl). Fifty-two healthy participants were randomly assigned to two conditions — either receiving 20 mg yohimbine or a placebo pill (double-blind) — prior to differential fear conditioning and MRI-scanning. We examined the strength of fear associations during acquisition and retention of fear (48 h later) by assessing the similarity of BOLD-MRI patterns and pupil dilation responses. Additionally, participants returned for a follow-up test outside the scanner (2—4 weeks), during which we assessed fear-potentiated startle responses. Replicating our previous findings, neural pattern similarity reflected the development of fear associations over time, and unlike average activation or pupil dilation, predicted the later expression of fear memory (pupil dilation 48 h later). While no effect of yohimbine was observed on markers of autonomic arousal, including salivary α-amylase (sAA), we obtained indirect evidence for the noradrenergic enhancement of fear memory consolidation: sAA levels showed a strong increase prior to fMRI scanning, irrespective of whether participants had received yohimbine, and this increase correlated with the subsequent

* Corresponding author at: Department of Clinical Psychology, University of Amsterdam, Weesperplein 4, Room 5.11, 1018 XA Amsterdam, The Netherlands. Tel.: +31 020 525 6044/020 525 6810.
E-mail address: M.Kindt@uva.nl (M. Kindt).

http://dx.doi.org/10.1016/j.psyneuen.2015.01.021

expression of fear (48 h later). Remarkably, this noradrenergic enhancement of fear was associated with changes in neural response patterns at the time of learning. These findings provide further evidence that representational similarity analysis is a sensitive tool for studying (enhanced) memory formation.

1. Introduction

Whereas much of what we learn will be forgotten with the passage of time, emotional memory appears to be particularly resilient to forgetting (LaBar and Cabeza, 2006; McGaugh, 2000; McGaugh and Roozendaal, 2002; Pape and Paré, 2010). Long-lasting memory for emotional experiences is in principle adaptive, but can become dysfunctional as in the case of anxiety disorders.

The quintessential model for studying fear learning and memory is Pavlovian fear conditioning, describing how a previously neutral event (Conditioned Stimulus, CS) elicits fear responses after it has become associated with an aversive event (Unconditioned Stimulus, UCS). The traditional fear-conditioning paradigm was originally designed to explore normal processes of fear learning and memory. Given that anxiety disorders are characterized by the persistence of fear, and by generalization of fear to other stimuli and contexts in the absence of actual danger, abnormal fear memory as such cannot be defined at the time of learning. In laboratory settings, however, abnormal fear memory can become apparent at later retention tests. A key question is whether the processes that lie at the root of the development of abnormal fears are already active during the initial phase of associative fear learning, or whether they are predominantly active during the post-encoding consolidation phase.

To mimic abnormal processes of fear learning and memory, the noradrenergic system can be stimulated during or immediately after fear conditioning, either directly through the central administration of noradrenaline (LaLumiere et al., 2003), or indirectly through the administration of the α2-adrenoceptor antagonist yohimbine HCl (Gazarini et al., 2013, 2014; Soeter and Kindt, 2011, 2012). By blocking the α2-adrenergic autoreceptor, yohimbine interrupts the negative feedback control of noradrenaline release, thereby increasing noradrenergic activity (Hedler et al., 1981; Langer, 1974; Wemer et al., 1979). This experimental model has been shown to strengthen fear memory, characterized by the persistence and overgeneralization of fear (Gazarini et al., 2013, 2014; Soeter and Kindt, 2011, 2012). Interestingly, the effect of yohimbine administration prior to fear learning was not yet expressed at the time of learning (i.e., in more differential startle potentiation or elevated skin conductance; Soeter and Kindt, 2011, 2012), suggesting that post-learning processes accounted for this enhancement. Indeed, ample evidence supports the role of noradrenaline in synaptic changes, including long-term potentiation (LTP), underlying the stabilization of a memory trace after its acquisition (Cahill et al., 1994; Cahill and Alkire, 2003; Hurlemann et al., 2005; Southwick et al., 2002; Strange and Dolan, 2004).

A crucial question is, however, whether the most commonly used physiological measures in fear-conditioning paradigms are in this case reliable indicators of the formation of fear memory. An inherent restriction of all memory research, including fear conditioning, is that one cannot assess what is stored in memory until a memory is expressed. Yet, behavior during learning (e.g., freezing in rats, physiological responding in humans) is not necessarily predictive of long-term memory, as much of what we learn will be either lost over time or will not be consolidated in the first place. In order to establish whether the memory enhancing properties of noradrenaline are already at play during a learning experience, a reliable (neural) signature for the formation of fear memory is required. Ideally, such a marker would indicate during learning whether, and how well, information will subsequently be consolidated.

In a recent functional Magnetic Resonance Imaging (fMRI) study, we applied representational similarity analysis (Kriegeskorte et al., 2008; Visser et al., 2011) to show that changes in neural response patterns at the time of encoding were predictive of the long-term physiological (i.e., pupil dilation) expression of fear memory (Visser et al., 2013), while the physiological expression during fear learning did not predict the later expression of fear. Specifically, we showed that response patterns evoked by two unrelated stimuli became alike as a result of their association with an aversive outcome (between-stimulus pattern similarity). The degree to which these patterns became similar predicted differential pupil responses 2—6 weeks later. This suggests that part of the consolidation process, or the selection of information for subsequent consolidation, already occurs during learning, but cannot be observed in indices of peripheral nervous system activity. The question is whether noradrenergic *enhancement* of fear associations can also be observed in neural patterns at the time of learning, indicating that the transition from normal fear to excessive fear may start instantaneously.

Here, we further investigate whether changes in neural response patterns at the time of learning are (1) a marker for memory formation and (2) related to changes in noradrenergic activation. In previous studies, noradrenergic enhancement resulted in a stronger fear memory, expressed as increased fear responses, slower extinction of learned fear, stronger reinstatement and reacquisition of a successfully extinguished fear and (over)generalization of fear to other stimuli and contexts (Gazarini et al., 2013, 2014; Soeter and Kindt, 2011, 2012). In the current experiment, consisting of three sessions (separated by 48 h and 2—4 weeks, respectively), we incorporated similar tests of long-term fear memory, including extinction, reinstatement and generalization of fear (Session 2) and renewal and reacquisition of fear (Session 3). We measured BOLD activation, pupil dilation (Sessions 1 and 2), and fear-potentiated startle responses (Session 3), in order to assess at what point in time

it is possible to detect the enhancing effects of noradrenaline on fear memory consolidation.

We predicted that the increases in noradrenergic activity would be observed on different measures of long-term fear memory, both at the behavioral level (i.e., differential pupil dilation and fear-potentiated startle response) and the neural level (i.e., differential activation and differential pattern similarity). Crucially, we predicted that changes in similarity of neural response patterns (between-stimulus pattern similarity) would already signal the enhancement of fear at the time of learning.

Although our initial aim was to experimentally modulate the strength of fear by enhancing noradrenergic activation, our findings did not show any effects of the oral administration of 20 mg yohimbine HCl prior to fear conditioning on markers of noradrenergic activation (i.e., salivary alpha-amylase; sAA). In fact, both experimental groups showed a strong increase in sAA levels prior to scanning, which implies a failed manipulation (for further details see Supplementary material). We therefore collapsed the data across groups, and explored whether individual differences in noradrenergic activation during fear conditioning were related to the long-term expression of fear and were reflected in neural response patterns at the time of encoding.

2. Methods

2.1. Participants

Fifty-two healthy undergraduate students were recruited by means of advertisements at the university website. During the period of data collection, 11 participants were excluded for several reasons: equipment failure ($n = 1$), not finishing the experiment ($n = 2$), excessive sleepiness during one of the experimental phases (judged on the basis of eye-tracker data combined with self-report, $n = 5$), or excessive head motion ($n = 3$). For the fMRI-analyses, the final sample consisted of 41 participants (11 male, 32 right-handed) between 18 and 24 years of age (mean 20.6 ± 1.8 S.D.). As described in more detail in the sections below, some of these 41 participants missed reliable saliva samples ($n = 3$—5, depending on the time point, Section 2.2.3), part of the pupil data ($n = 2$, Section 2.2.4), or EMG-data ($n = 1$, Section 2.2.5). The list-wise inclusion of data points yielded different sample sizes, depending on which variables are analyzed or combined (e.g., individuals that lack pupil data do not also lack saliva samples), as will be specified for each analysis. All participants were free from any condition contraindicative to the administration of 20 mg yohimbine (Soeter and Kindt, 2011; Supplementary material). Participants earned partial course credit or €60, for their participation. All participants gave their written informed consent before participation, had normal or corrected-to-normal vision, and were naive to the purpose of the experiment. Procedures were executed in compliance with relevant laws and institutional guidelines, and were approved by the University of Amsterdam's ethics committee (2013-CP-2387).

