Classically conditioned fear responses are preserved following unilateral temporal lobectomy in humans when concurrent US-expectancy ratings are used

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1. Introduction

Over the past decades, much progress has been made in understanding the neural circuit underlying fear and fear acquisition. Until today, most of the evidence stems from animal research, using a traditional fear conditioning paradigm in which an originally neutral stimulus (conditioned stimulus, CS) is paired a few times with an aversive event (e.g., electric shock, unconditioned stimulus, US). These studies, mostly conducted in rats, identified the amygdala, a brain area located in the anterior medial temporal lobes, as the key structure in the acquisition, storage, and expression of conditioned fear responses (e.g., Davis, 2000; LeDoux, 1996). In the late nineties, the introduction of brain imaging technology confirmed that the human amygdala is also involved in fear acquisition (Büchel, Dolan, Armony, \\& Friston, 1999; Büchel, Morris, Dolan, \\& Friston, 1998; LaBar, Gatenby, Gore, LeDoux, \\& Phelps, 1998). LaBar et al. (1998), for example, observed increased amygdala activation in response to a neutral stimulus (CS+) that was previously paired with an aversive event (US) compared to a second neutral stimulus (CS−) that never co-appeared with an aversive stimulus (US). Additionally, the conditioned skin conductance response (SCR) measured during CS+ presentations correlated positively with the magnitude of amygdala activation, suggesting that the amygdala plays an important role in fear conditioning in humans. Interestingly, however, amygdala activity was limited to the early stages of fear learning, when stimulus contingencies change (Büchel et al., 1998, 1999; Knight, Smith, Cheng, Stein, \\& Helms, 2004; LaBar et al., 1998).

Although imaging studies offer support for a role of the amygdala in fear learning in humans, lesion studies examining uni-
laterally or bilaterally amygdala-damaged patients are required in order to indicate whether the amygdala is also critically involved in this process. LaBar, LeDoux, Spencer, and Phelps (1995) were the first to present a classical fear conditioning task to a group of patients who sustained a unilateral temporal lobe resection. In line with animal research, the patient group showed diminished conditioned SCRs to an aversively conditioned stimulus compared to a healthy control group. Remarkably, most of the patients demonstrated normal unconditioned responses (URs) to the unconditioned stimulus (US) and acquired correct declarative knowledge about the association between the CS and the US. Corroborating evidence came from Bechara et al. (1995) who observed a complete absence of conditioned SCRs, despite intact knowledge of the CS–US association and normal URs in a bilaterally amygdala-damaged patient. In contrast, however, a patient with bilateral damage of the hippocampus was reported to show normal conditioned SCRs, while being unable to report the stimulus association. Together, these findings suggest a crucial role of the amygdala in the generation of conditioned autonomic responses, whereas the hippocampus is more important for the acquisition of declarative knowledge regarding the CS–US association. Finally, results of a more recently conducted lesion study (Weike et al., 2005) further confirmed an impairment of conditioned SCRs in unilaterally amygdala-damaged patients during classical fear conditioning. Different from LaBar et al. (1995), however, the patient group was less contingency aware than the controls. Moreover, the extent of differential SCRs in the patient as well as in the control group appeared to be modulated by the acquisition of declarative knowledge regarding the CS–US association. More specifically, controls as well as patients who correctly recognized the stimulus contingency exhibited intact conditioned SCRs, whereas those who were unable to verbalize the correct CS–US association showed impaired conditioned SCRs. Therefore, the findings of Weike et al. (2005) seem to suggest that the reduction of differential SCRs observed in unilaterally amygdala-damaged patients was actually caused by the fact that they were less contingency aware than controls. An important addition of the Weike et al. (2005) study is that next to the SCRs, startle responses were recorded. Irrespective of stimulus contingency knowledge, conditioned startle potentiation was diminished in all patients, suggesting that the amygdala is critically involved in the production of conditioned startle responses during fear learning. According to Weike et al. (2005), these data reveal a dissociation between fear learning as indexed by conditioned startle potentiation and explicit learning of the stimulus relationship as indexed by conditioned SCRs, supporting a two-level account of human fear conditioning (Ohman & Mineka, 2001). Based upon this account, fear learning can occur at a lower and higher level, with the former referring to a basic associative level of learning in which subjects learn to fear the CS. The higher level refers to learning of the stimulus contingency at a more cognitive level in which participants learn that one CS predicts the occurrence of the US (propositional learning, Lovibond & Shanks, 2002). Moreover, the dual-level theory proposes that low level learning is mediated by the amygdala, whereas high level learning is centered on the hippocampus for simple CS–US associations and the prefrontal cortex for more complicated stimulus relationships (Ohman & Mineka, 2001).