2.2. Apparatus and materials

2.2.1. Stimuli

The experiment consisted of three sessions: Session 1 and Session 2 (2 days later) were conducted during fMRI-scanning, while Session 3 was conducted in a physiological lab, 14—27 days later (mean 17.4 ± 3.4 S.D. days; Fig. 1). For the learning phase, a differential fear-conditioning paradigm was used, with delay conditioning and partial reinforcement. Two pictures of neutral faces, derived from the Karolinska Directed Emotional Faces dataset (Lundqvist et al., 1998; pictures AM34NES and AM23NES), and two pictures of houses, derived from the Web, were separated from their background and converted to greyscale, and served as the to-be conditioned stimuli (CS). Each stimulus was presented 13 times for 4.5 s. One face and one house were followed by a mild electrical stimulus in 6 out of 13 presentations (46%, CSs+). The electrical stimulation served as an unconditioned stimulus (UCS) and was delivered at CS+ offset for 2 ms, by a Digitimer DS7A through MRI-compatible carbon electrodes attached to the right shinbone. The other two stimuli were never reinforced (CSs−).

2.2.2. Subjective assessments and retrospective UCS-expectancy ratings

Prior to the experiment, trait anxiety and anxiety sensitivity were assessed with the Trait Anxiety inventory (STAI-T; Spielberger, 1983) and the Anxiety Sensitivity Index (ASI; Peterson and Reiss, 1992) respectively. At time point T0, T3, T4, T5, and T6 state anxiety was assessed with the State Anxiety inventory (STAI-S; Spielberger, 1983). At time point T3, T5 and T7 retrospective UCS-expectancies ratings (''How much did you expect the shock when this picture was presented?'') were obtained for each stimulus on a 9-point scale for different stages during the experiment.

2.2.3. Saliva sampling and analysis

The salivary enzyme α-amylase (sAA) is an indicator of noradrenergic activation (Nater and Rohleder, 2009). At time point T0, T2 and T3 saliva samples were collected, using cotton salivette collection devices (Sarstedt, Nümbrecht, Germany). Participants were instructed to place the swab in their mouth until it was soaked. Each sampling was preceded by a 5-min rest period. To allow for controlled saliva collection, participants were asked to refrain from caffeine, alcohol and excessive exercise starting 12 h before the beginning of the experiment, and to refrain from food, drinks (except for water), chewing gum, cigarettes and teeth brushing two hours prior to testing (Bosch et al., 2011). Saliva samples were stored at −30 °C until biochemical analysis was performed, which was conducted independently in the lab of Dr. Bosch at our university (assay RE80111; http://www.ibl-japan.co.jp/datasheet/82671_j.pdf) and in the lab of Prof. Kirschbaum at the Technische Universität Dresden, Germany.

Salivettes that did not contain enough saliva for a reliable assessment were excluded from analyses, leaving 36—38 participants for the separate time points (Table 1). Assays of sAA corresponded well between the lab of Dr. Bosch and the lab of Prof. Kirschbaum ($r = 0.973$, $r = 0.957$ and $r = 0.937$, for the three time points, respectively). Reported here are

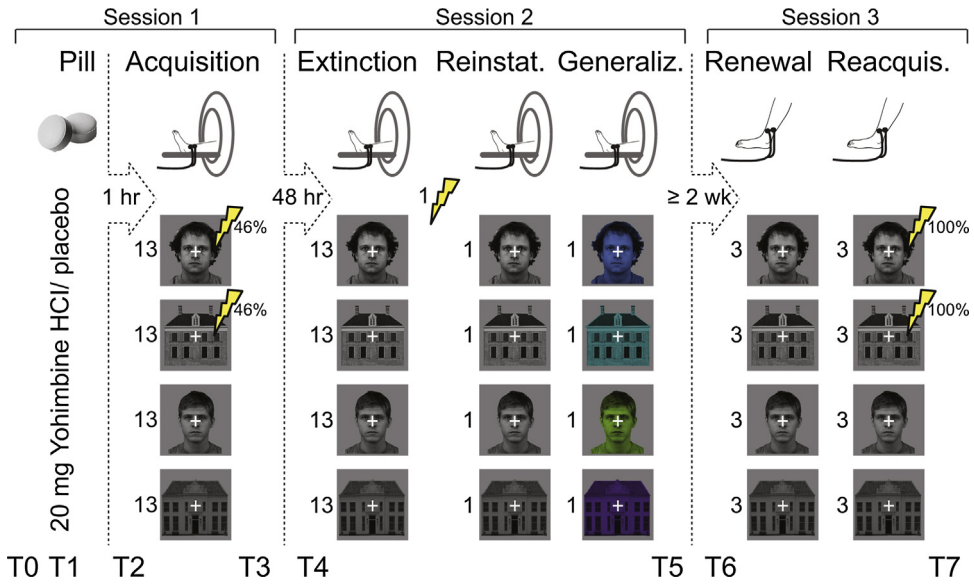

Figure 1 Mixed within-between-subject design, consisting of three sessions, with fear learning and several tests of retention of fear. As the pharmacological manipulation (T1) failed (Supplementary material), data are collapsed across groups. We measured BOLD activation, pupil dilation (Sessions 1 and 2), and fear-potentiated startle responses (Session 3), in order to assess at what point in time it is possible to detect the enhancing effects of noradrenaline on fear memory consolidation. Reinst., reinstatement; Generaliz., generalization; Reacquis., reacquisition.

the values that were obtained in the Kirschbaum lab. To assess individual changes in sAA, we calculated the percentage change in sAA levels, from T0 to T2 and from T0 to T3 (yielding 34 valid cases list-wise for both time points). Values that exceeded three standard deviations above or below group average were regarded as outliers and were removed ($n = 1$ for change from T0 to T2 and $n = 1$ for change from T0 to T3), leaving 33 participants for both time points to correlate with neural measures (Table 1), 31 of which had valid pupil data as well.

2.2.4. Pupil dilation

Pupil dilation and eye-movements were recorded continuously throughout MRI-scanning, using a remote non-ferromagnetic infrared Eyelink-1000 Long Range Mount eye-tracker (SR Research). Before task onset, a nine-point calibration procedure was performed.

Data were sampled at 1000 Hz. Samples around series of missing samples were regarded as unreliable and were removed (100 ms before and 100 ms after each series of 10 missing samples) and replaced by a linear trend at point, using the entire time series. Next, the interpolated pupil time series were low-pass filtered (third order Butterworth, 4 Hz). The baseline pupil diameter was the average value during the 500 ms prior to each CS onset. The pupil response to the CS was calculated as the peak change from baseline in a window from 0 to 4 s after picture onset (a window of 3—4 s — instead of 0—4 s — yielded identical results, indicating that the results are not affected by initial dilation or restriction induced by changes in luminance). Trials that suffered substantial signal loss, affecting more than 50% of either the baseline samples or the 4 s after stimulus onset were eliminated and replaced entirely by estimating the linear trend at point over trials for each condition separately. Participants that ended up missing more than 15% of the trials were excluded from further analyses of pupil data ($n = 2$), leaving 39 participants in the final sample (0—10.7% replaced trials per participant, median = 0%). Next, data were *Z*-transformed for each of the two experimental sessions separately, to reduce between-subjects variability.

In both sessions, participants were instructed not to move their eyes, but instead fixate on the center of the screen for as long as a stimulus was presented. Prior to stimulus onset, a white fixation cross appeared on the screen for 3 s, remaining visible for the duration of the stimulus.

Table 1 Mean values ± S.D. of self-reported anxiety sensitivity (ASI), trait anxiety (STAI-T), state anxiety (STAI-S) and salivary α-amylase (sAA).

	n	Mean (±S.D.)
ASI	41	10.6 (5.2)
STAI-T	41	34.7 (8.8)
STAI-S T0	41	32.3 (8.3)
STAI-S T3	41	35.0 (8.9)
STAI-S T4	41	32.7 (8.0)
STAI-S T5	41	30.1 (6.0)
STAI-S T6	41	31.6 (8.1)
Raw sAA levels (U/ml) T0	36	53.8 (67.7)
Raw sAA levels (U/ml) T2	37	78.4 (84.6)
Raw sAA levels (U/ml) T3	38	101.7 (88.6)
Change in sAA from T0 at T2 (%)	33	191 (147)
Change in sAA from T0 at T3 (%)	33	329 (323)

2.2.5. Fear-potentiated startle (FPS)

During the third session, startle responses were measured by means of electromyography (EMG) of the right orbicularis

oculi muscle. The startle probe eliciting the eye blink reflex was a 104 dB, 40 ms burst of broadband white noise with near instantaneous rise time, delivered binaurally through headphones (Sennheiser, model HD 25-1 II).