In sum, the lesion studies described so far (Bechara et al., 1995; LaBar et al., 1995; Weike et al., 2005) yield relatively inconsistent findings concerning the amygdala’s role in conditioned SCRs during fear learning. The findings of LaBar et al. (1995) and Bechara et al. (1995) suggest that the amygdala is crucial for the production of conditioned SCRs irrespective of stimulus contingency awareness, whereas the data of Weike et al. (2005) propose that the impaired conditioned SCRs observed in the unilaterally amygdala-damaged patients were related to their reduced CS–US knowledge rather than to their damaged amygdala. However, Weike et al. (2005) used a postexperimental questionnaire for the assessment of contingency awareness, which was administered only once at the end of the conditioning task and after a relatively long extinction phase. Forgetting or new learning may have interfered with recalling the CS–US association, especially in the patient group, as they can be forgetful after their surgery. As such, the retrospective test used could have been somewhat insensitive, underestimating the actual number of participants that was aware during acquisition. As suggested by Lovibond and Shanks (2002), a concurrent contingency awareness measure administered during the conditioning phase itself would be a more adequate method for assessing CS–US awareness.

Recently, Carter, O’Doherty, Seymour, Koch, and Dolan (2006) examined the neurological basis of fear conditioning in a functional imaging scan using such a concurrent awareness measure. Next to SCRs, contingency awareness was registered continuously throughout the whole experiment via US-expectancy ratings. The primary goal of Carter et al. (2006) was to identify brain regions that were specifically related to the explicit acquisition of contingency awareness on the one hand and the generation of conditioned SCRs on the other hand. The fMRI data revealed that activation in the middle frontal gyrus and the parahippocampal gyrus correlated with contingency awareness, whereas amygdala activation correlated with conditioned SCRs. According to Carter et al. (2006), these findings dissociate distinct roles of the middle frontal gyrus and the amygdala during fear conditioning, suggesting that the amygdala is especially involved in generating conditioned SCRs. Consistent imaging findings were obtained by Cheng, Knight, Smith, and Helmstetter (2006), who used a differential fear conditioning task, with SCRs and US-expectancy ratings being registered online. Prior to the imaging analysis and based upon the SCRs registered, all CS+ trials were classified as either trials eliciting increased SCRs (CS+response) or trials eliciting no increased SCRs (CS+non-response). Subsequent analyses revealed that amygdala activity increased during CS+response trials, but not during CS+non-response trials, suggesting that activity in the amygdala reflects the expression of learned autonomic responses. Interestingly, although differential responding in the amygdala was found for CS+response and CS+non-response trials, increased US-expectancy ratings were obtained on both trial types, implying that metabolic changes in the amygdala were more closely related to the observed SCR pattern than to the US-expectancy ratings given. Relatedly, Tabbert, Stark, Kirsch, and Viati (2006) manipulated awareness of the stimulus contingency association, creating an aware and unaware group. Similar to Weike et al. (2005), differential SCRs were obtained in the aware group, but not in the unaware group. Moreover, CS+ trials elicited enhanced responding of the amygdala in the unaware group, but increased activation of the anterior cingulate and the medial prefrontal cortex in the aware group. According to Tabbert et al. (2006), the CS+ trials differentially activated the fear network for unaware, but not for aware participants, supporting a model of fear conditioning that distinguishes between a more cognitive level of learning and activation of a fear network.

The general purpose of the current experiment is to gain more insight into the role of the amygdala in the generation of conditioned SCRs during human fear learning. In a previous study (Coppens, Van Paesschen, Vandenbulcke, & Vansteenkoven, unpublished data) we were able to replicate the earlier findings on impaired SCRs in unilaterally amygdala-damaged patients during a classical fear conditioning task (LaBar et al., 1995; Weike et al., 2005). Similar to Weike et al. (2005), however, fewer patients than controls were able to report the correct stimulus contingencies. In the present lesion study, we aimed to examine the amygdala’s role in the production of conditioned SCRs during a more explicit fear learning paradigm, when the presented CS–US association was more manifest. During the task, SCRs were recorded...
and participants were asked to predict the occurrence of the US continuously throughout the whole task. Methodologically, this has proven to be a more appropriate manner to measure stimulus contingency awareness (Lovibond & Shanks, 2002), allowing us to examine when CS–US knowledge is acquired in time. Additionally, such concurrent US-expectancy ratings direct participants’ attention towards the CS–US association, increasing chances for explicit learning to occur (Lovibond & Shanks, 2002). Based upon the recent fMRI findings, which show no amygdala involvement in the generation of US-expectancy ratings (Carter et al., 2006; Cheng et al., 2006), we expect patients with unilateral amygdala damage to show normal US-expectancy learning. Concerning the conditioned SCRs in the patient group, two opposing hypotheses can be formulated based upon the available literature. First, according to the imaging studies of Carter et al. (2006) and Cheng et al. (2006), the amygdala is activated during the production of SCRs when a more explicit fear learning task is used. As such, we expect unilaterally amygdala-damaged patients to show impaired or at least reduced conditioned SCRs compared to a healthy control group. Second, the dual-level account of Ohman and Mineka (2001) proposes that fear learning when occurring on a more cognitive level does not depend on the amygdala. As a consequence, unilaterally amygdala-damaged patients are supposed to show intact conditioned SCRs.

2. Method

2.1. Participants

The study included 16 patients who had undergone a standard unilateral anterior temporal lobectomy (at the University Hospital Gauthuysberg in Leuven) for the relief of intractable mesial temporal lobe epilepsy associated with unilateral hippocampal sclerosis (Van Paesschen, 2004) (13 left, 3 right; 6 females, 7 males). The medial resection of the anterior temporal lobe included the amygdala and 3.5 cm of the anterolateral part of the hippocampus (Fig. 1). Postoperatively, every patient underwent an MRI of the brain to confirm that the amygdala was removed. A pathological assessment of the resected brain tissue, however, revealed the presence of hippocampal sclerosis in every patient. All patients, who had experienced epileptic seizures from birth or early childhood, were since their operation seizure free for at least 2 years prior to the current study. On average, 4 years and 8 months (SD = 17.80 months) had elapsed between the chirurgical resection the patients underwent and their participation in the study. Although some patients were still on low to moderate doses of anticonvulsant medication at the time of testing, this appeared to have no influence on the patients’ conditioning performance. Twenty healthy participants who were matched to the lesion group with respect to age formed the control group. An ANOVA further confirmed that the difference in age between the lesion (M = 43.60 and SD = 7.86) and the control group (M = 45 and SD = 7.48) was statistically unreliable, F < 1. For one patient and three controls no SCRs were registered due to equipment failure. Hence they were omitted from the SCRs analyses. Finally, the experiment was approved by the Research and Ethics Committee of the University of Leuven, and all participants gave written informed consent before starting the experiment.

2.2. Stimulus material

Two pictures each depicting a geometric figure (square and trapezoid) served as the conditioned stimuli (CSs). They had a height of 24 cm and length of 16 cm each and were presented for 8 s against the black background of a 17 in. computer monitor (75 Hz) with a resolution of 640 × 480 pixels.

The unconditioned stimulus (US) consisted of a 500 ms electric pulse generated by a commercial stimulator (D57A, Digitimer, Welwyn Garden City, England) and delivered by Sensoromedics electrodes, which were filled with KY gel and attached to the participants’ right wrist. The intensity of the US was set at an idiosyncratic level that the subject experienced as “unpleasant and demanding some effort to tolerate.” Stimulus presentation as well as the registration of response latencies was controlled by Affect 4.0 (Hermans, Clarysse, Baeyens, & Spruyt, 2005; Spruyt, Clarysse, Vansteennonk, Baeyens, & Hermans, in press) and the experiment was run on an AMD Athlon processor 1.6 GHz.

2.3. Physiological recordings

Electrodermal activity was recorded from the hypothenar palm of the left hand, which was cleaned with tap water. A Coulbourn Instruments skin conductance coupler (V71-23) provided a constant voltage of 5 V across two standard Ag/AgCl electrodes (1 cm diameter), which were filled with KY gel. The signal was processed with a resolution of 0.01 μS and sampled with a rate of 10 Hz.

2.4. Procedure

On arrival at the laboratory, participants were informed that shocks and visual stimuli would be presented during the experiment. After signing the informed consent form, the sensors for physiological data recording were attached to the participant’s left hand palm and a short description concerning the recordings was given. Next, the electrodes for generating the electric stimulation were attached to the participant’s right wrist and the intensity of the US was adjusted individually to a level that participants described as highly annoying, but not painful. Participants were informed that during the experiment two pictures would be presented in randomized order and that one stimulus would sometimes be followed by an electric pulse (CS+), whereas a second picture would never be followed by an electric pulse (CS−). Finally, subjects were asked to relax and not to move during the test session.

The fear conditioning task consisted of three phases: A pre-acquisition phase, an acquisition phase and a post-acquisition phase. During the pre-acquisition phase, the CS+ and the CS− were presented each once, without US delivery. Subsequently, the acquisition phase followed, which consisted of eight CS+ trials, in which the CS was always followed by a US, and eight CS− trials, in which the CS was never paired with the US. On CS+ trials, the US appeared immediately at the offset of the CS (non-overlapping). The order of CS presentations was randomized with the restriction that no more than two trials of the same type were presented successively. Finally, in the post-acquisition phase, participants received once more a CS+ and a CS− presentation, here again without US occurrence. Every CS lasted for 8 s and between the CS presentations the intertrial interval (ITI) varied across trials from 15 till 19 s with a mean ITI of 17 s. During all phases, SCRs were recorded. Additionally, participants were asked to indicate continuously throughout the whole experiment to what extent they expected the shock to occur, using a custom-built dial operated by the participant’s right hand. The pointer of the dial could be turned 180° from 0 to 100, with 0 indicating that participants did not expect a shock at all and 100 that participants certainly expected the shock to occur. The expectancy dial generated an online analogue signal that was digitized at 10 Hz by an A/D converter and stored by the computer. Finally, at the end of the conditioning task, participants were asked to indicate on a scale ranging from −10 to +10 how intense, startling, and unpleasant they experienced the electric stimulation. In addition, unpleasantness ratings were obtained for the CS+ and the CS− as well.