The strength of the eye blink reflex was measured by two 7 mm Ag/AgCl electrodes filled with electrolyte gel (Signa Gel, Parker), positioned approximately 1 cm below the pupil and 1 cm below the lateral canthus (outer corner of the eye; Fridlund and Cacioppo, 1986). A ground electrode was placed on the participant's forehead (Blumenthal et al., 2005). The electrodes were connected to a custom made bipolar EMG amplifier with an input resistance of 1 GPhm and a bandwidth of 5—1000 Hz. To remove unwanted interference, a notch filter was set at 50 Hz. The integrated EMG signal was sampled at 1000 S/s (National Instruments, NI-USB6210). Raw EMG data were band-pass filtered (28—500 Hz, 4th order Butterworth, Blumenthal et al., 2005), and peak amplitudes of the blink reflex were identified within a 0—175 ms latency window following probe onset using custom-made software (VSRRP98 v9.0b, 1998, University of Amsterdam). Data from one participant were lost due to equipment failure, leaving 40 participants in the final sample. Fear-potentiated startle responses that exceeded three standard deviations above or below individual average peak amplitude were regarded as outliers (seven trials in total; 0.6%). Those values were replaced by the mean-plus-three standard deviations calculated over all trials from that individual, excluding the previously defined outlier trial(s). The data were subsequently *Z*-transformed to reduce between-subjects variability.

2.2.6. Image acquisition

Scanning was performed on a 3T Philips Achieva TX MRI scanner using a 32-channel head-coil. Functional data were acquired using a gradient-echo, echo-planar pulse sequence (TR = 2000 ms; TE = 27.63 ms; FA = 76.1°; 37 axial slices with ascending acquisition; 3 mm × 3 mm × 3.3 mm voxel size; 80 × 80 matrix; 240 × 133.98 × 240 FoV) covering the whole brain. Session 1 consisted of 636 volumes and Session 2 of 740 volumes. Foam pads minimized head motion. Online motion correction was applied by comparing each recorded volume to the initially recorded volume and adjusting the plane of recording with the displacement. A high-resolution 3D T1-weighted image (TR = 8.11 ms, TE = 3.73 ms, FA = 8°; 1 mm × 1 mm × 1 mm voxel size; 240 × 220 × 188 FoV) was additionally collected for anatomical visualization. Stimuli were backward-projected onto a screen that was viewed through a mirror attached to the head-coil.

2.2.7. Pre-processing

FEAT (FMRI Expert Analysis Tool) version 6.0, part of FSL (Oxford Centre for Functional MRI of the Brain [FMRIB] Software Library [www.fmrib.ox.ac.uk/fsl]) was used to analyze the (f)MRI data. We applied the same pre-processing and registration as in our previous study (Visser et al., 2013) in order to optimize comparability of the results. Pre-processing included slice-time correction, motion correction, spatial smoothing using a 5 mm full-width-at-half-maximum Gaussian kernel, high-pass filtering in the temporal domain (σ = 100 s) and pre-whitening (Woolrich et al., 2001). Structural images were co-registered to the functional images and transformed to MNI standard space (Montreal Neurological Institute) using FLIRT (FMRIB's Linear Image Registration Tool, FSL). The resulting normalization parameters were applied to the functional images.

2.2.8. Region of interest selection

Regions of interest (ROI) were selected based on their role in fear learning and (extinction) memory and included the anterior cingulate cortex (ACC, 9213 voxels), the insula (6591 voxels), amygdala (2967 voxels), hippocampus (5837 voxels) and ventromedial prefrontal cortex (vmPFC, 4160 voxels). We additionally included the superior frontal gyrus (SFG, 18946 voxels), a region outside the salience network, for its large fear acquisition effect as revealed by previous similarity analysis, in the absence of differences in average activation (Visser et al., 2011, 2013). ROIs were obtained from the Harvard-Oxford cortical and subcortical structural atlases (Harvard Center for Morphometric Analysis).

2.3. Experimental procedure

Upon arrival participants were screened (T0) and saliva samples were collected. Approximately 20 min later, the intensity of the electric stimulus was determined by adapting the level individually to be aversive but not painful (intensity range 8—60 mA, mean 26.5 ± 13.3 S.D.). Participants were told that two out of four stimuli might be followed by the electric stimulation, whereas the other two would never be followed by the electric stimulation. They were instructed to learn and remember the specific contingencies. Prior to structural scanning, when participants were already lying inside the scanner, a second saliva sample was collected (T2). After scanning, participants filled out questionnaires (T3) and another saliva sample was collected.

During the second session, 2 days later, the shock intensity was explicitly set at the individual level as determined in the previous session, but none of the CS stimuli were reinforced. Near the end of this session, one unpredictable shock was delivered to reinstate the memory of the CS-UCS contingencies. This session finished with the presentation of the CS-stimuli in different colors, to test for generalization of fear. Prior to this second session (T4) participants were instructed to remember what they had learned during acquisition, to prevent participants from erroneously expecting a different contingency scheme. After scanning, participants filled out questionnaires (T5).

During the third session, 2—4 weeks later, participants returned for a test of fear retention in a different context (i.e., a physiological lab, T6), conducted by a different experimenter. Each of the CS-stimuli was presented six times, of which the first three times were unreinforced (renewal test) and the last three times were reinforced (reacquisition of fear), while fear-potentiated startle responses were measured. Participants were told that they would be seeing the same stimuli and were reminded that the same two stimuli would never be followed by the shocks. After the experiment, participants filled out questionnaires (T7).

Stimulus presentation was similar to previous designs (Visser et al., 2011, 2013), i.e., inter-stimulus intervals were fixed (19.5 s, Fig. 2a) and each trial onset was triggered

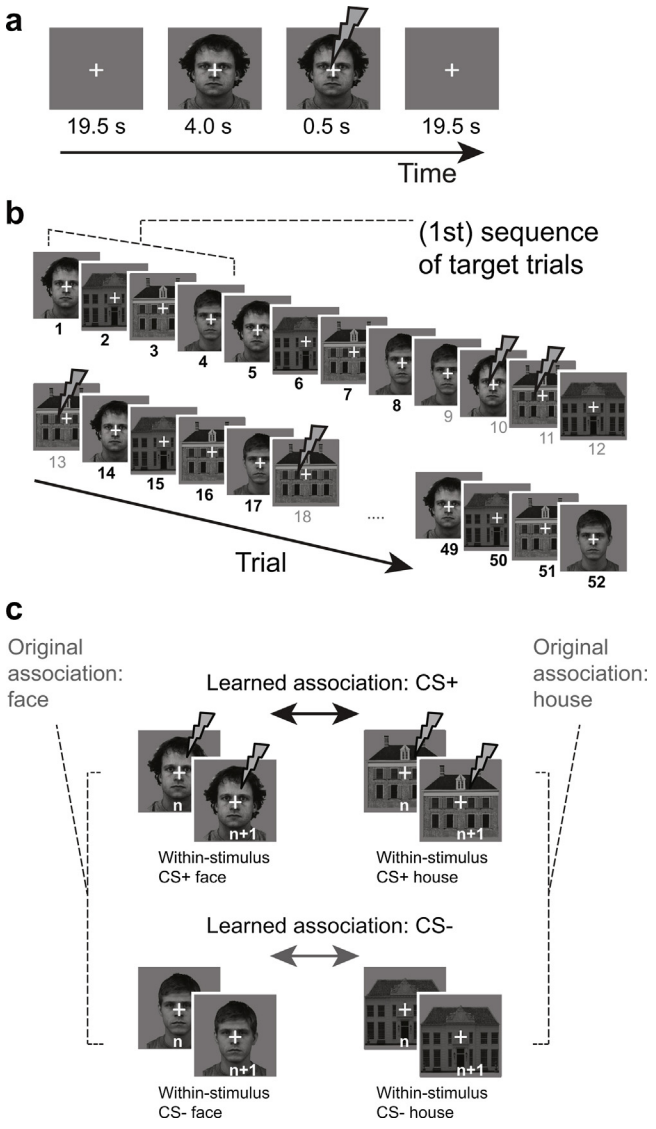

Figure 2 (a) Stimulus presentation with fixed inter-trial intervals and partial reinforcement. (b) The paradigm consisted of repeating sequences of target trials, presenting the four stimuli in a fixed order such that the time between two consecutive target trials was equal over the four conditions. In between, the semi-random presentation of filler trials ensured the unpredictability of stimuli. Administration of a UCS was restricted to filler trials. The first paired CS+ trials were presented between the second and third sequence of target trials. (c) In order to assess neural pattern similarity, correlations were calculated between patterns evoked by consecutive trials of the same stimulus (within-stimulus), trials of stimuli that share (non)reinforcement and trials of stimuli that were originally associated. Pupil dilation responses, startle responses, average activation, within-stimulus correlations and correlations for original associations were averaged over face- and house stimuli. This was done to reduce the number of comparisons and because we were not interested in the difference between faces and houses with regard to the experimental manipulation. Images are not to scale.

by the start of the acquisition of a BOLD-MRI volume. The order of stimulus presentation was fixed (counterbalanced across participants) and consisted of a repeating sequence of four target trials, with filler trials of the same stimuli in between (Fig. 2b). In total, the acquisition-phase consisted of 52 trials: 28 target trials (seven per stimulus type) and 24 filler trials (six per stimulus type), including all CS+-trials that co-terminated with a shock. The extinction-phase also consisted of 52 trials: 28 target trials (seven per stimulus type) and 24 filler trials (six per stimulus type), followed by four trials to test for reinstatement of fear (one per stimulus) and four trials to test for generalization of fear (one per stimulus). For each run, we constrained our analyses to target trials, to be certain that (a) shock-related activity did not confound CS-related activity (during the acquisition-phase) and (b) that the time between two consecutive target trials was equal over the four CS types (for both acquisition- and extinction-phase), while filler trials ensured that the stimulus presentation remained unpredictable for the participant (Visser et al., 2011, 2013). The distinction of target and filler trials was irrelevant for the third session, where no fMRI (hence no similarity analysis) was conducted, meaning that all 24 trials (six per stimulus type) from this session were analyzed. Similarly, the relatively high temporal resolution of the pupil dilation response allowed for the analysis of reinforced trials, so pupil data from the first two sessions include both filler and target trials.

2.4. Trial-by-trial similarity

For the trial-by-trial representational similarity analysis each trial was modeled as a separate regressor in a voxelwise whole-brain analysis using a general linear model (GLM), including six motion parameters as regressors of no interest. The resulting parameter estimates were transformed into *t*-values to down-weight noisy voxels. To this end, each voxel's parameter estimate was divided by the standard error of that voxel's residual error term after fitting the first-level GLM. The resulting *t*-values were converted to *Z*-values. In Matlab (version 8.0; MathWorks) we created for each participant, for each ROI a vector containing *Z*-values per voxel, for a particular trial (i.e., the 'spatial representation' of that trial). Next we calculated pair-wise Pearson correlations (i.e., 'representational similarity') between all vectors of all single trials, disregarding filler trials, resulting in a similarity matrix containing correlations among trials, for each participant, for each ROI. From this matrix, three different types of correlations were selected (Fig. 2c). The strength of these correlations was used as a metric of similarity. First, we examined *within-stimulus* correlations on consecutive target trials. Second, we examined *between-stimulus* correlations, both between adjacent target trials of original-related stimuli (original associations: faces and houses) and correlations between adjacent target trials that shared (non)reinforcement (learned associations: CS+ face with CS+ house and CS− face with CS− house). Of the between-stimulus correlations, the 'learned associations' are characterized by a focus on a common outcome and putatively reflect a type of higher-order fear learning (Visser et al., 2011, 2013). Note that the number of between-stimulus correlations is equal to the number of target trials,

whereas the number of within-stimulus correlations is equal to the number of target trials minus one. Correlations were Z-transformed for each experimental session separately.

2.5. Univariate analysis

To visualize trial-by-trial changes in average activation, we analyzed data as described in Section 2.4, except that when we analyzed the normalized single-trial parameter estimates, we averaged across voxels in an ROI. Thus, instead of preserving the spatial information by creating a vector of voxels per ROI, we obtained one value per ROI (average response amplitude), which we then Z-transformed across trials for each experimental session separately, to reduce between-subject variability.

To explore fear-related activation outside the selected regions of interest, we ran a standard voxelwise whole-brain analysis, modeling all target trials within a condition as one regressor and including filler trials, motion parameters and temporal derivatives as regressors of no interest. Higher-level mixed effects analyses were conducted to explore group differences (Supplementary material).

2.6. Statistical analyses

Z-transformed pupil dilation responses, startle responses, average activation, within-stimulus correlations and correlations for original associations were averaged over face- and house stimuli. This was done to reduce the number of comparisons and because we were not interested in the difference between faces and houses with regard to the experimental manipulation.

Statistical comparisons of the learned associations were performed by within-subjects Analysis of Variance (ANOVA), using Statistical Package for the Social Sciences (SPSS, version 21; SPSS Inc.). Statistical tests are equivalent for behavioral and neural measures, the only difference being the number of trials that is included in the analysis (e.g., for the learning phase this is 13 for pupil dilation, 6 for within-stimulus similarity, 7 for between stimulus similarity and average activation, as described in Sections 2.2.4, 2.2.5, 2.4 and 2.5) and stimulus type (2 levels [CS+ and CS−, averaged over faces and houses] for within-stimulus similarity, average activation, pupil dilation and EMG data, and three levels [CS+, CS− and original associations, the latter averaged over faces and houses] for between-stimulus similarity).

Differential fear learning was assessed by the interaction of all acquisition trials × stimulus type, but was only tested when there was also a significant main effect of stimulus type. Retention of fear memory was assessed by a main effect of stimulus type during the first three trials from the extinction-phase (Visser et al., 2013), henceforth referred to as 'retention trials', and if significant, extinction was assessed by a significant interaction between all extinction trials and stimulus type. Reinstatement of fear was assessed by an interaction of stimulus type and trial (last extinction trials and reinstatement trial), and was only assessed if there was significant retention and extinction of fear. Furthermore, to measure the extent to which fear generalized to similar stimuli we compared responses on the generalization trial. Note that for the assessment of within-stimulus pattern similarity at least two trials for each stimulus are needed (Fig. 2c). Given that there was only one trial to test for reinstatement and one trial to test for generalization of fear, assessment of within-stimulus pattern similarity was not possible at these retention tests. Also, renewal of fear could not be assessed directly, as the measures differed between the second and third experimental session. Hence, for the third phase, retention of fear was assessed by a main effect of stimulus type during the three renewal trials, and reacquisition of fear was assessed by comparing the last two trials of the reacquisition phase (when differential fear should be re-acquired) with the last renewal trial (when differential fear should be re-extinguished).

To explore individual differences in fear learning and memory we first calculated difference scores for each individual trial in each of the three phases: *CS+ minus CS−* for within-stimulus correlations, single-trial activation data, EMG data and pupil data; *2(CS+) minus CS-minus original association* for between-stimulus correlations. For the 'conditioning'-index we subtracted the average difference over the first trials (when participants did not know the contingencies; Fig. 2b) from the average difference over the later trials (when contingencies could have been learned), yielding an index that expressed the relative increase of the CS+ responses over the course of learning. Similarly 'retention'-indices were calculated as the average difference between CS+ and CS− on the three retention trials (Session 2) and the first three trials of the renewal phase (Session 3). The different indices were used for the continuous assessment (i.e., correlation analysis) of the predictive value of the different measures. For example, to test whether pattern similarity during learning predicts the behavioral expression of fear 2 days later, the conditioning-index calculated for pattern similarity was correlated with the retention-index calculated for pupil dilation.

Predictions were tested while correcting for multiple comparisons (6 ROIs) by limiting the false discovery rate (FDR; Benjamini and Hochberg, 1995). Prior to each test, normality was assessed by visually inspecting the data and a Kolmogorov—Smirnov test. In case that the assumption of sphericity was violated a Greenhouse—Geisser correction was applied. All *p*-values are reported two-sided, with the significance level set at $\alpha = 0.05$.

3. Results

3.1. Subjective assessments

All participants were aware of the contingencies immediately after fear conditioning and all remembered the contingencies 48 h later. At follow-up (>2 wk), 36 out of 41 participants still remembered the contingencies. Table 1 presents an overview of self-reported trait and state anxiety.

3.2. Pupil dilation responses

As an independent index for the expression of conditioned fear (Reinhard et al., 2006; Visser et al., 2013), differential pupil dilation responses were compared over trials for the first two phases ($n = 39$, Fig. 3a). Consistent with previous work, successful fear conditioning was evident from a