2.5. Data reduction and response definition

Skin conductance responses were visually inspected and corrected for artifacts before they were analyzed statistically. Conditioned SCRs were defined as the largest increase in conductance between 1 and 4 s after picture onset (first interval response) (Proksa & Kumpfer, 1973). The unconditioned response was scored as the largest increase in responding starting between 1 and 4 s after the onset of the electric stimulus. Zero responses were included in all analyses. To reduce interindividual variability that was not related to the conditioning task, conditioned responses were range corrected by dividing each individual score by the participant’s maximum response elicited by the US (Lykken & Venables, 1971). Unconditioned responses were not range-corrected. Finally, a square-root transformation was performed on the range-corrected conditioned responses and the unconditioned responses in order to normalize the distribution (Levy, 1980).

The US-expectancy ratings were measured during all trials at a sample rate of 10 Hz, meaning that every second the position of the expectancy dial was registered. A mean was calculated over the 10 US-expectancy ratings recorded during the last second of the CS presentation.

2.6. Data-analysis

The US-expectancy ratings and the physiological data were analyzed at the trial level for each phase separately, which allowed us to examine more accurately the development of expectancy learning and SCR conditioning in time. The expectancy ratings and conditioned SCRs recorded in the pre- and post-acquisition phases were subjected to a 2 (CS: CS+ vs. CS−) × 2 (Group: controls vs. patients) mixed ANOVA with CS acting as a within-subjects variable and Group as a between-subjects variable. Additionally, since the ratio of left and right damaged patients was unequally distributed (13 left vs. 3 right), and the number of

2 An unpublished study, performed at our department, demonstrated that operating an expectancy dial does not increase skin conductance responding.
right-damaged patients was very limited; some non-parametric tests were executed on the conditioned SCRs and the US-expectancy ratings obtained in the acquisition phase in order to provide a preliminary notion of the effect of lesion site. Moreover, effects of gender were not obtained and hence are discarded from the results section. Finally, for all statistical tests a .05 level of statistical significance was used and violations of sphericity were corrected using the Greenhouse–Geisser correction.

3. Results

3.1. Unpleasantness ratings of the CS

A CS × Group mixed ANOVA on the CS+ and CS− unpleasantness ratings demonstrated a main effect of CS, F(1,34) = 14.52, p < .05, suggesting that the CS+ was experienced as less pleasant than the CS− after fear learning. Crucially, the two-way interaction between CS and Group failed to reach significance, F(1,34) = 1.11, p = .84. The patient group as well as the control group thought of the CS+ as less pleasant than the CS−, F(1,34) = 7.24, p < .05 and F(1,34) = 7.33, p < .05, respectively.

3.2. US-expectancy ratings

3.2.1. Pre-acquisition phase

According to the CS × Group mixed ANOVA, the main effect of CS failed to reach significance, F < 1, suggesting that the expectancy ratings on CS+ and CS− trials differed not significantly from each other prior to conditioning. Also, the two-way interaction between CS and Group yielded no significance, F < 1. Neither the control group nor the lesion group showed differential expectancy ratings on CS+ and CS− trials in the pre-acquisition phase, with F < 1 for both contrasts (see Fig. 2a and b).

3.2.2. Acquisition phase

The CS × Group × Acquisition Trial mixed ANOVA yielded significant main effects of CS, F(1,34) = 106.30, p < .001, and of Acquisition Trial, F(4,140) = 5.44, p < .001. Also the interaction between CS and Acquisition Trial was reliable, F(4,136) = 22.85, p < .001. As expected, on CS+ trials US expectancies increased significantly from the first to the last acquisition trial, F(1,34) = 65.13, p < .001, whereas on CS− trials US-expectancies decreased significantly from the first to the last acquisition trial, F(1,34) = 6.83, p < .05. The crucial two-way interaction between CS and Group failed to reach significance F(1,34) = 1.55, p = .22, suggesting that the extent of expectancy learning was not modulated by the between-subjects variable Group. Planned comparisons further confirmed that the patient group and the control group both showed higher expectancy ratings on CS+ trials as compared to CS− trials, F(1,34) = 35.22, p < .001 and F(1,34) = 80.13, p < .001, respectively. Finally, the three-way interaction between CS, Group, and Acquisition Trial was not significant, F < 1, suggesting that expectancy learning developed similarly in patients and controls (see Fig. 2a and b).