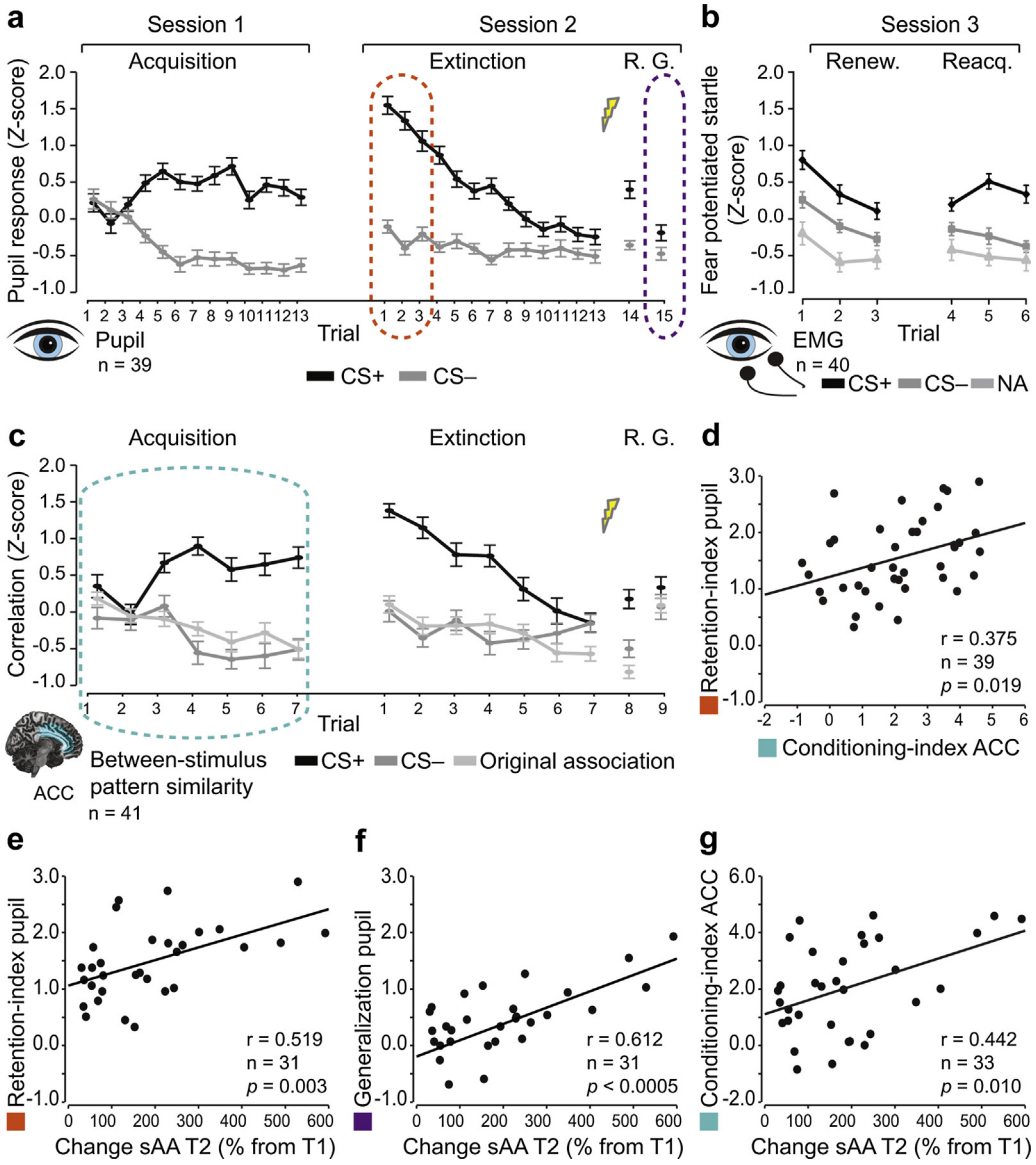

Figure 3 (a) Pupil dilation responses for the learning phase, the extinction phase, the reinstatement test (R.) and the generalization trial (G.) show strong acquisition, retention and reinstatement of fear, but weak generalization of fear. (b) Fear-potentiated startle responses for the renewal and the reacquisition phase 2—4 weeks later demonstrate retention of fear. (c) Between-stimulus pattern similarity shows strong acquisition, retention and reinstatement of fear, but weak generalization of fear. Error bars represent S.E.M. (d) Differential pattern similarity during fear conditioning predicted the later expression of fear. Similarly, a rise in salivary alpha-amylase (sAA) levels prior to fear conditioning predicted both (e) the retention of fear and (f) generalization of fear as indexed by differential pupil dilation. (g) Finally, a relation was observed between differential pattern similarity and percentage change in sAA from T0 to T2, suggesting that changes in neural response patterns at the time of learning reflect the noradrenergic enhancement of fear memory consolidation. ACC, anterior cingulate cortex.

trial-by-trial change in pupil dilation in response to the CS+, relative to the CS− ($F_{12,\ 456} = 13.98$, $p < 0.0005$, $\eta_P^2 = 0.27$). Follow-up tests revealed strong main effects of stimulus type ($F_{1,\ 38} = 117.86$, $p < 0.0005$, $\eta_P^2 = 0.76$) indicating that pupil size increased when a CS+ was presented. Retention of fear was evident from a main effect of stimulus type on the three retention trials ($F_{1,\ 38} = 206.03$, $p < 0.0005$, $\eta_P^2 = 0.84$). Extinction of fear was evident from a significant interaction of all extinction trials x stimulus type ($F_{\ 12,\ 456} = 16.03$, $p < 0.0005$, $\eta_P^2 = 0.30$). Finally there was reinstatement of fear ($F_{1,\ 38} = 6.95$, $p = 0.012$, $\eta_P^2 = 0.16$), and generalization of fear ($F_{1,\ 38} = 6.49$, $p = 0.015$, $\eta_P^2 = 0.15$).

3.3. Fear-potentiated startle responses

As a second index for the retention of conditioned fear differential startle responses were assessed during Session 3 (weeks 3—5) through a test of renewal and reacquisition of fear ($n = 40$, Fig. 3b). Retention of fear was evident

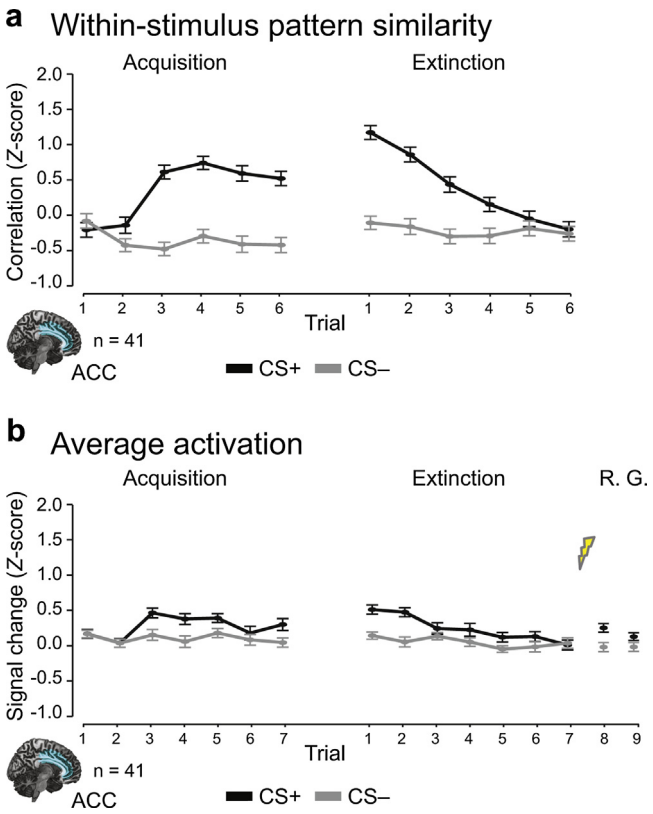

Figure 4 (a) Within-stimulus pattern similarity and (b) average activation showing clear acquisition and extinction of fear in the anterior cingulate cortex (ACC), although effect sizes are substantially smaller for average activation than for pattern similarity. Note that for the assessment of within-stimulus pattern similarity at least two trials for each stimulus are needed (Fig. 2c). Given that there was only one trial to test for reinstatement (R.) and one trial to test for generalization (G.) of fear, assessment of within-stimulus pattern similarity was not possible at these retention tests. We did not find a relation between within-stimulus pattern similarity or average activation and indices of noradrenergic activation (i.e., salivary alpha-amylase). Error bars represent S.E.M.

from a main effect of stimulus type on the renewal trials ($F_{1,\,39} = 22.22$, $p < 0.0005$, $\eta_P^2 = 0.36$). Fear seemed to persist in the reacquisition phase, as evidenced by strong main effects of stimulus type ($F_{1,\,39} = 49.39$, $p < 0.0005$, $\eta_P^2 = 0.56$).

3.4. Neural pattern similarity

As a neural marker for the formation and retention of fear associations, changes in similarity between patterns of BOLD MRI were examined over trials ($n = 41$). Consistent with previous work (Visser et al., 2011, 2013), the application of trial-by-trial similarity analysis revealed clear learning curves that indexed the formation of fear (for example the ACC, Figs. 3c and 4a and Supplementary Figs. 2 and 3), as evidenced by greater pattern similarity within and between CS+ stimuli compared to CS− stimuli in all ROIs (main effect of stimulus type: p's < 0.0005, η_P^2 ranging from 0.24 to 0.75). The interaction of stimulus type and learning trials reached significance in ACC, insula and SFG (p's < 0.0005, η_P^2 ranging from 0.12 to 0.32). Retention of fear was evident in all ROIs (p's < 0.0005, η_P^2 ranging from 0.34 to 0.68) and an interaction of stimulus type and extinction trials was observed in all areas (p's ≤ 0.044, η_P^2 ranging from 0.04 to 0.24), except for between-stimulus similarity in the vmPFC ($p = 0.100$). Reinstatement of fear (only tested for between-stimulus pattern similarity, see Section 2.6) was observed in amygdala and insula ($p = 0.04$ and $p = 0.013$, respectively), but did not survive correction for the number of ROIs tested. In contrast with the pupil data, no area showed a significant difference between CS+ and CS− on the generalization trial (p's > 0.258). For an overview of the statistics per ROI see Supplementary Tables 1–3.

3.5. Univariate analyses

Single-trial univariate analysis ($n = 41$, Fig. 4b) and univariate whole-brain analysis (Supplementary Tables 4 and 5) showed a typical activation in areas in the 'salience-network' in response to the CS+ compared to the CS−. During fear acquisition, higher activation was observed in response to CS+ compared to CS− stimuli in ACC (Fig. 4b) and insula (p's < 0.0005, $\eta_P^2 = 0.37$ and 0.41, respectively), as well as an interaction of stimulus type and trials (p's ≤ 0.042, $\eta_P^2 = 0.05$ and 0.08, respectively). During Session 2, retention of fear was observed in ACC, amygdala and insula (p's ≤ 0.004, η_P^2 ranging from 0.19 to 0.58) as well as an interaction of stimulus type and extinction trials (p's < 0.014, η_P^2 ranging from 0.06 to 0.14). For an overview of the statistics per ROI see Supplementary Tables 1–3. The finding that effect sizes were substantially smaller for average activation than for pattern similarity is consistent with previous results (Visser et al., 2011, 2013). This again shows the high sensitivity of pattern analysis compared to analysis of average activation for quantifying the changes in fear associations over time.

3.6. Markers for future fear memory

Consistent with previous findings (Visser et al., 2013), the behavioral expression of fear memory (i.e., differential pupil dilation during Session 2) was predicted from between-stimulus pattern similarity during learning, in ACC ($n = 39$, Fig. 3d; $r = 0.375$; $p = 0.019$), insula ($r = 0.299$; $p = 0.064$) and vmPFC ($r = 0.330$; $p = 0.040$). However, the effects were smaller in the current experiment and did not survive correction for the number of tested ROIs (for an overview of the statistics per ROI see Supplementary Tables 6 and 7). While in our previous study (Visser et al., 2013) pupil dilation during learning was unrelated to pupil dilation at test, we observed a trend significant relation between the two in the current study ($r = 0.278$; $p = 0.086$). These discrepancies are likely due to the differences in interval between learning and testing (2 days in the current study versus 2–6 weeks in Visser et al., 2013). In line with this, only 3 out of the 39 participants showed a stronger pupil response to a CS− compared to a CS+ at the retention trials, whereas 16 out of 38 participants showed this reverse responding in our previous study (Visser et al., 2013). This suggests that the short interval between learning and testing resulted in a ceiling effect. Notably, pattern

similarity was not related to differential startle responses (all $p > 0.091$), indicating that neural pattern similarity is not predictive of all types of fear memory and illustrating once more that response systems tend to dissociate (e.g., Bradley et al., 2008; Mauss and Robinson, 2009; Soeter and Kindt, 2010; Shackman et al., 2013; Weike et al., 2007).

3.7. Relation between noradrenergic activation and fear memory

We conducted exploratory analyses to investigate the relation between noradrenergic activity and associative fear learning and memory. A strong increase was observed from T0 to T2 and from T0 to T3 (Table 1), indicating that the scanner and/or the conditioning procedure itself might have provoked psychological stress (Bosch et al., 1996; Chatterton et al., 1996; Van Stegeren et al., 2006, but see Bosch et al., 2011 for a comprehensive review on the interpretation of sAA values). It is conceivable that scanner-induced stress increased noradrenergic activity and obscured any additional effects of the yohimbine manipulation on sAA levels, brain activation and behavioral measures of fear (Supplementary material).

The question that remained was whether this increase in noradrenergic activity, either manipulated or induced by the scanning procedure, had a role in the formation of fear associations. To answer this question, we correlated percentages change in sAA with conditioning- and retention-indices calculated for pupil dilation responses ($n = 31$). This revealed a positive correlation between percentage increase in sAA levels from T0 to T2 and retention (Fig. 3e; $r = 0.519$, $p = 0.003$), and generalization of fear (Fig. 3f; $r = 0.612$; $p < 0.0005$). Interestingly, this increase in sAA was unrelated to the conditioning-index ($p = 0.108$), indicating that an increase in noradrenergic activation was not *behaviorally* expressed (i.e., pupil dilation) at the time of learning. On the other hand, percentage increase in sAA levels from T0 to T2 correlated with *neural* conditioning-indices calculated for between-stimulus pattern similarity in ACC (Fig. 3g) and insula ($n = 33$; $r = 0.442$, $p = 0.010$ and $r = 0.387$, $p = 0.026$, respectively, uncorrected for the number of tested ROIs), but not with within-stimulus pattern similarity (p's > 0.087) or average activation (p's > 0.190).

To further examine the association between noradrenergic activation and between-stimulus pattern similarity in relation to memory strength, we performed a standard multiple regression with pupil dilation as the dependent variable (retention and generalization of fear in two separate models), and pattern similarity and percentage change in sAA from T0 to T2 as independent variables (Table 2). When both independent variables are included in the model, only sAA levels contribute significantly to the regression. This suggests that the noradrenergic modulation of fear memory may indeed be (partially) reflected in changes in pattern similarity at the time of encoding.

Finally, we explored the relation between noradrenergic activation *after* encoding and later fear memory. As the assumption of normality was violated for percentages increase in sAA levels from T0 to T3, we performed a Spearman correlation, revealing a relation with generalization ($\rho = 0.39$; $p = 0.028$), but not with retention ($\rho = 0.131$) of fear, suggesting that rises in noradrenaline *after learning* only had a moderate effect on the strength of memory. Where T3 marked the end of the first session, T2 marked the start of the scanning procedure and sAA levels at that time point presumably reflect anticipatory arousal. It seems that this anticipatory arousal may have affected the strength of the fear memory traces, while simultaneously concealing the effects of the pharmacological manipulation.

4. Discussion

The aim of the current study was to investigate whether changes in neural response patterns at the time of learning are a marker for memory formation and whether these changes are related to noradrenergic activation. Replicating our recent findings (Visser et al., 2013), between-stimulus pattern similarity in ACC, insula and vmPFC predicted the behavioral expression of fear (pupil dilation) 2 days later. Furthermore, despite our failed attempt to experimentally manipulate noradrenergic activation, results showed that a rise in sAA levels prior to scanning predicted the later expression of fear, which is in line with the putative role of noradrenaline in memory consolidation. Whereas a rise in sAA was not expressed in pupil dilation, within-stimulus pattern similarity or average activation at the time of encoding, it seemed that the noradrenergic modulation of later memory expression was already reflected in between-stimulus pattern similarity at that time. These results tentatively suggest that changes in neural response patterns during learning, specifically those that reflect higher-order fear learning (between-stimulus pattern similarity), uniquely mark the subsequent changes in synaptic structure that underlie (enhanced) consolidation.

These data again underscore the superior sensitivity of multi-voxel pattern analysis (MVPA) compared to analysis of average activation (Haxby et al., 2001; Haynes and Rees, 2005), and illustrate its heuristic value for the study of memory (Rissman and Wagner, 2012, for a review) and fear learning in particular (Bach et al., 2011; Dunsmoor et al., 2013; Li et al., 2008; Visser et al., 2011, 2013). Furthermore, the superior sensitivity of MVPA allows us to monitor the development of fear associations on a trial-by-trial basis. Whereas single-trial analysis is standard for behavioral data in the majority of animal and human fear-conditioning studies, it is still very uncommon in neuroimaging research due to the low signal-to-noise ratio of the BOLD-signal. Having a neural measure that is sensitive enough for single-trial analysis brings us one step closer to bridging the gap between behavioral and neuroimaging studies on associative fear learning and memory. In general, these findings emphasize the utility of neuroimaging research in the search for markers for memory.

A limitation of the current study is that we cannot make any causal inferences with regard to the relation between memory strength, changes in response patterns and noradrenergic activation, as our pharmacological manipulation failed (see Supplementary material). Although the dosage of yohimbine HCl was the same as used in lab experiments (Soeter and Kindt, 2011, 2012) and scanner experiments (van Stegeren et al., 2010) only systolic blood pressure and not diastolic blood pressure increased over time in the

Table 2 Standard multiple regression ($n = 31$) of between-stimulus pattern similarity and percentage change in salivary α-amylase (sAA) on retention and generalization of fear (pupil dilation).

	Bivariate correlation with retention pupil	p	β	sr^2 (semipartial correlation, unique)	p
Between-stimulus similarity ACC	0.448[a]	0.011	0.267	0.055	0.137
Change in sAA from T0 at T2 (%)	0.519	0.003	0.397	0.125	0.031
			Full model: $F_{2,28} = 6.75$, $p = 0.004$, $R^2 = 0.33$		
	Bivariate correlation with generalization pupil	p	β	sr^2 (semipartial correlation, unique)	p
Between-stimulus similarity ACC	0.425[a]	0.017	0.183	0.027	0.136
Change in sAA from T0 at T2 (%)	0.612	<0.0005	0.528	0.221	0.003
			Full model: $F_{2,28} = 9.39$, $p = 0.001$, $R^2 = 0.40$		

Two separate regression models are presented, with retention of fear (pupil dilation; top) and generalization of fear (pupil dilation; bottom) as the dependent variables. In both models the independent variables are between-stimulus pattern similarity and percentage change in salivary α-amylase (sAA).