3.2.3. Post-acquisition phase

The CS × Group mixed ANOVA demonstrated that expectancy learning remained significant in the post-acquisition phase, F(1,34) = 82.92, p < .001. Similar to the acquisition phase, the two-way interaction between CS and Group was not reliable, F < 1, suggesting a similar level of expectancy learning in both test groups in the post-acquisition phase (see Fig. 2a and b). The patient group as well as the control group still showed differential responding, with respectively F(1,34) = 34.23, p < .001 and F(1,34) = 51.61, p < .001.

![Fig. 1. Standard temporal lobe resection for intractable temporal lobe epilepsy. T1-weighted MPRAGE sequence, sagittal view of mesial temporal region. (A) The preoperative MRI showed the amygdala, indicated by three small, bold arrows, lying above and in front of the hippocampus (small, long white arrow). (B) The postoperative MRI showed that the amygdala was removed and replaced with cerebrospinal fluid, which is black on a T1-weighted image (marked with ×).](image)

![Fig. 2. (a) Expectancy ratings registered during CS+ and CS− trials in the pre-acquisition phase (PRE), the acquisition phase (trials A1–A8), and the post-acquisition phase (POST), for the patient group. (b) Expectancy ratings registered during CS+ and CS− trials in the pre-acquisition phase (PRE), the acquisition phase (trials A1–A8), and the post-acquisition phase (POST), for the control group.](image)
3.3 Skin conductance responses

3.3.1 Unconditioned responses
A one-way ANOVA on the postexperimental US ratings revealed that patients and controls evaluated the US as equally intense, unpleasant, and startling, all Fs < 1. Nevertheless, an ANOVA performed on the unconditioned responses recorded during the acquisition phase demonstrated that the SCRs elicited by the electric shock were significantly higher in the patient group than in the control group, F(1,34) = 12.13, p < .01.

3.3.2 Pre-acquisition phase
A CS × Group mixed ANOVA on the conditioned SCRs revealed that there were no differential SCRs on CS+ and CS− trials prior to conditioning, F < 1. In addition, the two-way interaction between CS and Group failed to reach significance, F < 1. Neither of the two test groups showed differential SCRs in the pre-acquisition phase, F < 1 for both contrasts (see Fig. 3a and b).

3.3.3 Acquisition phase
A CS × Group × Acquisition Trial mixed ANOVA showed significant main effects of CS and Acquisition Trial, F(1,30) = 18.55, p < .001 and F(6,165) = 2.30, p < .05, respectively. Conditioned SCRs were significantly higher during CS+ trials as compared to CS− trials, suggesting that aversive conditioning emerged. According to the main effect of Acquisition Trial, conditioned SCRs declined significantly from the first to the last acquisition trial. The main effect of Group reached marginal significance, F(1,30) = 4.05, p = .05, suggesting that conditioned SCRs were more pronounced in the patient group than in the control group. The crucial two-way interaction between CS and Group, failed to yield significance, F < 1, suggesting that the extent of aversive conditioning was comparable in the two test groups (see Fig. 3a and b). Planned comparisons further confirmed that the control group, F(1,30) = 10.18, p < .01, as well as the lesion group, F(1,30) = 8.58, p < .01, showed reliable differential SCR. Additionally, the two-way interaction between CS and Acquisition Trial lacked significance, F(6,178) = 1.14, p = .34, indicating that the extent of conditioned SCR was comparable in size across the eight acquisition trials. Finally, also the three-way interaction between CS, Group, and Acquisition Trial was not reliable, F(6,178) = 1.21, p = .30, suggesting that conditioned SCRs developed in patients and controls in a similar way.

3.3.4 Post-acquisition phase
Finally, a CS × Group mixed ANOVA demonstrated that the main effect of CS failed to reach significance, F < 1, suggesting that differential SCRs disappeared in the post-acquisition phase. Also the two-way interaction between CS and Group yielded no significance, F < 1. Neither the control group, nor the lesion group showed differential SCRs in the post-acquisition phase, with F < 1 for both contrasts (see Fig. 3a and b).

3.4 Effect of lesion site
The ratio of left and right damaged patients was unequally distributed and the number of right damaged patients was very small. Nevertheless, we examined whether the degree of fear conditioning differed in left and right damaged patients in order to obtain a preliminary notion in regard to the role of the left and right amygdala in fear learning. For the dependent variable, two SCR means were calculated: one across the eight CS+ acquisition trials and one across the eight CS− acquisition trials. Subsequently, the mean of the CS− acquisition trials was subtracted from the mean of the CS+ acquisition trials, creating a differential conditioned SCR factor. Eventually, a non-parametric Wilcoxon signed-rank test was executed on this dependent variable showing that there was no significant difference in differential SCR between left and right damaged patients, Z = −1.07, p = .29. To test for possible effects of lesion site on the US-expectancy ratings given during acquisition, a similar method was used, creating a differential US-expectancy factor. Again, the Wilcoxon signed-rank test revealed no difference in differential US-expectancy ratings between left and right amygdala-damaged patients, Z = −.07, p = .95.

4. Discussion
Previous lesion studies suggested that the amygdala plays a crucial role in the generation of conditioned SCRs in a classical fear conditioning task in humans (Bechara et al., 1995; LaBar et al., 1995; Weike et al., 2005). The purpose of the current experiment was to expand our knowledge concerning the amygdala’s role in fear learning, by investigating whether this anterior temporal lobe region is also critically involved in the production of conditioned SCRs during a more explicit form of fear learning. A differential fear conditioning task was presented to a unilaterally amygdala-damaged patient group and a control group with SCRs and US-expectancy ratings being registered online throughout the whole task. As such, attention was directed towards the CS–US association, rendering the fear conditioning task more explicit. The results revealed that patients and controls rapidly acquired contingency knowledge as measured by the online US-expectancy ratings. Both test groups gave increased US-expectancy ratings during CS+ presentations and decreased US-expectancy ratings during CS− presentations. However, when examining the US-expectancy ratings carefully, one may notice that neither the controls, nor the patients showed maximized US-expectancy on CS+ trials. According to this latter finding, participants were not completely CS–US
aware, but acquired a reliable level of stimulus contingency awareness instead. More importantly, differential US-expectancy ratings were equally large in both groups and learning proceeded equally rapid in the patient group as in the control group. In sum, these findings suggest that unilateral damage to the amygdala caused no deficit, no reduction, and no retardation in the acquisition of stimulus contingency knowledge. Crucially, the introduction of online US-expectancy ratings had an enormous impact on the autonomic responses registered during fear learning in the unilaterally amygdala-damaged patient group. Contrary to earlier findings, the patient group as well as the control group showed increased conditioned SCRs during CS+ trials as compared to CS− trials. As for the US-expectancy ratings, differential SCRs were in both groups comparable in size, suggesting that conditioned SCRs were not even reduced after a unilateral temporal lobe resection. Additionally, differential autonomic responses developed similarly in patients and controls, as the pattern of conditioned SCRs was identical in both groups. Finally, according to the unpleasantness ratings of the CS+ and the CS− figures given at the end of the fear learning task, patients and controls rated the CS+ as less pleasant than the CS−, suggesting that aversive learning was established in both groups.

In the present study, we did not obtain any significant differences in conditioned SCRs between left and right amygdala-damaged patients. However, as the number of right amygdala-damaged patients was very small we should be careful in placing too much weight upon these findings. Nevertheless, earlier research by LaBar et al. (1995) and Weike et al. (2005) support this observation, as they similarly obtained no difference in autonomic fear conditioning in left and right temporal lobectomy groups. In addition, a lesion study conducted earlier by Peper, Karcher, Wohlfarth, Reinschagen, and LeDoux (2001), which was exclusively designed to examine the contribution of the left and right temporal lobe in fear learning, also reported no effects of laterality.

Except for these clear-cut results, two unusual findings emerged. First, the unconditioned SCRs that were registered during the acquisition phase appeared to be more pronounced in the unilaterally amygdala-damaged patient group than in the control group, suggesting that patients reacted stronger to the electric pulse than controls. In the previous study of Weike et al. (2005), where an electric pulse also acted as US, no such finding was observed. One possible explanation is that the differential URs obtained for patients and controls were caused by a different intensity of the electric pulse chosen at the beginning of testing by both test groups. Unfortunately, due to equipment failure the selected intensity of the electric pulse was not registered for the patient group, precluding us to verify this explanation. Nonetheless, we are convinced that in spite of this observed difference in URs between patients and controls, both groups experienced the task as equally sensitive. Especially, the finding that patients and controls gave comparable ratings on the US intensity, unpleasantness, and startling measures supports this idea. Finally, we would like to emphasize that for the reported conditioned responses, this difference in URs between both groups was taken into account. All conditioned responses were range-corrected by dividing them by the participant’s maximum response elicited by the US. This way the conditioned SCRs obtained during the acquisition phase could impossibly be influenced by the different URs for patients and controls.

Secondly, in both test groups the conditioned SCRs observed during fear acquisition were immediately extinguished in the post-acquisition phase. However, an important aspect of elicited SCRs is that their amplitude declines and eventually disappears with repeated stimulus presentations (Dawson et al., 2000). In the lesion studies reported so far (Bechara et al., 1995; LaBar et al., 1995; Weike et al., 2005) conditioned SCRs were not analyzed at the trial level as was done in our experiment. The conditioned SCRs recorded during acquisition were typically averaged over trials and reduced to no more than three or four acquisition blocks. By using the latter method, however, response habituation becomes less visible. Nevertheless, LaBar et al. (1995) also report a trend of response habituation in the control group late in acquisition.