[a] These correlation coefficients deviate from the values reported in the main text and Fig. 3d and g, due to the list-wise inclusion of participants that have data for all three variables (i.e., $n = 31$). Bivariate correlations between both independent variables ($r = 0.457$, $p = 0.010$) and between each independent variable and the dependent variable remain significant. ACC, anterior cingulate cortex.

yohimbine group. And even though there was an increase in sAA over time in the yohimbine group, this increase was equally strong in the placebo group, which clearly deviates from what has been observed before (Soeter and Kindt, 2011, 2012). The question is why the placebo group showed this steep increase in sAA levels. It may be that the MRI-scanning procedure in itself already evoked a stress response, as supported by previous studies that found rises in sAA and/or cortisol (e.g., Eatough et al., 2009; Muelhan et al., 2011; Peters et al., 2011). Specifically, other studies on emotional memory have observed moderate (van Stegeren et al., 2010) to strong (van Stegeren et al., 2006) rises in sAA levels in the placebo group prior to scanning, accompanied by a lack of an effect of yohimbine alone (i.e., without cortisol administration) on declarative memory performance (van Stegeren et al., 2010), which the authors attributed to scanner-related sympathetic arousal. Compared to solely being exposed to emotional pictures, the anticipation of a fear-conditioning procedure may be even more arousing for naïve participants. Very few studies have combined fear conditioning (using electrical stimulation), MRI scanning and a pharmacological manipulation. This combination may have created a stressful environment, thereby boosting noradrenaline levels and concealing any additional effects of the pharmacological agent.

A putative ceiling effect in noradrenergic activation could also explain the lack of group effects on the dependent variables of interest. Both pupil dilation and neural pattern similarity showed strong differential fear responses during each experimental phase. In previous reports, extinction of fear was completed after two trials in the placebo condition (Soeter and Kindt, 2011, 2012), versus 6 (Soeter and Kindt, 2012) or 11 (Soeter and Kindt, 2011) in the yohimbine condition. In comparison, extinction in the current study required eight trials in the placebo condition versus 10 trials in the yohimbine condition. These observations suggest that it is not the absence of a strong fear memory in the yohimbine condition, but rather a stronger-than-expected fear memory in the placebo condition that accounts for the negative results. Future studies should consider implementing a training phase inside a (mock) scanner prior to the actual experiment to reduce scanner related fear responding (Chapman et al., 2010; Lueken et al., 2012).

Another issue that may be marked as a potential weakness of the study is that the pupil dilation response is not a specific index of fear, as pupils have been found to dilate in response to positively arousing pictures as well (Bradley et al., 2008). We acknowledge that most physiological measures used in fear-conditioning paradigms (e.g., skin conductance, acoustic startle reflex, heart rate) measure certain aspects of the defensive response system, and may not necessarily relate to conscious feelings or other (behavioral) aspects of fear (e.g., Beckers et al., 2013; LeDoux, 2014; Mauss and Robinson, 2009). As it is unlikely that our experiment induced positive emotions, we do believe that in this particular setting pupil dilation responses reflected a type of negative autonomic arousal that is at least related to fear, regardless of whether these responses indexed the actual subjective experience of fear, increased orienting to threatening stimuli, or perhaps even action preparation (e.g., Reinhard and Lachnit, 2002). In any case, it seems that pupil dilation does not merely reflect contingency learning, as no pupil dilation was observed for a control stimulus (CS that co-terminated with a neutral sound) in our previous study (Visser et al., 2013). This is not to say that neural pattern similarity is an exclusive marker for the formation

of fear memory. Future research should establish whether neural pattern similarity is predictive of all types of arousing memories.

To recap, although our pharmacological manipulation failed, we obtained indirect evidence for the noradrenergic enhancement of fear memory at the time of encoding. These results may have important clinical implications. Based on the assumption that the transition from normal fear to pathological fear occurs in the aftermath of the actual learning experience, many contemporary interventions are aimed at secondarily preventing the development of disorders such as post-traumatic stress disorder (PTSD), for example by pharmacologically disrupting post-trauma consolidation processes (Steckler and Risbrough, 2012, for a review). These interventions are, however, not always effective (Cohen et al., 2011; Hoge et al., 2012; Stein et al., 2007) and require that people have access to treatment within a few hours after the trauma. The present findings suggest that the *strengthening* of a fear memory is already initiated at the time of encoding and support the view that time-dependent stages of memory formation are based on independent processes acting in parallel (McGaugh, 2000). Moreover, these parallel processes seem independently influenced by phasic increases in noradrenergic activity (McGaugh and Roozendaal, 2002; Sara, 2009). This raises the question whether post-trauma interventions may in fact be too late to interfere with the cascade of events that may eventually lead to psychopathology. Further research is needed to establish whether primary interventions (e.g., keeping noradrenaline levels as low as possible when the risk of experiencing aversive events is high) are a promising alternative in the prevention of PTSD.

Role of the funding sources

This study was funded by a Vici grant (Merel Kindt, #453-07-006) from the Netherlands Organization for Scientific Research (NWO) and the Research Priority Area on Brain and Cognition of the University of Amsterdam.

Conflict of interest statement

The authors declare no conflict of interests.

Acknowledgments

The authors would like to thank M. Spaan and B. Molenkamp for technical support, J. Bosch and C. Kirschbaum for the saliva-analysis, P. Vonk for supervising the medical aspects of the study and A. Eigenhuis for blinding and counterbalancing of the experimental procedure and statistical advice.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2015.01.021.

References

Bach, D.R., Weiskopf, N., Dolan, R.J., 2011. A stable sparse fear memory trace in human amygdala. J. Neurosci. 31, 9383—9389.

Beckers, T., Krypotos, A.-M., Boddez, Y., Effting, M., Kindt, M., 2013. What's wrong with fear conditioning? Biol. Psychol. 92, 90—96.

Benjamini, Y., Hochberg, Y.J., 1995. Controlling the false discovery rate — a practical and powerful approach to multiple testing. J. R. Stat. Soc. B: Methodol. 57, 289—300.

Blumenthal, T.D., Cuthbert, B.N., Filion, D.L., Hackley, S., Lipp, O.V., van Boxtel, A., 2005. Committee report: guidelines for human startle eyeblink electromyographic studies. Psychophysiology 42, 1—15.

Bosch, J.A., Brand, H.S., Ligtenberg, T.J., Bermond, B., Hoogstraten, J., Nieuw Amerongen, A.V., 1996. Psychological stress as a determinant of protein levels and salivary-induced aggregation of *Streptococcus gordonii* in human whole saliva. Psychosom. Med. 58, 374—382.

Bosch, J.A., Veerman, E.C., de Geus, E.J., Proctor, G.B., 2011. α-Amylase as a reliable and convenient measure of sympathetic activity: don't start salivating just yet! Psychoneuroendocrinology 36, 449—453.

Bradley, M.M., Miccoli, L., Escrig, M.A., Lang, P.J., 2008. The pupil as measure of emotional arousal and autonomic activation. Psychophysiology 45, 602—707.

Cahill, L., Alkire, M.T., 2003. Epinephrine enhancement of human memory consolidation: interaction with arousal at encoding. Neurobiol. Learn. Mem. 79, 194—198.

Cahill, L., Prins, B., Weber, M., McGaugh, 1994. Beta-adrenergic activation and memory for emotional events. Nature 371, 702—704.

Chapman, H.A., Bernier, D., Rusak, B., 2010. MRI-related anxiety levels change within and between repeated scanning sessions. Psychiatry Res. Neuroimaging 182, 160—164.

Chatterton Jr., R.T., Vogelsong, K.M., Lu, Y.C., Ellman, A.B., Hudgens, G.A., 1996. Salivary alpha-amylase as a measure of endogenous adrenergic activity. Clin. Physiol. 16, 433—448.

Cohen, H., Kaplan, Z., Koresh, O., Matar, M.A., Geva, A.B., Zohar, J., 2011. Early post-stressor intervention with propranolol is ineffective in preventing posttraumatic stress responses in an animal model of PTSD. Eur. Neuropsychopharmacol. 21, 230—240.

Dunsmoor, J.E., Kragel, P.A., Martin, A., LaBar, K.S., 2013. Aversive learning modulates cortical representations of object categories. Cereb. Cortex, 1—14.

Eatough, E.M., Shirtcliff, E.A., Hanson, J.K., Pollak, S.D., 2009. Hormonal reactivity to MRI scanning in adolescents. Psychoneuroendocrinology 34, 1242—1246.

Fridlund, A.J., Cacioppo, J.T., 1986. Guidelines for human electromyographic research. Psychophysiology 23, 567—589.