Taken together, the current data extend previous research by demonstrating that robust conditioned SCRs can be obtained in unilaterally amygdala-damaged patients, under conditions that promote the acquisition of explicit knowledge regarding the stimulus association. Although our findings suggest that the amygdala plays no critical role in a more explicit fear learning task, we cannot fully exclude its involvement in the task. Other brain areas such as the contralateral intact amygdala or cortical regions may have compensated for the brain damage in our patient group, preventing us from detecting any effect of lesion (Coppens et al., 2006, 2007).

Next, our findings are discussed in function of the functional imaging studies and lesion studies on fear learning which were performed earlier. Overall, our SCR data were not completely in line with the hypotheses we formulated based upon the functional imaging results of Carter et al. (2006) and Cheng et al. (2006). Carter et al. (2006) observed that US-expectancy ratings and conditioned SCRs were based upon a different neurological circuitry, with the former correlating with activity in the middle frontal gyrus and the parahippocampal gyrus, and the latter correlating with amygdala, hippocampus, and occipital cortical activation. Consistent with the findings of Carter et al. (2006), Cheng et al. (2006) reported that metabolic changes in the amygdala were more closely related to the observed SCR pattern than to US-expectancy ratings. Consequently, we expected our patient group to show normal US-expectancy ratings, but reduced conditioned SCRs compared to the control group. Although the US-expectancy data were consistent with our initial hypotheses, the SCR findings were not, as they appeared equally pronounced in the patient and control group. Nevertheless, our findings are not necessarily at odds with those of Carter et al. (2006) and Cheng et al. (2006), as the observed correlation between amygdala activity and conditioned SCRs not automatically implies that this brain region is also essential for this process. In that sense, Carter et al. (2006) demonstrated that except for amygdala activation, activity in other brain regions, like the hippocampus and the occipital cortex, also correlated with the generation of conditioned SCRs. In other words, it is possible that the amygdala was still involved in the current task but to a lesser degree. In that case, the involvement of other presumably cortical areas may have overshadowed the possibility for detecting any observable effect of amygdala damage in the conditioned SCRs.

At first glance, the current finding of intact conditioned SCRs in unilaterally amygdala-damaged patients during fear conditioning also seems to challenge the findings of earlier conducted lesion studies (Bechara et al., 1995; LaBar et al., 1995; Weike et al., 2005), which report on amygdala-damaged patients failing to show conditioned SCRs during a fear learning task. Procedurally, however, the CS–US association presented in the current study was more explicit than in previous experiments. In the study of Bechara et al. (1995), for example, four different CSs were presented during the acquisition phase, with one of them (CS+) being followed by a startling sound (US) 50% of the time. This increased number of CSs and the use of a partial reinforcement schedule possibly made the task more complex and the stimulus contingency less apparent compared to our procedure. Moreover, the studies of LaBar et al. (1995) and Weike et al. (2005) included no explicit instructions regarding the presented stimulus association and no online US-expectancy ratings were collected, again, making the CS–US association less manifest. Altogether, without being contradictory, the lesion studies performed thus far suggest that conditioned SCRs during fear learning can arise via two pathways depending upon task instructions and explicitness of the presented stimulus contingency. The first route passes through the amygdala and is mainly applied dur-
ing traditional fear learning (Bechara et al., 1995; LaBar et al., 1995; Weike et al., 2005), whereas the second route bypasses the amygdala and is used primarily during more explicit fear learning.

A paradigm similar to ours was employed earlier by Funayama et al. (2001), who presented an instructed fear learning task to a unilaterally amygdala-damaged patient group and a healthy control group. Prior to experimentation, all participants were informed that throughout the course of the study at least one, but not more than three electric pulses would be delivered. Moreover, the shock would follow one particular stimulus (CS+) and never the other (CS−). In reality, only one shock was delivered, which occurred during the final CS+ presentation. Consequently, conditioned startle responding, when observed throughout acquisition, could only have resulted from linguistic/cognitive representations acquired through language. Thus, similarly to the explicit fear learning task we used, the CS−US association presented in the Funayama et al. (2001) study was very salient. Nonetheless, conditioned startle responses appeared to be impaired in left amygdala-damaged patients, but not in right amygdala-damaged patients or healthy controls. Again, these findings seem to contradict our results at first sight, as most of our patients sustained a left temporal lobe resection and nevertheless demonstrated normal conditioned SCRs. However, the use of a different dependent measure, conditioned startle responding instead of conditioned SCR, may account for these opposing findings. According to Hamm and Weike (2005), both responses index different aspects of learning and possibly rely on different neural circuits. More important, however, is that preliminary research indicates that in humans increased startle responding during the observation of aversive stimuli is diminished after a unilateral temporal lobe resection (Buchanan et al., 2004; Funayama et al., 2001). As a consequence, when observing impaired startle responding in unilaterally amygdala-damaged patients during fear learning, this could be caused by a general disturbance of startle responding instead of by a fear learning impairment.

Although the current findings differ significantly from those obtained earlier, we demonstrated that they are not necessarily at odds with them. In addition, our data are in line with the hypotheses we formulated based upon the dual-level account of Öhman and Mineka (2001). Basically, the two-level account proposes that fear learning can occur at a lower and higher level, with the former referring to a basic associative level of learning as appears in a traditional fear conditioning task and the latter comprising learning of the stimulus association at a more cognitive level. With respect to the neural circuitry involved, low level learning is considered to be mediated by the amygdala, whereas high level learning is thought to be centered on the hippocampus for simple CS−US associations and the prefrontal cortex for more complicated stimulus relationships. Moreover, Öhman and Mineka (2001) suggest that fear learning in humans can occur at either of these two levels depending upon task requirements, attentional factors or complexity of the CS−US association. Besides, according to the original model, conditioned startle potentiation has been proposed as a reliable index for low level learning, whereas conditioned SCRs have been considered as an index for high level learning. Regarding this latter assumption, however, we would like to emphasize that until today no conclusive evidence is available of whether conditioned SCRs are exclusively related to high level learning (Lovibond and Shanks, 2002). Therefore, we propose that conditioned SCRs not necessarily have to be related to high level learning, but could reflect low learning level as well.

When adjusting this last assumption, the two-level model of Öhman and Mineka (2001) fits well with the lesion data obtained so far. According to the theory, fear learning in humans can occur at either a low or high learning level depending upon explicitness of the CS−US association. In the previous studies of Bechara et al. (1995), LaBar et al. (1995), and Weike et al. (2005) the presented CS−US association was not explicitly accentuated, leading conditioned SCRs to result from a low level of learning, centered on the amygdala. By introducing US-expectancy ratings in the present experiment, the CS−US association became more manifest, triggering the more cognitive level of learning. Consistent with the idea that high level learning is independent of amygdala involvement, unilaterally amygdala-damaged patients showed normal conditioned SCRs.

Eventually, we would like to call attention on two unresolved issues within the human conditioning literature. First, at present a lot of inconsistency exists in regard to what is being indexed by conditioned SCRs during Pavlovian fear learning. On the one hand, many neuropsychological studies examining the neurological basis of fear conditioning refer to conditioned SCR as an implicit conditioning response, occurring rather automatically and independently from stimulus contingency awareness (e.g. Carter et al., 2006; Cheng et al., 2006; Peper et al., 2001). On the other hand, most of the evidence available today in the human conditioning literature is consistent with the position that conditioned SCRs and stimulus contingency awareness are strongly related to each other. More precisely, awareness is generally considered as a necessarily but insufficient prerequisite for the occurrence of conditioned SCRs. The two-level account of fear learning (Öhman and Mineka, 2001; Weike et al., 2005) which we referred to earlier, suggests that conditioned SCR forms a reliable index for high level learning, whereas conditioned startle responding reflects low level learning. We would like to underline that currently, the issue of whether conditioned SCR is exclusively related to contingency learning occurring at a high learning level (Lovibond and Shanks, 2002) or under some circumstances might be an index for low level processing as well (Öhman and Soares, 1994) is still under discussion. Similarly, whether conditioned startle responding only results from low level learning or could also be the product of cognitive learning still remains to be solved, although the earlier findings of Weike et al. (2005) and Funayama et al. (2001) suggest that conditioned startle responses seem rather unaffected by CS−US awareness or explicitness of the CS−US association.

Second, until today, a massive debate is going on in the human conditioning literature between opponents of the single-process theory on the one hand and the dual-process theory on the other hand. According to the former account, all learned behavior is determined by a single propositional reasoning system, which requires cognitive resources and attention. The dual-process approach on the other hand incorporates all the reasoning processes of the propositional approach plus an additional automatic link formation mechanism in which learning about relationships occurs automatically via the formation of links between mental representations of events (for an overview, see Lovibond and Shanks, 2002). The dual-level account of fear learning (Öhman and Mineka, 2001), which we referred to earlier, is one such example of a dual-process approach. Up till now, no evidence exclusively in line with one of the two approaches is available and the dispute between both theories continues. In this regard, we would like to emphasize that although our findings fit nicely within a dual-process theory, they not necessarily contradict a single-process theory either.

In conclusion, the current findings extend previous findings. In particular, by using online US-expectancy ratings, we demonstrate that robust SCR conditioning can be obtained in patients with unilateral amygdala damage as long as they are able to acquire stimulus contingency awareness. This is slightly in contrast with previous research (Bechara et al., 1995; LaBar et al., 1995; Weike et al., 2005), which demonstrated that unilateral amygdala-damaged patients show impaired conditioned SCRs during classical fear conditioning. Therefore, the present findings suggest that the amygdala is less involved in the generation of conditioned SCRs in a more explicit form of fear learning than it is in the production of con-
tioned SCRs during traditional fear conditioning. A dual-process theory is proposed in order to explain for the present and previous findings.

References