Gazarini, L., Stern, C.A., Carobrez, A.P., Bertoglio, L.J., 2013. Enhanced noradrenergic activity potentiates fear memory consolidation and reconsolidation by differentially recruiting α1- and β-adrenergic receptors. Learn. Mem. 20, 210—219.

Gazarini, L., Stern, C.A., Piornedo, R.R., Takahashi, R.N., Bertoglio, L.J., 2014. PTSD-like memory generated through enhanced noradrenergic activity is mitigated by a dual step pharmacological intervention targeting its reconsolidation. Int. J. Neuropsychopharmacol. 18.

Haxby, J.V., Gobbini, M.I., Furey, M.L., Ishai, A., Schouten, J.L., Pietrini, P., 2001. Distributed and overlapping representations of faces and objects in ventral temporal cortex. Science 293, 2425—2430.

Haynes, J.D., Rees, G., 2005. Predicting the orientation of invisible stimuli from activity in human primary visual cortex. Nat. Neurosci. 8, 686—691.

Hedler, L., Stamm, G., Weitzell, R., Starke, K., 1981. Functional characterization of central α-adrenoceptors by yohimbine diastereomers. Eur. J. Pharmacol. 70, 43—52.

Hoge, E.A., Worthington, J.J., Nagurney, J.T., Chang, Y., Kay, E.B., Feterowski, C.M., Katzman, A.R., Goetz, J.M., Rosasco, M.L., Lasko, N.B., Zusman, R.M., Pollack, M.H., Orr, S.P., Pitman, R.K., 2012. Effect of acute posttrauma propranolol on PTSD outcome and physiological responses during script-driven imagery. CNS Neurosci. Ther. 18, 21—27.

Hurlemann, R., Hawellek, B., Matusch, A., Kolsch, H., Wollersen, H., Madea, B., Vogeley, K., Maier, W., Dolan, R.J., 2005. Noradrenergic modulation of emotion-induced forgetting and remembering. J. Neurosci. 25, 6343—6349.

Kriegeskorte, N., Mur, M., Bandettini, P., 2008. Representational similarity analysis — connecting the branches of systems neuroscience. Front. Syst. Neurosci. 2, 1—28.

LaBar, K., Cabeza, R., 2006. Cognitive neuroscience of emotional memory. Nat. Rev. Neurosci. 7, 54—64.

LaLumiere, R.T., Buen, T.V., McGaugh, J.L., 2003. Post-training intra-basolateral amygdala infusions of norepinephrine enhance consolidation of memory for contextual fear conditioning. J. Neurosci. 23, 6754—6758.

Langer, S.Z., 1974. Presynaptic regulation of catecholamine release. Biochem. Pharmacol. 23, 1793—1800.

LeDoux, J.E., 2014. Coming to terms with fear. Proc. Natl. Acad. Sci. U. S. A. 111, 2871—2878.

Li, W., Howard, J.D., Parrish, T.B., Gottfried, J.A., 2008. Aversive learning enhances perceptual and cortical discrimination of indiscriminable odor cues. Science 319, 1842—1845.

Lueken, U., Muehlan, M., Evens, R., Wittchen, H.-U., Kirschbaum, C., 2012. Within and between session changes in subjective and neuroendocrine stress parameters during magnetic resonance imaging: a controlled scanner training study. Psychoneuroendocrinology 37, 1299—1308.

Lundqvist, D., Flykt, A., Öhman, A., 1998. The Karolinska Directed Emotional Faces — KDEF. CD ROM from Department of Clinical Neuroscience, Psychology Section, Karolinska Institutet, ISBN 91-630-7164-9.

Mauss, I.B., Robinson, M.D., 2009. Measures of emotion: a review. Cogn. Emot. 23, 209—237.

McGaugh, J.L., 2000. Memory — a century of consolidation. Science 287, 248—251.

McGaugh, J.L., Roozendaal, B., 2002. Role of adrenal stress hormones in forming lasting memories in the brain. Curr. Opin. Neurobiol. 12, 205—210.

Muelhan, M., Lueken, U., Wittchen, H.U., Kirschbaum, C., 2011. The scanner as a stressor: evidence from subjective and neuroendocrine stress parameters in the time course of a functional magnetic resonance imaging session. Int. J. Psychophysiol. 79, 118—126.

Nater, U.M., Rohleder, N., 2009. Salivary alpha-amylase as a noninvasive biomarker for the sympathetic nervous system: current state of research. Psychoneuroendocrinology 34, 486—496.

Pape, H.C., Paré, D., 2010. Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. Physiol. Rev. 90, 419—463.

Peters, S., Cleare, A.J., Papadopoulos, A., Fu, C.H.Y., 2011. Cortisol responses to serial MRI scans in healthy adults and in depression. Psychoneuroendocrinology 36, 737—741.

Peterson, R.A., Reiss, S., 1992. Anxiety Sensitivity Index Revised Test Manual. International Diagnostic Systems, Worthington, OH.

Reinhard, G., Lachnit, H., 2002. Differential conditioning of anticipatory pupillary dilation responses in humans. Biol. Psychol. 60, 51—68.

Reinhard, G., Lachnit, H., Konig, S., 2006. Tracking stimulus processing in Pavlovian pupillary conditioning. Psychophysiology 43, 73—83.

Rissman, J., Wagner, A.D., 2012. Distributed representations in memory: insights from functional brain imaging. Annu. Rev. Psychol. 63, 101—128.

Sara, S.J., 2009. The locus coeruleus and noradrenergic modulation of cognition. Nat. Rev. Neurosci. 10, 211—223.

Shackman, A.J., Fox, A.S., Oler, J.A., Shelton, S.E., Davidson, R.J., Kalin, N.H., 2013. Neural mechanisms underlying heterogeneity in the presentation of anxious temperament. Proc. Natl. Acad. Sci. U. S. A. 110, 6145—6150.

Soeter, M., Kindt, M., 2010. Dissociation response systems: erasing fear from memory. Neurobiol. Learn. Mem. 94, 30—41.

Soeter, M., Kindt, M., 2011. Noradrenergic enhancement of associative fear memory in humans. Neurobiol. Learn. Mem. 96, 263—271.

Soeter, M., Kindt, M., 2012. Stimulation of the noradrenergic system during memory formation impairs extinction learning but not the disruption of reconsolidation. Neuropsychopharmacology 37, 1204—1215.

Southwick, S.M., Davis, M., Horner, B., Cahill, L., Morgan III, C.A., Gold, P.E., Bremner, J.D., Charney, D.C., 2002. Relationship of enhanced norepinephrine activity during memory consolidation to enhanced long-term memory in humans. Am. J. Psychiatry 159, 1420—1422.

Spielberger, C.D., 1983. The Manual for the State-Trait Inventory. Consulting Psychologists Press, Palo Alto, CA.

Steckler, T., Risbrough, V., 2012. Pharmacological treatment of PTSD — established and new approaches. Neuropharmacology 62, 617—627.

Stein, M.B., Kerridge, C., Dimsdale, J.E., Hoyt, D.B., 2007. Pharmacotherapy to prevent PTSD: results from a randomized controlled proof-of-concept trial in physically injured patients. J. Trauma. Stress 20, 923—932.

Strange, B.A., Dolan, R.J., 2004. β-Adrenergic modulation of emotional memory-evoked human amygdala and hippocampal responses. Proc. Natl. Acad. Sci. U. S. A. 101, 11454—11458.

van Stegeren, A.H., Rohleder, N., Everaerd, W., Wolf, O.T., 2006. Salivary alpha amylase as marker for adrenergic activity during stress: effect of betablockade. Psychoneuroendocrinology 31, 137—141.

van Stegeren, A.H., Roozendaal, B., Kindt, M., Wolf, O.T., Joels, M., 2010. Interacting noradrenergic and corticosteroid systems shift human brain activation patterns during encoding. Neurobiol. Learn. Mem. 93, 56—65.

Visser, R.M., Scholte, H.S., Beemsterboer, T., Kindt, M., 2013. Neural pattern similarity predicts long-term fear memory. Nat. Neurosci. 16, 388—390.

Visser, R.M., Scholte, H.S., Kindt, M., 2011. Associative learning increases trial-by-trial similarity of BOLD-MRI patterns. J. Neurosci. 31, 12021—12028.

Weike, A.I., Schupp, H.T., Hamm, A.O., 2007. Fear acquisition requires awareness in trace but not delay conditioning. Psychophysiology 44, 170—180.

Wemer, J., Van der Lugt, J.C., De Langen, C.D., Mulder, A.H., 1979. On the capacity of presynaptic alpha receptors to modulate norepinephrine release from slices of rat neocortex and the affinity of some agonists and antagonists for these receptors. J. Pharmacol. Exp. Ther. 211, 445—451.

Woolrich, M., Ripley, B., Brady, J., Smith, S., 2001. Temporal autocorrelation in univariate linear modelling of FMRI data. Neuroimage 14, 1370—1386.